EVALUATION OF SALIVARY FLOW RATE, GUSTATORY PERCEPTION AND CANDIDAL CARRIAGE IN PATIENTS WITH ORAL SUBMUCOUS FIBROSIS – AN OBSERVATIONAL ANALYTICAL CASE CONTROL STUDY

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LIST OF ABBREVIATIONS

Abbreviation	Full Form
OSMF	Oral submucous fibrosis
SFR	Salivary flow rate
C.	Candida
OPMDs	Oral potentially malignant disorders
OSCC	Oral squamous cell carcinoma
MST	Modified schirmer test
mm	Millimeters
RSFR	Resting salivary flow rate
SDA	Sabouraud’s dextrose agar
KOH	Potassium hydroxide
CFU	Colony forming units
ANOVA	Analysis of variance
SD	Standard deviation
P-value	Probability value
NS	Statistically not significant
HS	Statistically Highly significant
S	Statistically significant

Introduction

Hippocrates has rightly said :

"There are in fact two things, science and opinion; the former begets knowledge, the latter ignorance."

"Every human being is the author of his own health and disease."

– Siddhartha Gautam Buddha.

Oral submucous fibrosis (OSMF) is a well-known chronic debilitating disorder affecting any part of the oral cavity. **Schwartz in 1952** was the first to describe OSMF. He termed the lesion as 'Atrophia idiopathica (tropica) mucosa oris'. This was later designated as OSMF by **Joshi in 1953**. OSMF has been known under various terminologies such as 'Idiopathic scleroderma of the oral cavity', 'Sclerosing stomatitis', and 'Juxta epithelial fibrosis'. A literature search reveals that in **600 B.C.**,

a condition similar to OSMF was reported by **Sushruta** who termed it as 'VIDARI'.[1,2] The geographical extent of this chronic disorder is considered to affect mainly the people in Southeast Asia, especially the Indian subcontinent. The reason that has been stated for the occurrence of OSMF among the people in this regions of the world is mainly due to extensive use of Arecanut in various forms.[3] Arecanut is the endosperm of the fruit of the Areca catechu palm tree.[4] Arecanut and tobacco together are used in a smokeless form in paan (betel leaves), mawa, kharra, gutkha, zarda, khaini, niswar, scented supari or meethi supari etc.[5] Apart from this, various factors have also been stated such as genetic and immunologic processes, nutritional deficiencies, more consumption of chillies etc.[6] In Indian population, Arecanut chewing has been reported to be the fourth "dependent substance" which is a main cause for the occurrence of OSMF followed by nicotine, alcohol and caffeine.[7] Although a lot of literature about this disorder has been published in recent times, however, the definition given by **Pindborg in 1966** still stands apt for the description of OSMF both clinically and histologically. He stated OSMF as "an incidious, chronic disease affecting any part of the oral cavity and sometimes pharynx. Although occasionally preceded by and/or associated with vesicle formation, it is always associated with juxta-epithelial inflammatory reaction followed by fibroelastic changes in the lamina propria, with epithelial atrophy leading to stiffness of the oral mucosa causing trismus and inability to eat."[8] As far as the oral cavity is concerned the most commonly affected areas is the buccal mucosa followed by palate, retromolar area, faucial pillars and pharynx.[9] For dentists, OSMF is of concern and an important disease because of its potentially malignant behaviour. It is one of the most prevalent, potentially malignant and preventable disorder of oral mucosa in India.

Numbers of studies have been documented of varying results of OSMF turning into malignancy. The exact reason of the malignant transformation is not known. However, it is considered to be a disorder of collagen, the malignant potential of OSMF could be due to micro-trauma due to usage of arecanut and tobacco that is imposed onto the epithelium. This then makes it susceptible to oral cancers.[10,11]

Arecanut is used as the fourth most common psychoactive substance. Arecanut contains several components or substances such as carbohydrates, fats, protiens, crude fibers, alkaloids, polyphenols like flavonols and tannins and some mineral content, which all interfere with homeostasis of the extracellular matrix of oral tissue.[12,13] Arecoline, arecaidine, guvacoline and guvacine are the four major alkaloids of arecanut. These all have several biological and stimulating effect and are known to stimulate fibroblasts to produce collagen.[12]

Essentially, Kharra/Gutkha consists of arecanut (7-9 gms) with the combination of tobacco (2-3 gms) and few drops of slaked lime placed on cellophane paper and the contents are homogenized by rubbing. In the presence slaked lime (calcium oxide, turns to alkali calcium hydroxide in aqueous form), arecoline which has a parasympathomimmetic effect and guvacoline are effectively hydrolysed into arecaidine and guvacine respectively, which makes it alkaline facilitating absorption to give immediate euphoric effect. This cause amplified fibroblastic proliferation and increased collagen formation.[4,12]

Thus, in Kharra/Gutkha chewers, leaching of the chemicals of arecanut and minerals such as copper and iron in the saliva and oral mucosa have been noted, which alters the properties and composition of saliva. Because of the

parasympathomimetic effect on oral mucosa and saliva, it shows variation in salivary flow rate and taste parameters.[14]

In the oral cavity, Saliva is a critical and complex fluid produce by the salivary glands that preserves the oral cavity. It creates and regulates the healthy environment in the oral cavity by forming a film of fluid coating the teeth and mucosa. It has a primary role in dissolving the taste stimulus to taste buds.[13]

The Composition of Saliva is (Fig. no. 1):-

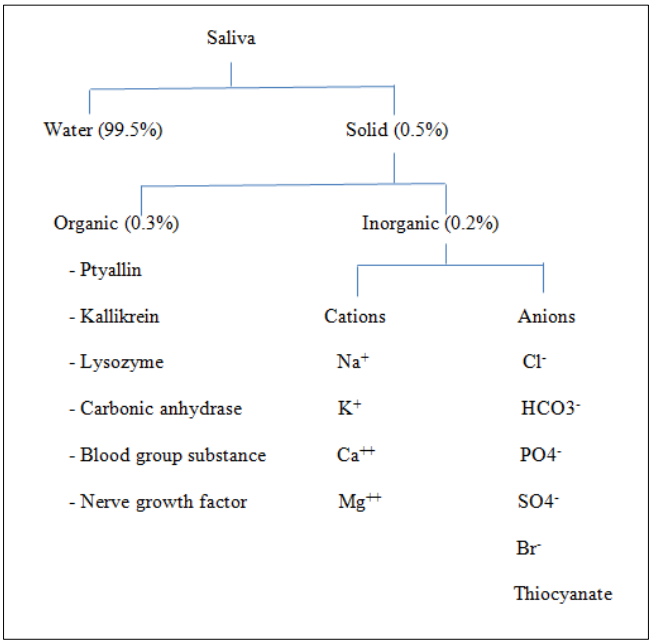


Fig. no. 1: Flowchart showing composition of saliva.[15]

There can be the variations in these components depending upon the nature, source of saliva, the intensity of the secretory stimulus and the time of the day. The total volume of saliva secreted is approximately 750 to 1000 ml per day by humans. The resting salivary flow rate of the whole saliva is 0.2 to 0.4 ml/min, parotid is 0.4 ml/min, submandibular is 0.1 ml/min. On stimulation the rate increases to 0.2 to 0.4 ml/min for the whole saliva, 1.0 to 2.0 ml/min for parotid and 0.8 ml/min for the

submandibular salivary gland.[15] Several studies and cases have reported among the OSMF affected individuals with the manifestation of altered salivary flow but studies do not clearly mention an increase or decrease in the salivary flow rate (SFR) and taste perception.

Taste sensation is perceived due to the presence of taste buds present over the dorsum of tongue, larynx and oesophagus.[16] The ability to taste or taste sensations occurs when the molecules are released during mastication, drinking or digestion.[17] There are various factors which alters the taste perception. This may be due to inflammation and infection in oral cavity which ultimately reduce the blood flow and lead to injury to taste buds or may cause the atrophy of papilla.[18]

Human beings are able to recognize the basic tastes i.e. sweet, salt, bitter and sour. As salivary flow has a function of dissolving the taste stimulus and carry it to the taste buds, an alteration in the saliva can affect the taste perception both qualitatively and quantitatively.[14] This may lead to either dysguesia (distorted normal taste) or aguesia (absence of taste). Alteration in properties of saliva i.e. salivary flow due to any reason can cause changes in its flow rate and consequently the taste perception which unfortunately will affect the quality of life.[19]

OSMF clinically present as a reduced mouth opening and protrusion of tongue, blanching of oral mucosa and burning sensations. There is atrophy of the epithelium marked in advanced stages of OSMF.[1] The common fungal infections in the oral cavity are caused by Candida species. Candida, the name is derived from an ancient custom in Rome for a candidatus, which is a candidate for public office, to

dress in white color. And Albico means "to be white", hence the term Candida Albicans. There are various pathognomic species like Candida (C.) albicans, C. tropicalis, C. parapsilosis, C. krusei, C. lusitaniae and C. glabrata. The primary cause of Oral candidiasis is C. albicans. These are the opportunistic organisms which shows lesions in patients with reduced salivary flow.[20,21] The presence of Candida in the mouth together with epithelial changes such as atrophy, hyperplasia and dysplasia, may compromise the mucosal barrier and facilitate candidal invasion in both immunocompetent and immunocompromised individuals by colonizing, invading and inducing lesions in any part of oral cavity.[22] Candida albicans is the predominant species isolated in potentially malignant and malignant diseases. This infection can induce epithelial atypia and can transform into malignancy or carcinoma by the release of chemical carcinogens such as nitrosamine compounds – Nitrosamine N-nitrobenzylmethylamine and carcinogenic acetaldehyde.[23] Therefore, Candida could also play a role in malignant transformation in this potentially malignant disorder, where there is the atrophy of the epithelium leading to excessive penetration of the carcinogen. But the biology behind this association is still unsolved.

OSMF has always been a challenging disease with high prevalence in India. Any alterations in the above parameters i.e. salivary flow rate, gustatory perceptions and candidal carriage will affect the overall quality of life of an individual as well as patients with OSMF, where malignant transformation rate has reported ranging from 7% to 13%.[24]

Therefore, the study is undertaken with a purpose to evaluate and compare the salivary flow rate, gustatory perceptions and candidal carriage among patients with OSMF and normal individuals. Management of these changes can be done together with physiotherapy, pharmacotherapy and follow up which can bring satisfaction with an improved quality of life to the patient.

Aim and Objectives

The present study will be an attempt to evaluate Salivary flow rate, Gustatory perception and Candida carriage in patients with Oral submucous fibrosis with the following aim:

AIM

To evaluate Salivary flow rate, Gustatory perception and Candida carriage in patients with Oral submucous fibrosis.

The aim will be fulfilled with the help of the following objectives:

OBJECTIVES

1. To evaluate the Salivary Flow Rate among OSMF patients.

2. To evaluate the Gustatory Changes among OSMF patients.

3. To evaluate the Candidal Carriage among OSMF patients.

4. To evaluate and compare all the 3 parameters among OSMF & Healthy individuals.

Review of Literature

Oral Potentially malignant disorders (OPMDs), the most dreaded diseases, have always been in the discussion due to its extensive spread in the World. Today, OPMDs remain as the greatest problem facing the pathologists and oncologists in India. Statistically speaking, tumors of the oral cavity and oropharyngeal region rank first among males and third among females while constituting approximately 30% of all cancers. It is a matter of regret that the mortality rate from oral cancer is still high. This is often due to late diagnosis and lack of prognostic variables. It is unlikely that Oral squamous cell carcinoma (OSCC) arise directly from the normal epithelium. The epithelium first in all probability goes through a series of stages of initiation and promotion leading to OPMDs. These lesions further produce morphological changes in the cells which results in clinically definable lesions.[25]

"Oral potentially malignant disorders", though the awkward phrase, the term clearly defines a group of lesions that carries an increased risk of cancer progression and underscores the complexity and hamstring for clinicians, pathologists, and their patients in assessing cancer risk. The 2017 World Health Organization (WHO) definition of OPMDs is "clinical presentations that carry a risk of cancer development in the oral cavity, whether in a clinically definable precursor lesion or in clinically normal mucosa." The term oral potentially malignant disorders describe a recognizable group of mucosal diseases that have a risk of progressing to squamous cell carcinoma.[26]

ORAL SUBMUCOUS FIBROSIS

Oral submucous fibrosis is a chronic, progressive scarring disease that predominantly affects the people of South-East Asian origin. The onset is insidious, over 2 to 5 years. Prodormal symptoms includes a burning sensation in the mouth on consumption of spicy food, appearance of vesicles over palate, ulcerations or recurrent generalized inflammation of the oral mucosa, altered salivation, defective gustatory sensation and dryness of the mouth. Focal vascular dilatations manifest clinically as petechiae in the early stages of the disease. **Rajendra et al. in 1994**, observed Petechiae in about 22% of OSMF cases, mostly on tongue followed by the labial mucosa with no sign of blood dyscrasias or systemic disorders. Pain is noticed in the areas of submucosal fibrotic bands when palpated, is a useful clinical test.

As the disease progress, the oral mucosa becomes blanched, slightly opaque and white fibrous bands appears. The buccal mucosa and lips may affect at the early stage but however, it was thought that the palate and faucial pillars are the first

involved sites. The oral mucosa is involved symmetrically and the fibrous bands in the buccal mucosa run in a vertical direction. The density of the fibrous bands deposits ranges from a slight whitish area on the soft palate, with no symptoms, to a dense fibrosis which cause fixation and shortening and even deviation of the uvula and the soft palate. In the cases of the dense fibrosis, the involved tissue around the pterygomandibular raphae, causes varying degrees of difficulty in the mouth opening. In some of the cases, the fibrosis spreads to the pharynx and down to the piriform fossae. There is restricted tongue movement with significant atrophy of the tongue papillae observed in the advanced stage of OSMF. With the progressing fibrosis, it leads to difficulty in opening the mouth, inability to blow out a candle or balloon or whistle and difficulty in swallowing (Fig. no. 2). When the fibrosis progresses to involve the nasopharynx, the patient may experience referred pain to the ear and a nasal voice as one of the later signs.[27]

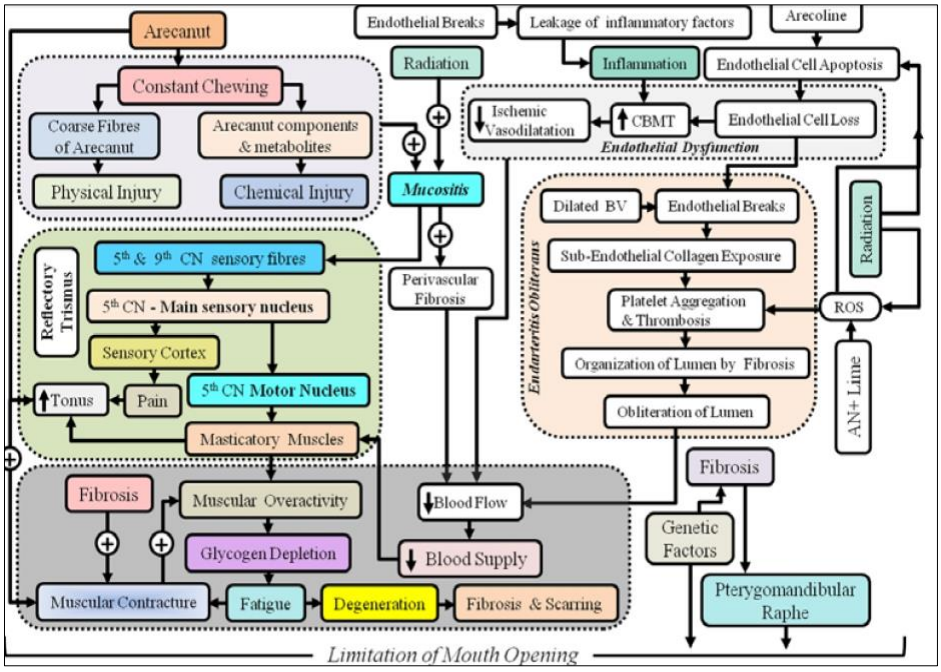


Fig no. 2: Schematic representation of limitation of mouth opening.[28]

CLASSIFICATION SYSTEMS

There are various classification systems of OSMF based on both clinical and histopathological aspects.

Table no. 1: Classification by Pindborg and Sirasat 1966, according to histopathology.[8]

Very early stage	Finely fibrillar collagen dispersed with marked edema. Plump young fibroblast containing abundant cytoplasm. Blood vessels dilated and congested with inflammatory cells mainly polymorphonuclear leukocytes.
Early stage	Early hyalinization of juxta-epithelial area. Collagen in thick bundles. Moderate no. of plump young fibroblast. Dilated and congested blood vessels with inflammatory cells primarily lymphocytes, eosinophils and occasional plasma cells.
Moderately advanced stage	Collagen is moderately hyalinized. Thickened collagen bundles are separated by slight residual edema. Fibroblastic response is less marked. Blood vessels either normal or compressed. Inflammatory response consists of lymphocytes and plasma cells.
Advanced stage	Collagen is completely hyalinised. Smooth sheets with no separate bundles of collagen seen. Edema absent. Hyalinised area is devoid of fibroblasts. Blood vessels are completely obliterated or narrowed. Inflammatory cells are lymphocytes and plasma cells.

Table no. 2: Classification by Pindborg 1989, according to clinical features.[29]

Stage I	Stomatitis includes erythematous mucosa, vesicles, mucosal ulcers, melanotic mucosal pigmentation and mucosal petechiae.
Stage II	Hallmark of this stage is Fibrosis occurring in healing vesicles and ulcers.
	i. Early lesions = blanching of oral mucosa.
	ii. Older lesions = verticle & circular fibrous bands in buccal mucosa around the mouth opening or lips.
	iii. This results in a mottled marble like appearance.
	iv. Specific findings = reduction in mouth opening, stiff and small tongue, blanched and leathery floor of the mouth, fibrotic and de-pigmented gingiva, rubbery soft palate, shrunken bud like uvula, sunken cheeks, not commensurate with age or nutritional status.

Stage III	Sequelae of OSMF are as follows:
	v. Leukoplakia found in more than 25% of individuals with OSMF.
	vi. Speech and hearing deficit may occur because of involvement of tongue and the Eustachian tube.

Table no. 3: Classification by Khanna JN and Andra de NN 1995, according to clinical and histopathological grouping.[30]

Group I Very early cases	Clinical features	Burning sensation, acute ulceration, recurrent stomatitis. Not associated with mouth opening limitation.
	Histopathology	Fine fibrillar collagen network interspersed with marked edema. Blood vessels dilated and congested. Plump aggregates, young fibroblast. Polymorphonuclear (PMN) leukocytes with few eosinophils. Normal epithelium.
Group II Early cases	Clinical features	Buccal mucosa marble like and motted. Widespread sheet of palpable fibrosis. Interincisal distance = 26-35 mm.
	Histopathology	Juxta-epithelial hyalinization present. Thick and separated collagen bundle. Dilated and congested blood vessels. Inflammatory cells (PMNs, eosinophils with plasma cells.)
Group III Moderately advanced cases	Clinical features	Trismus evident. Interincisal distance = 15-20 mm. Atrophied vermillion border, pale and firmly attached buccal mucosa to underlying tissue. Vertical fibrous bands palpable on soft palate, ptergomandibular raphae and anterior faucial pillar.
	Histopathology	Juxta-epithelial hyalinization present. Thick and separate collagen bundle with slight cellular oedema. Constricted blood vessels. Plasma cells and lymphocytes seen. Atrophied epithelium with loss of rete pegs. Interspersed muscle fibre with dense collagen bundle.
Group IV A. Advanced cases	Clinical features	Trismus is severe. Interincisal distance < 15 mm. Fauces thickened and shortened and firm on palpation. Uvula shrunken and appears as small fibrous bud. Limited tongue movement. Circular bands felt around entire mouth.

B. Advanced cases with malignant & premalignant changes.	Histopathology	Hyperkeratosis/leukoplakia, squamous cell carcinoma can be seen. Smooth hyalinised collagen. Extensive fibrosis. Obliterated blood vessel and reduced melanocytes. Fibroblast markedly absent in hyalinised zone. Total loss of epithelial rete pegs. Mild to moderate atypia. Extensive degeneration of muscle fiber.

Table no. 4: Classification by More C et al 2011, according to clinical and functional staging.[2]

Clinical staging

Stage I (S1)	Stomatitis and/or blanching of oral mucosa.
Stage II (S2)	Presence of palpable fibrous bands in buccal mucosa and/or oropharynx, with/without stomatitis.
Stage III (S3)	Presence of palpable fibrous bands in buccal mucosa and/or oropharynx, and in any other parts of oral cavity, with/without stomatitis.
Stage IV (S4)	1. Any one of the above stage along with other potentially malignant disorders, e.g. oral leukoplakia, oral erythroplakia, etc.
	2. Any one of the above stage along with oral carcinoma.

Functional staging

M1	Interincisal mouth opening upto or greater than 35 mm.
M2	Interincisal mouth opening between 25 and 35 mm.
M3	Interincisal mouth opening between 15 and 25 mm.
M4	Interincisal mouth opening less than 15 mm.

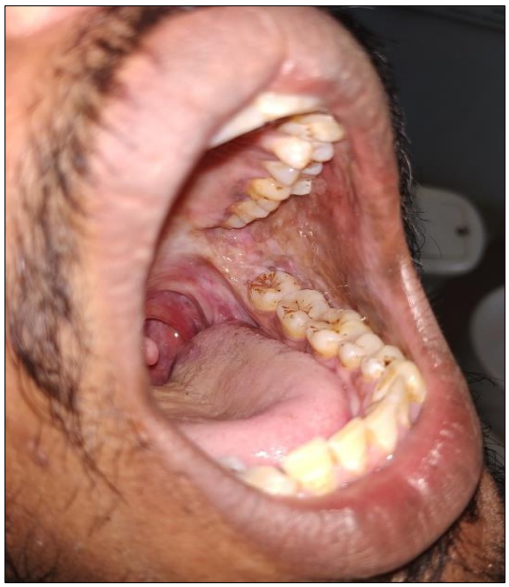


Fig. no. 3: Clinical photograph showing Stage I OSMF – not associated with mouth opening limitation.

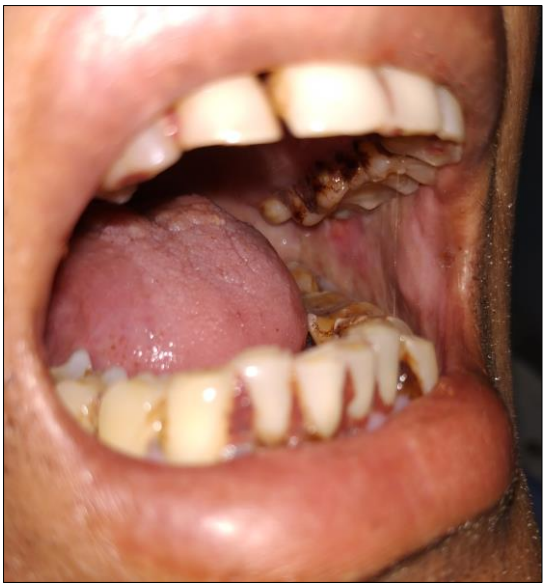


Fig. no. 4: Clinical photograph showing Stage II OSMF – interincisal distance 26 mm.

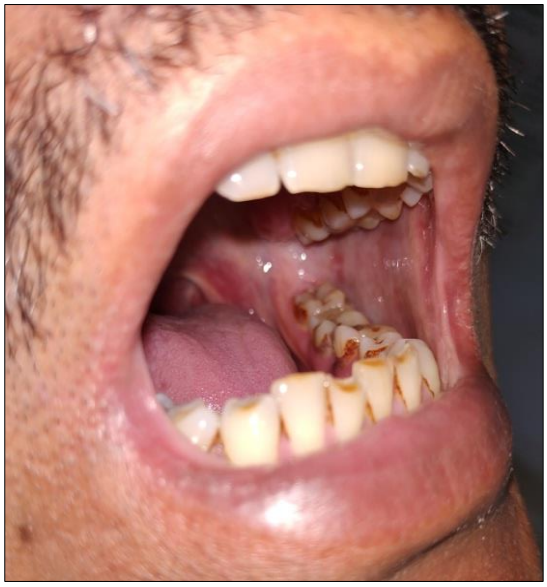


Fig. no. 5: Clinical photograph showing Stage III OSMF – interincisal distance 19 mm.

Some of the previous studies on the pathogenesis of OSMF suggested that the occurrence may be due to the following[27] : (Fig. no. 6)

1. **Harvey et al. 1986**, Stimulation of fibroblast proliferation and collagen synthesis by arecanut alkaloids.
2. **Meghji et al. 1987**, Clonal selection of the fibroblasts with the high amount of collagen production during the long term exposure to areca quid ingredients.
3. **Scutt et al. 1987**, By stabilization of collagen structure by catechin and tannins from the arecanut.
4. **Shieh et al. 1992**, By decreased secretion of collagenase.
5. **Ma et al. 1995**, By an increase in collagen cross-linkage as caused by upregulation of lysyl oxidase by OSMF fibroblasts.
6. **Kou et al. 1995**, By the production of collagen with a more stable structure (collagen type I trimer) by OSMF fibroblasts.
7. **Tsai et al. 1999**, Deficiency in collagen phagocytosis by OSMF fibroblasts.

8. **Haque et al. 2000**, By fibrogenic cytokines secreted by activated macrophages and T lymphocytes.

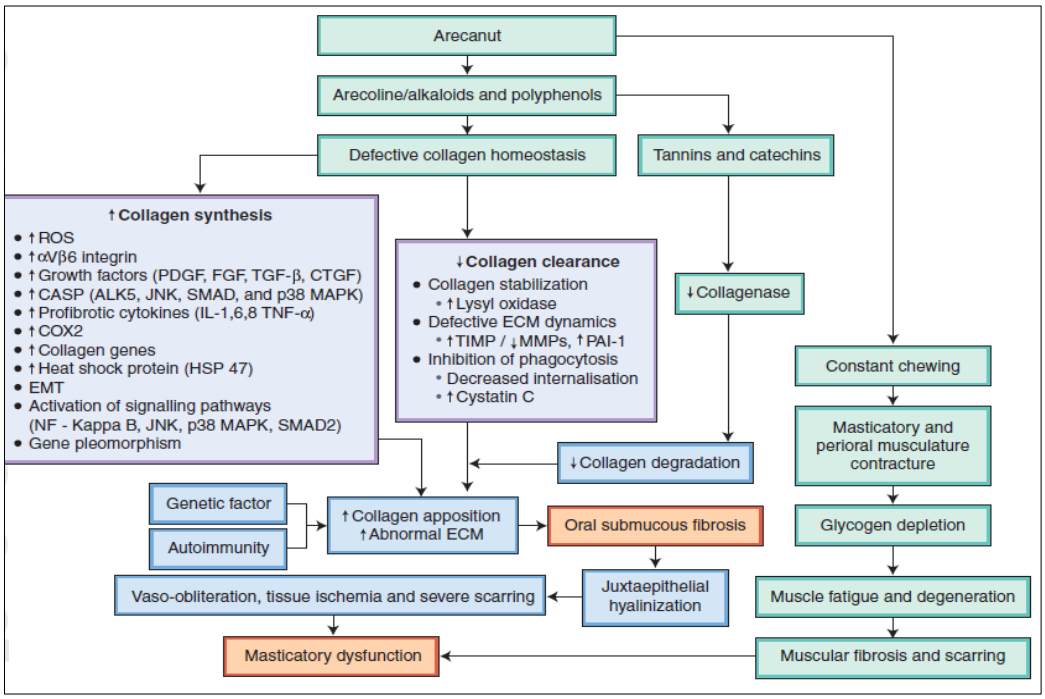


Fig. no. 6: Schematic representation of overview of the complex pathogenesis of OSMF.[4]

In OSMF, the incidence of dysplasia varies from 7-26%. However, the precise mechanism behind the malignant transformation is not clearly understood, but it may involve many promoting factors and co-factors and hence, the development into malignancy can be consider to be multifactorial.[31]

Though there are many factors involved, Arecoline is the most abundant areca alkaloid, suggested to be a possible carcinogen. It plays roles to be cytotoxic and genotoxic to oral mucosal fibroblasts, oral keratinocytes and also inhibits the growth, attachment, and matrix protein synthesis of cultured human gingival fibroblasts.[32] Arecoline induced effects in the mechanism of malignant transformation of OSMF is explained in fig. no. 7.

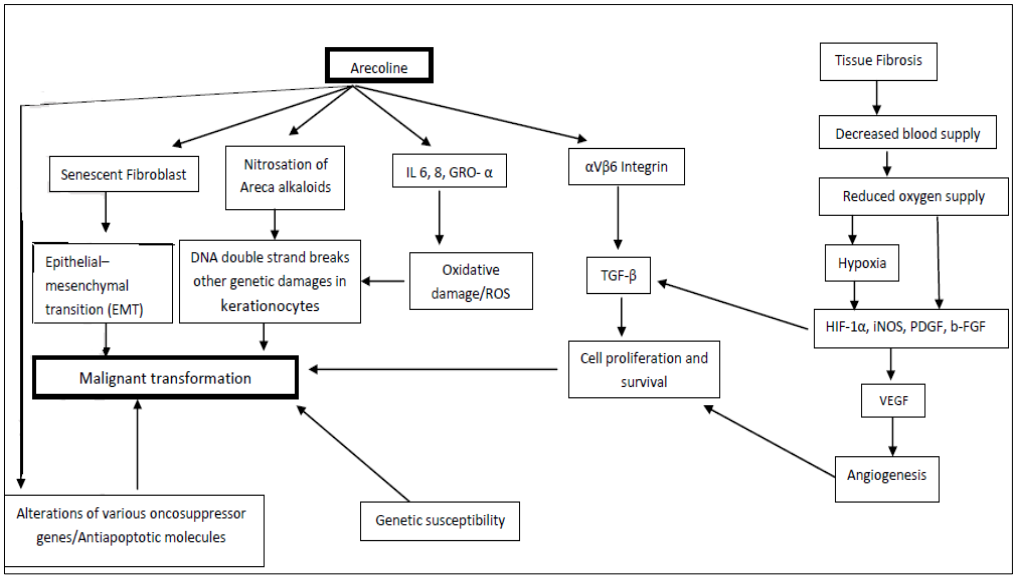


Fig. no. 7: Schematic representation of mechanism of arecoline induced malignant transformation of OSMF.[32]

Winkler et al. (1999)[33] reviewed depressed taste and smell in geriatric patients. They found that considerable differences exist between elderly people and young people in regards to sensory perception. They concluded that sensitivity to salty and bitter tastes declines with age but sweet and sour perceptivity does not. The authors also found that most of the studies reviewed suggested that the sense of smell is more impaired by aging as compared with the sense of taste. They concluded that smoking diminishes the taste of food and makes flavourful foods taste flat, while tongue brushing can increase taste sensation for geriatric patients.

Reichart et al. (2002)[34] studied betel quid-associated oral lesions and oral candida species in a female cambodian cohort by taking oral swabs (Fungiquick1, Hain Diagnostika, Germany) from tongue and palate of 48 Cambodian women with betel-quid chewing habit and 13 control subjects. They found candida species in 70.8% of the cases and 69.2% in controls. There was no significant difference in the

candidal carriage rate isolated between the study and the control group. They reported that the chemical constituents of betel quid have no overall effect on the oral carriage of candida and do not preferentially affect the specific candida species present in the oral environment.

Chen A et al. (2005)[35] had a preliminary study using the modified schirmer test (MST) to measure mouth dryness on 10 patients who had received head and neck radiation, 10 oral chronic graft versus-host disease and 41 healthy adult volunteers. They found that the mean reading for the control subjects at three minutes was 29.5 millimeters (mm), while that for the test subjects was 6.9 mm ($P = < 0.00005$). They concluded that the results of the MST were able to distinguish between healthy adult volunteers and subjects who experienced profound xerostomia and hyposalivation.

Rooban T et al. (2006)[14] studied the effect of habitual arecanut chewing on resting whole mouth salivary flow rate (RSFR) and pH in 110 chewers and 50 non-chewers. They found no significant difference between the mean SFR of chewers and non-chewers. But found a statistically significant difference between mean pH of chewers and non-chewers ($P = 0.02$). They concluded that with chewing raw arecanut, an increase in frequency and exposure time increased SFR and pH respectively and in processed arecanut chewers, increase in duration and frequency of consumption increased SFR and decreased pH respectively.

Ariyawardana A et al. (2007)[36] carried out a case control study, Oral submucous fibrosis and oral yeast carriage in Sri Lankan patients with 30 OSMF and 30 healthy subjects by oral rinse technique and cultured on sabouraud's agar media (SDA). Species formed were identified using API 32C AUX identification kits. They

found 19 (63.6%) of the test group and 15 (50%) of the control group having yeast isolated from their mouth. However, it was not statistically significant (x2 = 1.086, degree of freedom = 1, P = 0.29).

Zaidan F, Al-Omary W and Al-Sandook T (2008)[37] studied Threshold sensitivity of taste perception and the role of saliva and zinc level in some physiological & pathological conditions which included 218 individuals (35–80) years old divided into six groups; smokers, diabetics, haemodialysis patients and hypertensive patients on chronic use of captopril, 60 years aged subjects and controls. They found a significant increase in the taste detection and recognition thresholds of the four basic tastes i.e. sweet, salty, sour and bitter of all groups than in the control, except the salty taste thresholds of the haemodialysis group and the salty taste detection threshold of the diabetics group. And also a significantly decrease ($P < 0.001$) in saliva flow rates, serum and saliva zinc levels in study groups compared to the control group. They suggested that decreased saliva flow rate and saliva zinc concentration could be causative factors for hypogeusia.

Khan GJ, Javed M and Ishaq M (2010)[38] conducted a study on effects of smoking on SFR in 2 groups: 20 male smokers and control group by collection of saliva under resting condition and following application of crude nicotine and citric acid solution to the tip of the tongue. They found no statistical significance under resting condition, after stimulation with nicotine and following stimulation with citric acid in smokers as compared to controls ($P < 0.05$). They concluded that taste receptor responses in smokers were not adversely affected with long term tobacco smoking and hence not altering the SFR as compared to the control group.

Anila et al. (2011)[22] carried out a comparative study of candida in OSMF and healthy individuals in 20 OSMF and 20 healthy patients by oral rinse technique and cultured on SDA media. They found that the incidence and intensity of candida (primarily C. albicans) was higher in OSMF patients than in healthy controls, but these findings were within the normal limit (3-47%). They concluded that candida may not be an etiologic factor in malignant transformation.

Kamat et al. (2011)[39] studied oral candida carriage, quantification and species characterization in OSMF patients and healthy individuals in 30 clinically diagnosed OSMF cases and 20 healthy individual by sterile swab technique inoculated on SDA and CHROMagar culture media and candida species were identified using the KB006 candida identification. They found 11 (36.67%) cases in the study group and 2 cases (10%) in the control group yielded candida growth on culture, and the difference was statistically significant (v2-test = 4.4350, P = 0.0350). They concluded that OSMF favours the colonization of candida. Mucosal alterations due to underlying disease process or betel-quid chewing coupled with other factors might lead to candida colonization even in the absence of clinically related mycotic manifestation.

Sharma P and Saxena S (2011)[40] quantified the presence of C. albicans in 45 precancerous lesions (group I), 45 malignant lesions (group II) and 45 cases of precancerous conditions such as Lichen planus and OSMF (group III). They made three cytological smears from lesional tissue, one wet smear with 10% potassium hydroxide (KOH) solution and the two other smears with Gram's stain and periodic acid Schiff (PAS) stain. Colonies were cultured on SDA medium followed by quantification on a colony counter. They found statistically significant association

between C. albicans and precancer (P = <0.05, P = <0.01) and similarly in the frequency of oral yeast carriage (P = <0.05, P = <0.01) in the malignant lesions group than the OPMDs. They suggested a close correlation of candida infections to Leukoplakias and OSCC supporting an association between candida species and oral neoplasia.

Deeplaxmi et al. (2012)[41] studied altered taste perception in OSMF patients by analysing 15 OSMF patients and 15 healthy individuals using four different freshly prepared solutions for basic tastes (sweet, salty, sour, bitter) in increasing concentrations for localized testing and whole mouth rinse test. Sucrose for sweet (0.1-1.0 moles per litre), citric acid for sour (0.320-0.032 moles per litre), quinine hydrochloride for bitter (0.01-1.0 moles per litre) and sodium chloride for salty (0.01-1.0 moles per litre) were used. They found delayed perception with sweet taste followed by salt, bitter and sour in OSMF patients when compared to controls. They also observed altered taste perception increasing with severity of OSMF. There was early response to sour taste (93.3%) with both tests.

Shinozaki et al. (2012)[42] studied close association between oral candida species and oral mucosal disorders in patients with xerostomia in 48 patients with xerostomia and 15 controls by measuring it as colony forming units after propagation on selective medium and identified by polymerase chain reaction and restriction fragment length polymorphism analysis. They found significantly decreased SFR, increased rate of oral mucosal symptoms and higher numbers of candida in patients with xerostomia compared with control. They suggested that particular candida

species are involved in the pathogenesis of oral mucosal disorders in patients with xerostomia.

Nayak et al. (2012)[43] analysed candida carriage in OSMF by studying 15 OSMF patients and 15 normal patients by oral rinse technique. Presence of candida was demonstrated by 10% KOH, Gram's stain and culture on SDA. They found statistically significant colony forming units (CFU) when counted in OSMF as compared to controls. They concluded that increase in the candida carriage rate in OSMF patients may suggest the role of candida in its neoplastic transformation due to its known potential to produce acetaldehyde and nitrosamines.

Chaudhary et al. (2012)[44] studied candida carriage in OSMF patients by analysing 33 OSMF patients and 70 healthy individuals with age and sex matched using the conventional diagnostic microbial procedures and the final identification of each isolate was done by inoculating the organism suspension in KB006 HiCandida Identification Kit. They found predominant subspecies of C. albicans and C. parapsilosis in both OSMF (12.12%) and (3.03%) respectively and in healthy individuals (15.71%) and (2.85%) respectively. C. tropicalis (6.06%) and C. glabrata (3.03%) found only in OSMF patients. Therefore, they concluded that the rate of oral yeast carriage was significantly higher in OSMF (24.24%) as compared to healthy individuals (18.57%). The reason behind this could be xerostomia observed in OSMF patients and therefore a strict oral hygiene regime should be followed.

Khader N and Dyasanoor S (2015)[12] studied assessment of salivary flow rate and pH among areca nut chewers and OSMF subjects in 45 arecanut chewers, 45 OSMF patients and 45 controls. They found a statistically significant increase in SFR

(35.7 mm at 3rd minutes) among arecanut group and a decrease in SFR (23.4 mm at 3rd minutes) among OSMF group when compared to apparently healthy subjects (30.7 mm at 3rd minutes). And statistically insignificant mean pH among arecanut, OSMF and control groups which was 6.76, 6.82, and 6.74 respectively. They concluded that there was hypersalivation among arecanut chewers and hyposalivation among OSMF patients with no significant change in salivary pH when compared to healthy subjects.

Barman I and G Prasad U (2015)[45] assessed the salivary flow rate and pH among 20 arecanut chewer (raw/betel leaf), 20 tobacco users (smoking/smokeless) and 20 control group. Unstimulated saliva used for SFR and pH meter (HICOM, India) for salivary pH. They found a significant difference of salivary pH between arecanut chewers and non-chewers and insignificant salivary pH between tobacco users and non-chewers. And no significant value of SFR between smoking form of tobacco & non-chewers.

Khader N and Dyasanoor S (2015)[16] studied gustatory changes due to arecanut chewing and OSMF in 45 arecanut chewers, 45 OSMF patients and 45 controls. Taste determination was done by edible taste strips, sweet paper, salt paper (sodium benzoate), sour paper (PTC) and bitter paper (thiourea). They found statistically significant hyperguesia to sweet (28.89%) and bitter taste (35.56%) among areca nut group and hypoguesia to salty (62.2%) and dysguesia to sour taste (40%) among OSMF patients when compared to apparently healthy subjects.

Gupta B, Chandra S, Raj V and Gupta V (2015)[46] compared salivary flow and candida carriage in patients with oral submucous fibrosis in 30 OSMF patients

and 30 healthy individuals. They used preweighed cotton rolls placed at the openings of major salivary duct for 5 minutes for salivary flow rate and oral rinse technique for candida species on SDA. They found statistically significant (P = < 0.001) decrease in the salivary flow rate in OSMF individuals along with constant decrease in different grades as compared to the controls. They also found statistical significance with (P = < 0.001) absence of candida carriage in healthy individuals (control) as well as in grade I and grade IV OSMF patients when compared to grade II and grade III OSMF patients. Therefore, statistically significant difference was seen in the SFR and candidal carriage in the OSMF patients as compared to the healthy individual (P < 0.001). They concluded that salivary flow rate was constantly reduced with different grades of OSMF patients while candida carriage seen in grade II and grade III OSMF patients. They suggested that a higher candidal carriage in grade II and grade III OSMF patients could be related to decreased SFR.

Rehan F et al. (2016)[47] analysed resting mouth salivary flow rate and salivary pH of 70 tobacco chewers, 70 smokers and 70 non-consumers of tobacco. They used simple drooling method for 5 minutes for SFR and indicator paper strips (SIMPLEXTM) for pH. They found that there was no effect of tobacco consumption on RSFR but significant effect on salivary pH. Lesser pH levels were noted in habit groups compared to control group. They concluded that RSFR does not get affected by tobacco consumption however, salivary pH levels certainly decreases in tobacco consumers, especially smokers, which can lead to decreased salivary defence mechanism against various mucosal and dental diseases.

Qamar A et al. (2016)[48] studied the relationship between alterations in RSFR and pH among 354 tobacco chewers, having different forms of tobacco chewing habits like gutka, paan and niswar, which are the widely consumed chewable tobacco products in Pakistan. Resting saliva was used for SFR and pH indicator strips (pH 0-14, universal indicator, Merck, Germany). They found RSFR and pH significantly decreased with increase in packs consumed per day, duration of exposure and duration of usage. They concluded a significant negative correlation between RSFR and pH with tobacco chewing.

Dyasanoor S and Khader N (2016)[13] studied alteration in salivary properties and taste perception in OSMF among 45 OSMF patients with 14 stage I and 31 stage II and 45 controls. They executed their study with MST strips, pH strips and taste strips for SFR, pH and taste perception respectively. They found a statistically significant decrease in SFR among OSMF group (23.4 mm at 3rd min) and hypogeusia to salty (62.2%) and dysgeusia to sour taste (40%) when compared to controls (30.7 mm at 3rd min). It showed statistical significance ($P < 0.05\%$) inferring hyposalivation in Stage II OSMF (24.1 mm at 3rd min) juxtaposing with Stage I OSMF (31.4 mm at 3rd min). And statistically significant hypogeusia to salty ($n = 23$) and sweet ($n = 16$), and dysgeusia ($n = 14$) to sour among Stage II OSMF when differentiated with Stage I OSMF. While the mean pH among the OSMF and control groups was insignificant. They concluded that there is a marked decrease of SFR and taste perception of salty and sour among Stage II OSMF when compared to stage I OSMF subjects.

Kantak et al. (2017)[49] studied consequences of habitual arecanut chewing on unstimulated whole mouth salivary flow rate and PH among 44 arecanut/tobacco chewers and 30 non chewers. They collected saliva under resting condition for SFR and expressed in ml/min for 10 minutes and the pH was recorded by using a digital pH meter. They found the insignificant difference between the mean SFR for chewers and non-chewers (P = 0.5). And statistically significant difference between mean salivary pH for chewers and non-chewers (P = 0.05). They concluded that SFR is altered in a lesser extent while salivary pH is altered to a greater extent in arecanut chewers, because of the oral mucosa to be vulnerable to the toxic effects of the arecanut chewing.

Yadav M, Jaiswal S, Mishra A, Ramakant (2017)[50] studied taste alteration among 30 OSMF stage II patients and 30 healthy individuals by the use of four different freshly prepared solutions of the 4 basic tastes, sucrose for sweet (0.1-1.0 moles per litre), citric acid for sour (0.320-0.032 moles per litre), quinine hydrochloride for bitter (0.01-1.0 moles per litre) and sodium chloride for salty (0.01-1.0 moles per litre) in three different concentrations (low-C1, medium-C2 and high-C3). They performed two different tests, first being spatial or localized by using these four different tastants in three progressively increasing concentration directly over dorsum of the tongue with cotton swab for 5 seconds and second being whole-mouth rinse tests for 10 seconds. They found significant taste alteration with salt taste followed by sweet, bitter and sour. They concluded that besides from increasing risk of cancer the change in taste perception in OSMF patients often leads to depression.

Chaturvedi S, Nair P, Naik S and Patel R (2017)[51] studied isolation and identification of oral candida organism in precancerous and cancerous lesion of oral cavity among 18 OSMF, 18 OSCC and 18 healthy individuals on hichrom agar media. They found C. albicans (66.7%) in OSMF and OSCC group, followed by C. glabrata (5.6%) and C. albicans, C. glabrata and C. krusei accounting ach (11.1%) in healthy individuals. This was statistically significant (P = 0.008).

Gurudath et al. (2018)[52] assessed salivary flow rate and salivary pH in total of 377 subjects with smoking and smokeless form of tobacco habits and 60 controls. Schirmer tear strips and pH strips were used to assess SFR and pH. They concluded that on comparison between control group = 60 and habit groups consisting of 60 smokers, 115 smokeless, 202 combined habits, a statistically significant (P = < 0.001) reduction of SFR was observed in habit groups and a statistically significant reduction (P = < 0.004) was observed only in smokeless tobacco usage group when compared with control group. A significant reduction in SFR and borderline reduction in pH was observed in subjects with lesions (P = < 0.001).

Panditray S et al. (2018)[53] studied the prevalence of candida species and salivary flow rates in 42 patients diagnosed clinically as stage I to stage IV OSMF based on **More C et al (2011)**[2] criteria. Oral rinse technique and saliva collection technique was used for candida colony count and SFR respectively. They found highly statistically significant difference (P = 0.000). The mean CFU/mL in OSMF patients was (435.17 ± 381.863) more than controls (34.02 ± 80.510) and the mean SFR/min in OSMF was (.2455 ± .22283) less than controls (.6776 ± .40099). They concluded that the salivary flow rates progressively decreased from stage I to IV,

while CFUs were highest in stage III and lowest in stage I followed by stage IV and stage II OSMF.

More C et al. (2018)[54] conducted a case control study on association of candida species with 50 OSMF, 50 oral leukoplakia and 50 normal individuals. They collected swab and subjected for gram staining and SDA and positive cultured candida samples analyzed by using HiMedia M 1297 HiCrome candida agar plates for determining the different species. They found that the gram staining was positive in oral swabs of 14 (28%) participants of OSMF group, 20 (40%) participants of OL group and zero from normal group and similarly, the oral swabs in 28% and 40% of the participants of OSMF and oral leukoplakia group showed positive, when cultured on SDA medium. There was a statistically significant ($P < 0.001$) positive culture on HiMedia M 1297 HiCrome candida agar plates detected Candida albicans and non albicans in OSMF and oral leukoplakia group.

Deshpande A, Gokak K, Jalihal S and Bagewadi A (2019)[55] estimated salivary flow in oral submucous fibrosis patients using vibrotactile stimulation in 20 OSMF and 20 healthy individuals. The resting saliva was measured by spitting method after accumulation of saliva in the mouth for 3 min and the volume was compared with stimulated saliva which was measured immediately after masseter muscles stimulation by extraoral vibrations of 90 Hz frequency through the apparatus for 3 min. They concluded that although there is saliva blockage due to the fibrosis in minor salivary glands and ducts of major salivary glands in OSMF, there was increased salivary flow upon vibratory stimuli but noted more increased in control group. And the resting salivary secretion was decreased in OSMF group below the

normal range. Increase in salivation is assumed to be from tonic vibration reflex of muscles and conduction of vibrations by the bone till the major and minor salivary glands.

Gupta A, Reddy M and Kheur S (2019)[56] assessed the gustatory threshold and salivary flow rate in 70 patients diagnosed with very early OSMF having the history of arecanut habit and 30 healthy individuals with no history of habits. The patients were asked to sip and rinse with four blinded solutions each having sweet, sour, salty and bitter flavours for 1 min. And the SFR was assessed by placing sterilized cotton in the patient's mouth also for 1 min. The saliva-soaked cotton was weighted and the weight difference was taken as the SFR. They found no significant difference in the gustatory threshold between very early OSMF patients and the control group ($P > 0.05$). And the SFRs of very early OSMF (0.41 ml/min) patients also remained insignificant to that of the control group (0.55 ml/min) ($P > 0.05$). Therefore, they concluded that the gustation, similar to SFR, remains unaltered in the initial phase of OSMF and cannot be used as an early diagnostic indicator.

Kale et al. (2019)[57] evaluated and compared taste perception among 30 tobacco chewers and 30 non-chewers. Taste identification was done based on time using four aqueous solutions of basic tastes – sweet, salty, sour, and bitter and was recorded in seconds and compared between them. They found a significant increase in taste identification time for salty taste in tobacco chewers of 12.32 seconds compared with non-chewers of 10.21 seconds. They concluded that the average taste identification time was higher for tobacco chewers than non-chewers for sweet and salty taste. While the average taste identification time was lower for tobacco chewers

than non-chewers for sour and bitter taste. The results of their study demonstrated a noticeable decrease in taste perception to salty taste among tobacco chewers when compared with non-chewers.

Bangi B et al. (2019)[58] evaluated gustatory function and compared in 30 OSMF patients of stage I, II and III, 30 gutka chewers and 30 healthy individuals. The patients were enrolled by using filter paper strips impregnated with different taste qualities for assessing taste perception. They found significant changes in sour taste with 33.3% hypoguesia in OSMF subjects and 13.3% showed hypoguesia to all tastants in gutka chewers and hypoguesia to salt, sour and bitter in stage III compared to stage I and II. They concluded significant alterations to taste perception with sour, salt, and bitter and then to sweet in OSMF subjects.

Samatha S et al. (2019)[59] studied candida species in 20 OSMF and 20 healthy individuals. They collected samples by scrapping the superficial mucosal layer for estimation of candidal growth, quantification of candidal colony count and to speciate the different species of candida cultured on SDA and CHROM agar. They found 53.3% of OSMF patients and 6.7% of healthy controls having candida growth on culture. They concluded that the candidal colonies were higher in the OSMF group than compared to healthy controls. However, the candidal carriage in OSMF group was not statistically significant when compared with the control group ($P = 0.004$).

Roy S et al. (2019)[60] evaluated candidal species among 30 OPMDs, 40 OSCC and 25 healthy individuals. They collected swab samples for the microbiological culture and incubated on SDA and then only positive candidal colonies on CHROM agar for speciation followed by incisional biopsy for histopathological confirmation.

They found that on SDA medium in controls, OPMD and OSCC groups, candida was present in 6 (24%), 13 (43%) and 33 (82%) respectively. There was an extremely significant difference (P = 0.000) on intergroup and intragroup comparison like among both controls versus OSCC and OPMD versus OSCC. However, controls versus OPMD showed no significance (P = 0.1332).

Materials and Methods

The present study titled "Evaluation of Salivary flow rate, Gustatory perception and Candida carriage in patients with Oral submucous fibrosis" was carried out in the department of Oral pathology and microbiology as per the inclusion criteria. An informed consent was obtained from all the patients along with the explanation of the procedure that was performed. Parameters were then evaluated.

The study was approved by the institutional ethics committee.

TYPE OF STUDY / STUDY DESIGN: Observational, Analytical, Case control study.

STUDY DURATION: Study was carried out from May 2018 to October 2019 over a period of 18 months.

MATERIALS / DATA COLLECTION TOOLS

The armamentariums used for the study were as under:

1. Mouth mirror.
2. Tweezer/ forceps.
3. Schirmer strips.
4. Taste strips.
5. Sterile container.
6. Phosphate buffer saline. - For 1 Litre
    - Sodium dihydrogen phosphate - 3.4gm.
    - Disodium hydrogen phosphate- 12.0 gm.
    - Sodium chloride - 8.5 gm.
    - Distilled water - 1000 ml.
7. Sabouraud's dextrose agar medium (SDA).
8. Centrifuge machine.
9. Incubator. (37°C)
10. Microscope.

SAMPLE SIZE

Referring to the article by **Dyasanoor S. et al 2016**, the salivary flow rate for OSMF and Control groups were 23.4 ± 7.1 and 30.7 ± 2.5 respectively. Accordingly, the estimated effect size was 1.37, which was considered to be on higher side. Alternatively, to attain the effect size of 0.75, a sample of 29 (~**30**) per group was needed that can provide the desired effect with 95% confidence and 80% power.

The formulation used for estimating the sample size was:

$$n = \frac{2 * (z_{1-\alpha/2} + z_{1-\beta})^2 s^2}{\varepsilon^2}$$

Where, z1-/2 and z1- are the critical values for 95% confidence interval and 80% power, while *s* is the pooled standard deviation of the groups, represent the tolerable difference between the groups. The difference and pooled standard deviation leads to effect size.

SAMPLING TECHNIQUE

The criteria for inclusion and exclusion were decided. The individuals were selected randomly in each group subject to the fulfilment of inclusion and exclusion criteria and allocated as group A = Healthy individuals and group B = OSMF.

Group A: Healthy individuals (Control group)

Sample size: 30

Inclusion criteria

1. Subjects between the age group of 15-45 years.
2. No history of habits like arecanut/arecanut containing product chewing, smoking, consumption of alcohol.
3. Apparently healthy patients.

Group B: OSMF patients (OSMF group)

Sample size: 30

Inclusion criteria

1. Patients between the age group of 15-45 years.
2. Arecanut/arecanut containing product chewing habit.

3. Patients with clinically diagnosed OSMF as Stage I = 10 patients, Stage II = 10 patients and Stage III = 10 patients.

The criteria considered is mouth opening according to **Khanna J.N. and Andra de NN (1995)**[30] classification of OSMF as :-

Stage I : Not associated with mouth opening limitation.

Stage II : Inter-incisal distance is 26-35mm.

Stage III : Inter-incisal distance is 15-20mm.

Exclusion criteria

1. Individuals with other associated habits like smoking or consumption of alcohol.
2. Individuals with conditions other than OSMF which cause reduction in mouth opening – eg. Systemic sclerosis, Temporomandibular joint ankylosis, Impacted 3rd molar, Myofunctional pain disorder, infections, jaw fracture, etc.
3. Individuals with any known systemic disorders like hypertension, diabetes etc.
4. Individuals under any drugs like anti-cholinergics, diuretics, antihistamines, anti-hypertensives & psychoactive substance that might alter the salivary parameters.
5. Individuals who are pregnant and those who underwent radiotherapy.

STUDY GROUPS:

The samples were divided into two groups:

Group A	**Healthy individuals (control group)**	30 samples
Group B	**Oral submucous fibrosis patients**	30 samples

PLATE I

Fig. no. 8: Diagnostic instruments.

Fig. no. 9: Sterile container for saliva collection.

PLATE II

Fig. no. 10: Schirmer strips for determination of SFR.

Fig. no. 11: Taste strips with sweet, salty, bitter, sour and control.

PLATE III

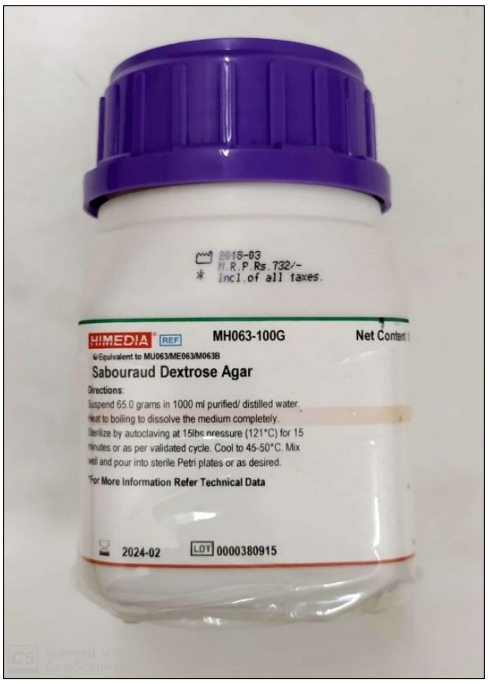


Fig. no. 12: Sabouraud's dextrose agar media.

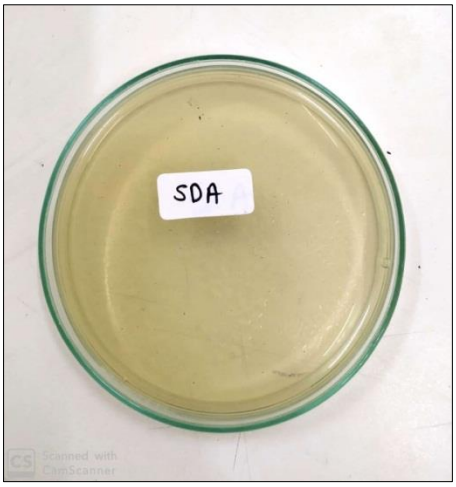


Fig. no. 13: Sabouraud's dextrose agar plate.

PLATE IV

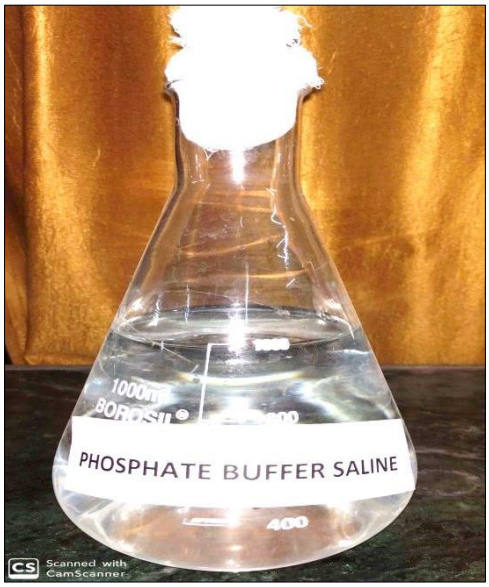


Fig. no. 14: Phosphate buffer saline solution.

Fig. no. 15: Centrifuge machine.

PLATE V

Fig. no. 16: Incubator.

Fig. no. 17: Binocular light microscope.

METHODS OF MEASUREMENT

1. Salivary flow rate determination: Modified Schirmer test procedure.[12]

1. The subjects were asked not to eat or drink 2 hours prior to the modified schirmer test. The test was carried out in the morning hours between 9 a.m. to 11 a.m. avoiding the circadian variation.
2. After a period of 3-5 minutes rest, the patient was asked to swallow all the saliva in the mouth prior to the test and not to swallow anymore during the test.
3. In addition, the patient was asked to rest the tongue on the hard palate.
4. The strip (Tear touch schirmer strips – Madhu Instruments Pvt. Ltd., New Delhi) was hold vertically and the rounded end of the strip was positioned at the floor of mouth.
5. When the round end of the strip contacted the moisture, the saliva travelled up the strip and its distance was read at 1, 2 and 3 minutes and recorded immediately.

2. Determination of Gustation/Taste: Taste Strip method.[13]

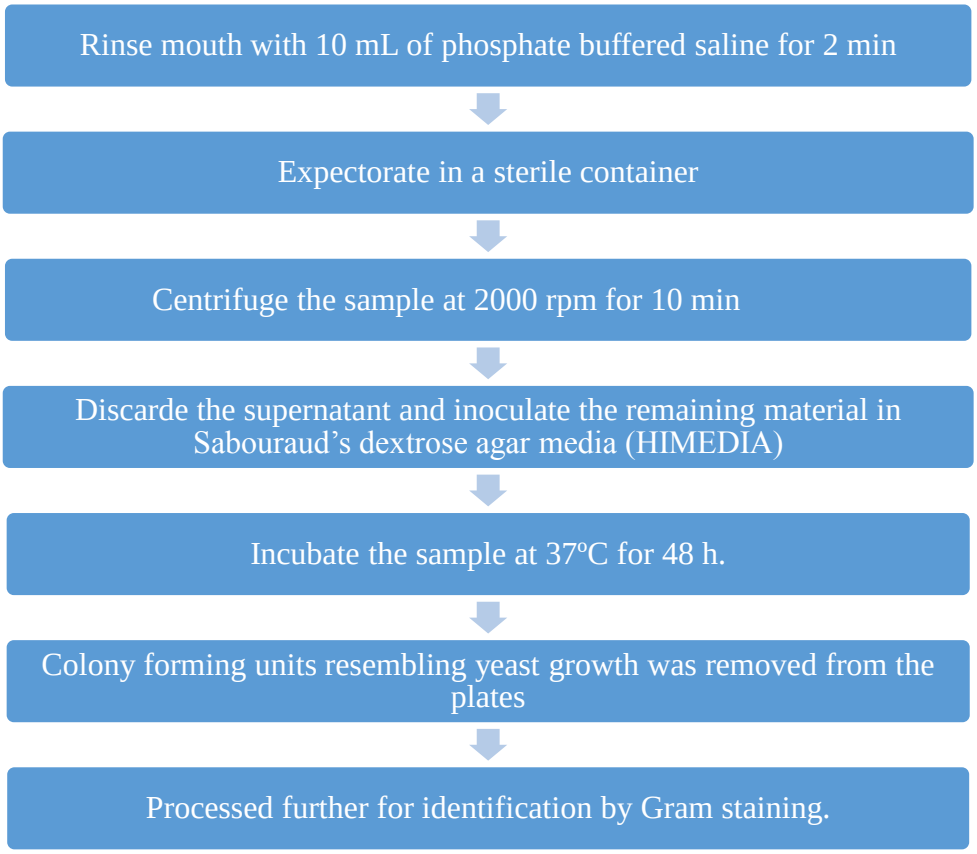
1. Before assessing the taste in the individuals, the subjects were asked not to eat or drink 1 hour prior of the procedure and asked to rinse the mouth in tap water.
2. The taste strips were 4 in numbers and control paper. The basic tastants, i.e, sweet (sweet paper), salty (sodium benzoate paper), bitter (thiourea paper), sour (PTC paper) and control paper (inert and tasteless) – PL Precision laboratories, U.S.A.

3. Each taste strip with specific taste was placed over the dorsum portion of the tongue, and the subject was asked to determine the taste of the strip.

4. The strip gradually dissolved in 2 min.

3. Determination of Candida: Saliva Culture technique for candida.

Salivary samples was collected by the oral rinse technique described by Samaranayake et al.[61] as follows :



METHODS OF DATA COLLECTION

1. Salivary flow rate assessment: The Salivary flow rate will be assessed in the distance covered in mm at 1, 2 and 3 minutes. [12, 13]

2. Gustation/Taste determination: The taste perceived by the subjects will be recorded as normal perception, dysgeusia and ageusia with the help of taste strips.[13,16]

3. Candida determination: The number of yeast colonies will be counted and expressed as colony-forming units per milliliter (CFU/mL) of the collected sample. [43]

STATISTICAL METHODS EMPLOYED

The data obtained was compiled on a MS Office Excel Sheet (v 2010) and was subjected to statistical analysis using SPSS v 14.0.

For all the statistical tests, $P < 0.05$ was considered to be statistically significant.

- Chi-square test was used to assess age and gender distribution across both the groups.
- Chi-square test was used to assess Gustatory perception across both the groups.
- Independent t-test was used to compare Salivary flow rate between OSMF and Control group.
- One-way analysis of variance (ANOVA) was used to assess comparison of Salivary flow rate of different Stages of OSMF at 1 min, 2 min and 3 minutes.
- Chi-square test was used to assess Candida culture across both the groups.

ETHICAL ISSUES INVOLVED – None

PLATE VI

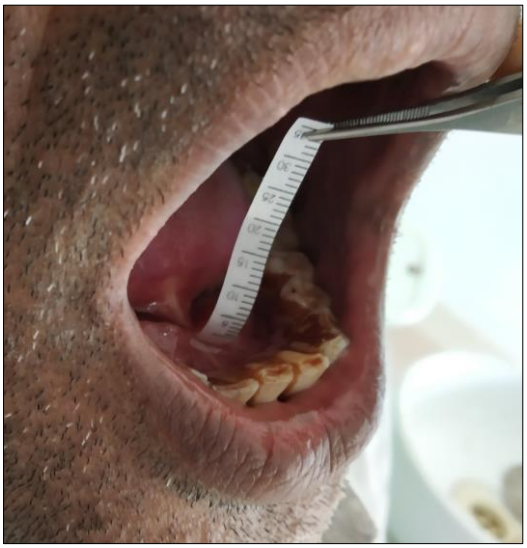


Fig. no. 18: Clinical photograph showing SFR determination using sterile schirmer strip.

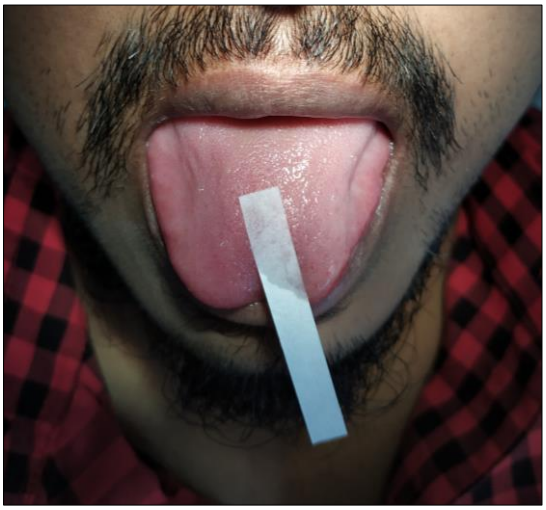


Fig. no. 19: Clinical photograph showing Gustatory perception determination using taste strip.

PLATE VII

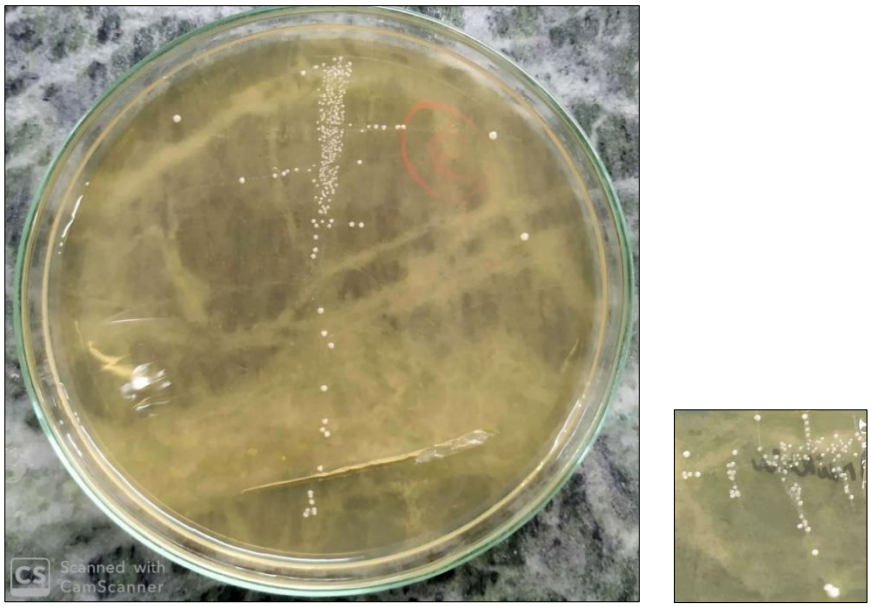


Fig. no. 20: White colored colonies showing Candidal growth on SDA culture plate in OSMF case.

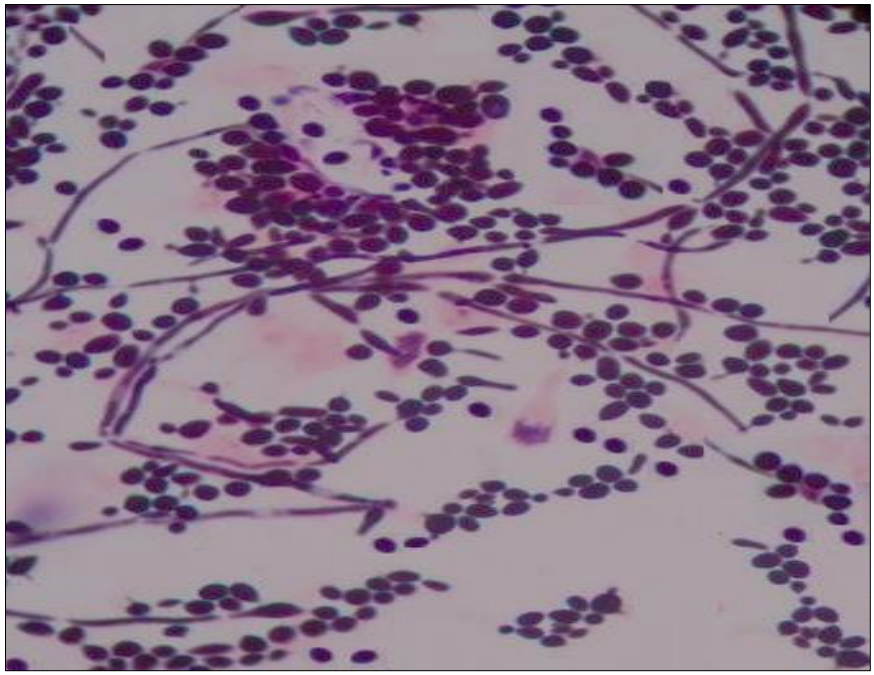


Fig. no. 21: Photomicrograph showing Candidal growth, on identification by Gram staining – 100x (Oil immersion).

Observations and Results

The present observational, analytical, case control study consisted of a total of 60 patients. Out of these, 30 were clinically diagnosed cases of Oral submucous fibrosis (OSMF) and 30 were healthy individuals, considered as controls for comparative purpose.

STUDY GROUPS

Group A – 30 Healthy individuals (Control group).

Group B – 30 OSMF patient.

Each study group was evaluated for Salivary flow rate, Gustatory perception and Candidal carriage. SFR distance (mm) was assessed at 1, 2 and 3 min, Gustatory perception as normal, dysgeusia and ageusia and Candidal carriage was counted and

expressed as colony-forming units per milliliter (CFU/mL) in controls and all the three stages of OSMF group.

The data collected was entered into Microsoft excel spreadsheet. Tables and Charts were prepared with the help of windows 10 word and excel software. Statistical analysis was done by Statistical software STATA Version 14.0 and $P < 0.05$ was considered as statistical significance.

The observations of these studies are explained in detail in the following sections.

Table no. 5: Age distribution across the study groups.

AGE IN YEARS	OSMF GROUP		CONTROL GROUP		P-VALUE
	FREQUENCY	PERCENT	FREQUENCY	PERCENT	
16 – 25	7	23.3	10	33.3	0.4173, NS
26 – 35	12	40.0	11	36.7	
36 – 45	11	36.7	9	30.0	
TOTAL	30	100	30	100	
MEAN AGE	31.83 ± 7.84		30.16 ± 7.95		

Degree of freedom=2, CHI-SQUARE=0.7729, P > 0.05, Not Significant (NS).

Comments (Table no. 5 and Graph no. 1)

Out of 30 OSMF group, 7 (23.3%) were between 16-25 years, 12 (40%) were between 26-35 years and 11 (36.7%) were between 36-45 years of age. Mean age in OSMF group was 31.83 years with standard deviation of 7.84 years. Out of 30 Control group, 10 (33.3%) were between 16-25, 11 (36.7%) were between 26-35

years and 9 (30%) were between 36-45 years of age. Mean age in Control group was 30.16 years with standard deviation of 7.95 years. The difference was statistically not significant with P = 0.4173 (>0.05).

Graph no. 1: Bar diagram of age distribution across the study groups.

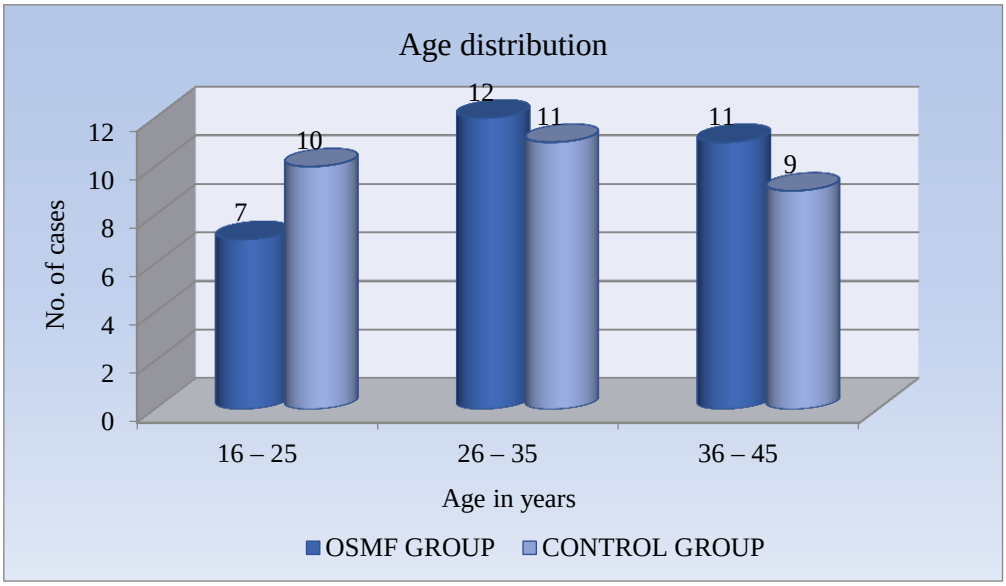


Table no. 6: Gender distribution across the study groups.

GENDER	OSMF GROUP		CONTROL GROUP		P-VALUE
	FREQUENCY	PERCENT	FREQUENCY	PERCENT	
MALE	26	93.7	20	67.7	0.067, NS
FEMALE	4	13.3	10	33.3	

Degree of freedom =1, CHI-SQUARE=3.3540, P > 0.05, Not Significant (NS)

Comments (Table no. 6 and Graph no. 2)

Out of 30 samples with OSMF, 26 (93.7%) were males and 4 (13.3%) were females and out of 30 samples with healthy individuals (controls), 20 (67.7%) were males and 10 (33.3%) were females. The difference was statistically not significant with P = 0.067 (>0.05). The statistical analysis was done using CHI-SQUARE TEST.

Graph no. 2: Bar diagram of gender distribution across the study groups.

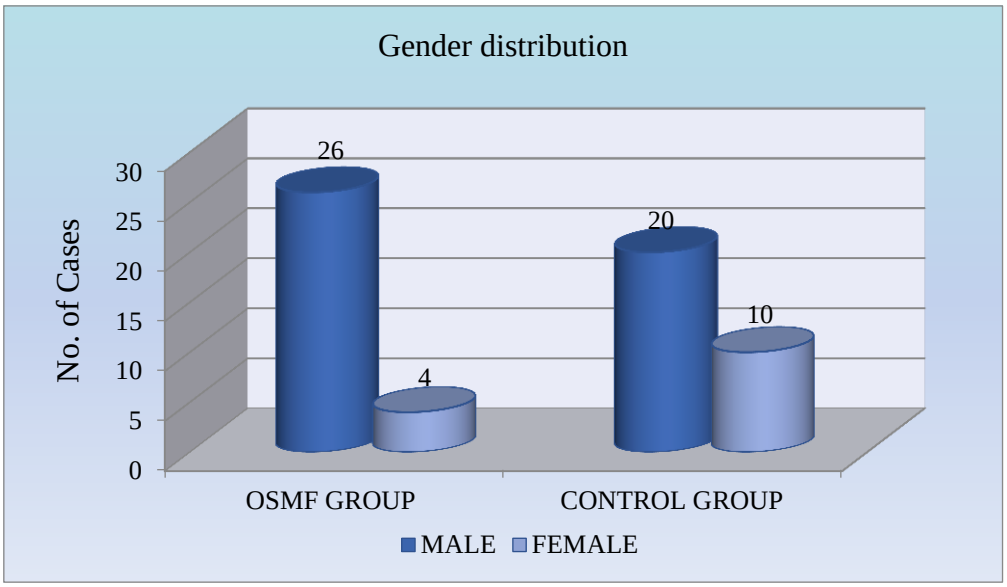


Table no. 7: Mean and SD values of Salivary flow rate distance (mm) at 1, 2 and 3 minutes in OSMF and control group.

TIME	OSMF GROUP		CONTROL GROUP		t-VALUE	P-VALUE
	MEAN	SD	MEAN	SD		
1 MIN	6.36	3.50	9.96	2.14	4.8065	<0.0001, HS
2 MIN	12.46	4.03	20.8	3.20	8.8769	<0.0001, HS
3 MIN	17.7	4.92	29.3	1.48	12.3664	<0.0001, HS

Degree of freedom=58, $P < 0.01$, Highly Significant (HS)

Comments (Table no.7 and Graph no. 3)

Comparison of the Salivary flow rate between study groups at 3rd minute revealed an overall mean of 17.7 mm with standard deviation of 4.92 mm for OSMF and overall mean of 29.3 mm with standard deviation of 1.48 mm for control groups. The differences between the subjects were highly significant with $P < 0.0001$.

Result: This is suggestive of marked reduction in Salivary flow rate among OSMF group as compared to the controls.

Graph no. 3: Line diagram of Mean values of Salivary flow rate distance (mm) at 1, 2 and 3 minutes in OSMF and control group.

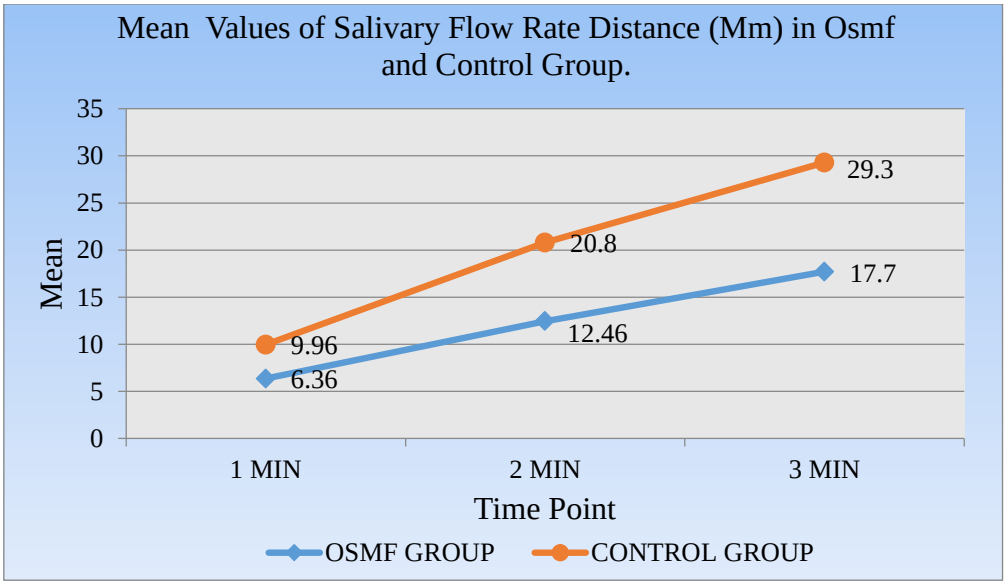


Table no. 8 : Comparison of salivary flow rate of different stages of OSMF at 1, 2 and 3 minutes.

TIME	STAGE-I		STAGE-II		STAGE-III		F-VALUE	P-VALUE
	MEAN	SD	MEAN	SD	MEAN	SD		
1 MIN	9.2	2.89	7.4	1.83	2.5	1.08	27.87	<0.0001, HS
2 MIN	15.1	2.18	14.0	3.55	8.3	2.31	17.55	<0.0001, HS
3 MIN	20.4	3.16	20.6	3.23	12.1	2.33	27.18	<0.0001, HS

P < 0.0001, Highly Significant (HS)

Comments (Table no. 8 and Graph no. 4)

Comparison of Salivary flow rate between stage I, II and III OSMF group at 3rd minute showed overall mean of 20.4 mm with standard deviation of 3.16 mm for stage I OSMF, mean of 20.6 mm with standard deviation of 3.23 mm for stage II OSMF and mean of 12.1 with standard deviation of 2.33 mm for stage III OSMF group. The difference was statistically highly significant with $P < 0.0001$. Statistical analysis was done using ONE-WAY ANOVA test.

Result: This is suggestive of decrease in Salivary flow rate among stage III OSMF group as compared to stage I and stage II OSMF. This could be concluded that the salivary flow rate decreases with increase in stages of OSMF.

Graph no. 4: Bar diagram of comparison of salivary flow rate of different stages of OSMF at 1, 2 and 3 minutes.

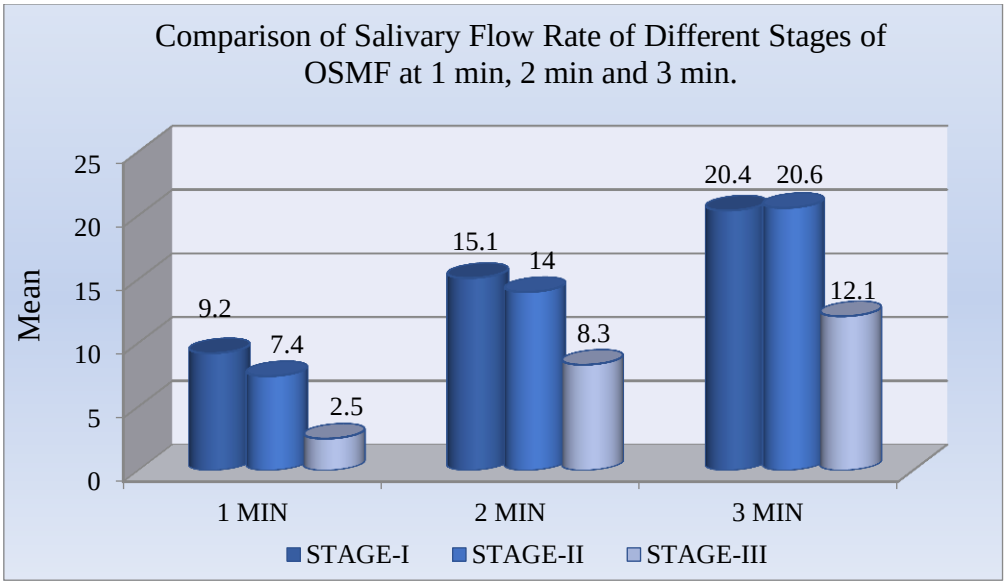


Table no. 9: Comparison of Gustatory perceptions among OSMF and control groups.

SWEET TASTE	OSMF GROUP		CONTROL GROUP		P-VALUE
	FREQUENCY	PERCENT	FREQUENCY	PERCENT	
NORMAL	17	56.67	30	100	CHI-SQ =16.5957 P<0.001, HS
DYSGEUSIA	5	16.67	0	-	
AGEUSIA	8	26.67	0	-	
SALTY TASTE					
NORMAL	9	30.0	28	93.33	CHI-SQ =25.8996 P<0.001, HS
DYSGEUSIA	9	30.0	0	-	
AGEUSIA	12	40.0	2	6.67	
BITTER TASTE					
NORMAL	16	53.33	28	93.33	CHI-SQ =12.2727 P<0.001, HS
DYSGEUSIA	7	23.33	1	3.33	
AGEUSIA	7	23.33	1	3.33	
SOUR TASTE					
NORMAL	7	23.33	25	83.33	CHI-SQ =22.9671 P<0.001, HS
DYSGEUSIA	17	56.67	2	6.67	
AGEUSIA	6	20.00	3	10.0	

Degree of freedom = 2, P < 0.001, Highly Significant (HS)

Comments (Table no. 9 and Graph no. 5,6,7,8)

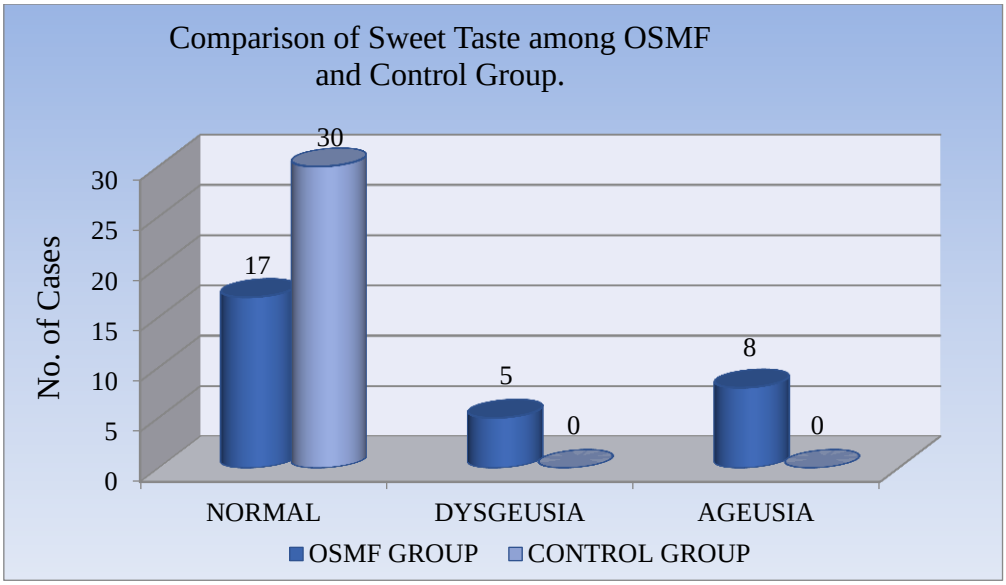
Comparison of taste perceptions among OSMF and control subjects were demonstrated. Out of 30 OSMF subjects; 17 (56.67%) normal, 5 (16.67%) showed dysgeusia and 8 (26.67%) showed ageusia to Sweet taste; 9 (30%) normal, 9 (30%) showed dysgeusia and 12 (40%) showed ageusia to Salty taste; 16 (53.33%) normal, 7 (23.33%) showed dysgeusia and 7 (23.33%) showed ageusia to Bitter taste; 7

(23.33%) normal, 17 (56.67%) showed dysgeusia and 6 (20%) showed ageusia to Sour taste perception were observed.

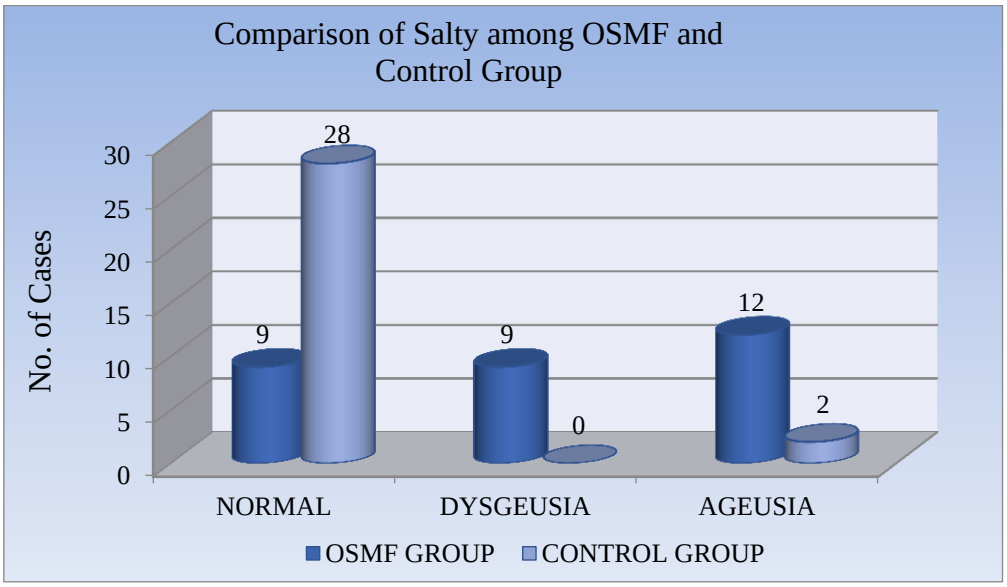
Out of 30 control subjects; all 30 (100%) showed normal with no subjects to dysgeusia and ageusia to Sweet taste; 28 (93.33%) normal, 2 (6.67%) showed ageusia and no subjects showed dysgeusia to Salty taste; 28 (93.33%) normal, 1 (3.33%) showed dysgeusia and 1 (3.33%) showed ageusia to Bitter taste; 25 (83.33%) normal, 2 (6.67%) showed dysgeusia and 3 (10.0%) showed ageusia to Sour taste perception were observed. The difference between them showed statistically highly significant result with $P < 0.001$ (<0.05) for all taste perceptions. Statistical analysis was done using CHI-SQUARE TEST.

Result: This is suggestive of Ageusia to Salty taste and Dysgeusia to Sour taste among OSMF group. Normal taste perception among Control group.

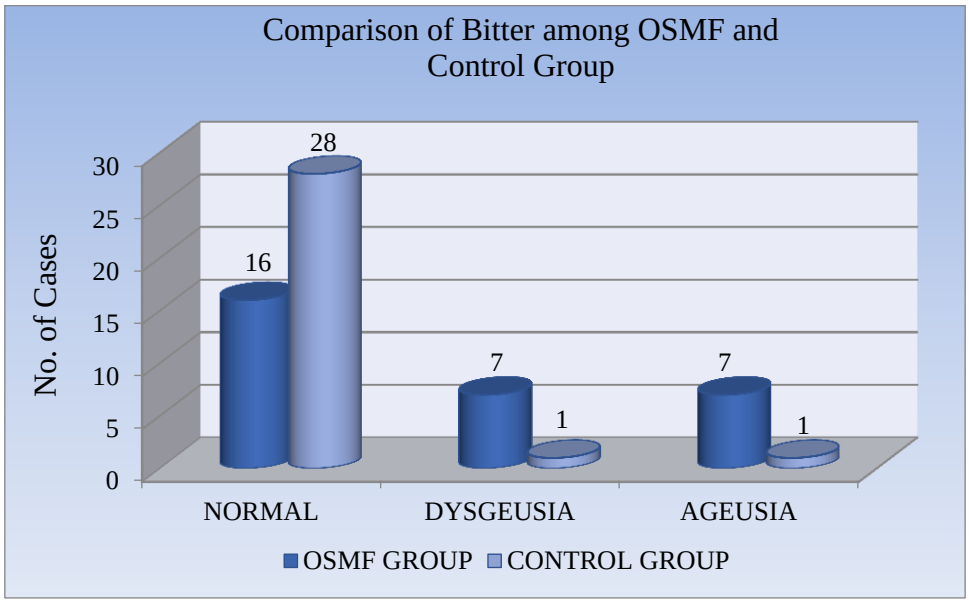
Graph no. 5: Bar diagram of comparison of Sweet taste among OSMF and control groups.



Graph no. 6: Bar diagram of comparison of Salty taste among OSMF and control groups.



Graph no. 7: Bar diagram of comparison of Bitter taste among OSMF and control groups.



Graph no. 8: Bar diagram of comparison of Sour taste among OSMF and control groups.

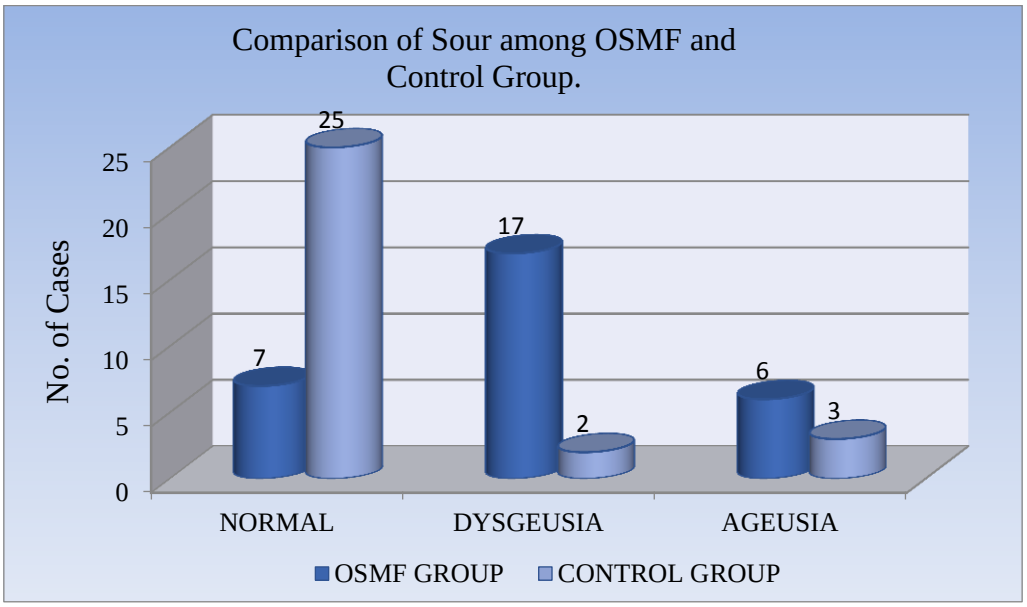


Table no. 10: Comparison of Gustatory perceptions between stage I, stage II and stage III OSMF.

OSMF GROUP	SWEET TASTE			P-VALUE
	NORMAL	DYSGEUSIA	AGEUSIA	
STAGE-I	9 (90%)	0	1 (10%)	CHI-SQ = 7.8441 P=0.090, NS
STAGE-II	5 (50%)	2 (20%)	3 (30%)	
STAGE-III	3 (30%)	3 (30%)	4 (40%)	
	SALTY TASTE			
STAGE-I	7 (70%)	1 (10%)	2 (20%)	CHI-SQ = 12.6667 P=0.024, S
STAGE-II	1 (10%)	5 (50%)	4 (40%)	
STAGE-III	1 (10%)	3 (30%)	6 (60%)	
	BITTER TASTE			
STAGE-I	7 (70%)	2 (20%)	1 (10%)	CHI-SQ = 4.7679 P=0.346, NS
STAGE-II	6 (60%)	1 (10%)	3 (30%)	
STAGE-III	3 (30%)	4 (40%)	3 (30%)	
	SOUR TASTE			
STAGE-I	3 (30%)	5 (50%)	2 (20%)	CHI-SQ = 5.8151 P=0.236, NS
STAGE-II	2 (20%)	4 (40%)	4 (40%)	
STAGE-III	2 (20%)	8 (80%)	0	

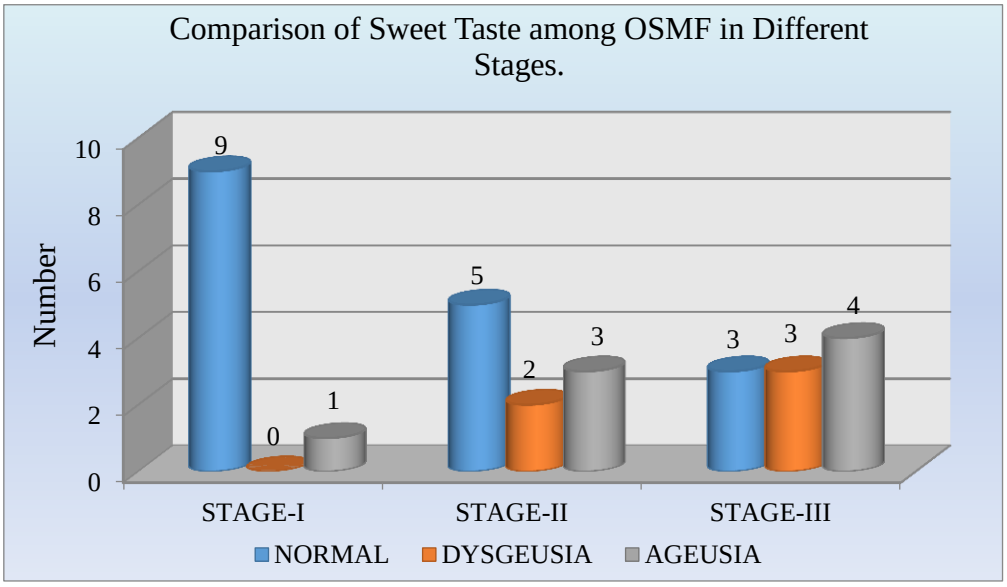
Degree of freedom = 4, P > 0.05 Not Significant (NS), P < 0.05 Significant (S)

Comments (Table no. 10 and Graph no. 9,10,11,12)

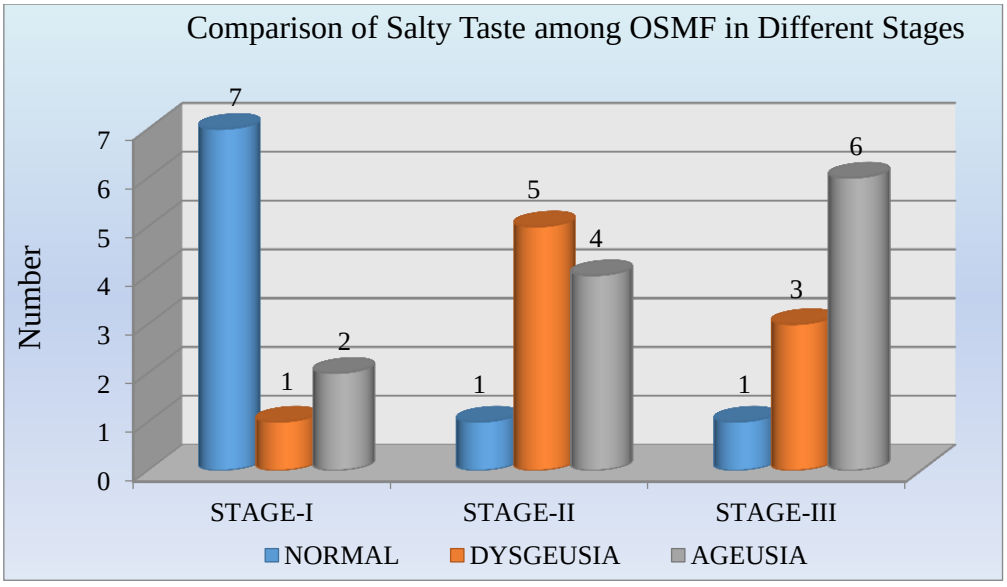
Comparison of taste perception among the various tastants between the 3 stages of OSMF inferred 9 (90%) normal and 1 (10%) ageusia to Sweet taste; 7 (70%) normal, 1 (10%) dysgeusia and 2 (20%) ageusia to Salty taste; 7 (70%) normal, 2 (20%) dysgeusia and 1 (10%) ageusia to Bitter taste; 3 (30%) normal, 5 (50%) dysgeusia and 2 (20%) ageusia to Sour taste among 10 stage I OSMF subjects. 10 subjects of stage II OSMF displayed 5 (50%) normal, 2 (20%) dysgeusia and 3 (30%) ageusia to Sweet taste; 1 (10%) normal, 5 (50%) dysgeusia and 4 (40%) ageusia to Salty taste; 6 (60%) normal, 1 (10%) dysgeusia and 3 (30%) ageusia to Bitter taste; 2 (20%) normal, 4 (40%) dysgeusia and 4 (40%) ageusia to Sour taste. 10 subjects of stage III OSMF displayed 3 (30%) normal, 3 (30%) dysgeusia and 4 (40%) ageusia to Sweet taste; 1 (10%) normal, 3 (30%) dysgeusia and 6 (60%) ageusia to Salty taste; 3 (30%) normal, 4 (40%) dysgeusia and 3 (30%) ageusia to Bitter taste; 2 (20%) normal and 8 (80%) dysgeusia to Sour taste. The difference between them showed no statistical significance with P = 0.090 (>0.05) for Sweet taste, P = 0.346 (>0.05) for Bitter taste and P = 0.236 (>0.05) for Sour taste. There was statistical significance with P = 0.024 (<0.05) for Salty taste. Statistical analysis was done using CHI-SQUARE TEST.

Result: This is suggestive of Ageusia to Salty taste among stage III. Dysgeusia to Sour taste among stage III OSMF noted, however was not significant.

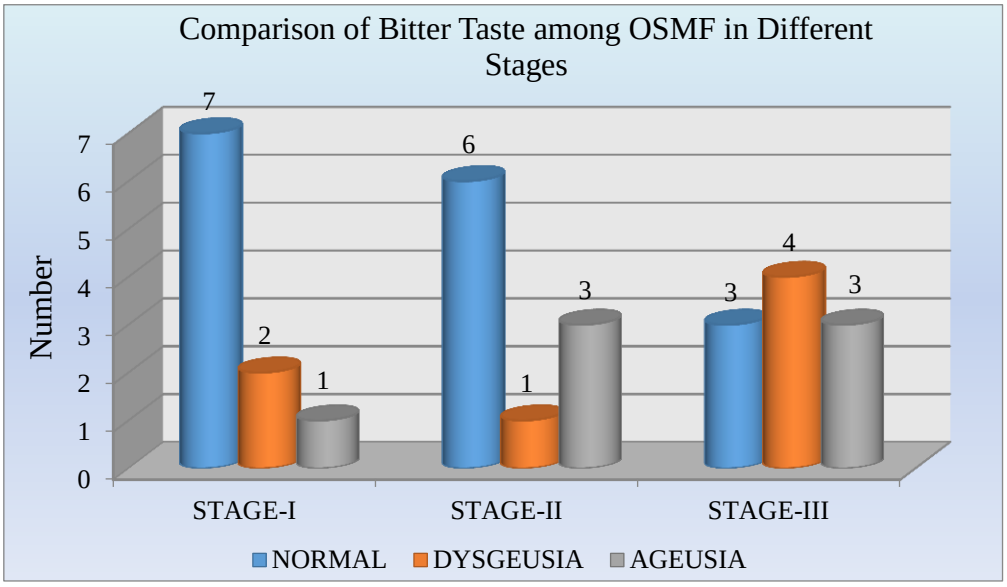
Graph no. 9: Bar diagram of comparison of Sweet taste among OSMF in stage I, stage II and stage III.



Graph no. 10: Bar diagram of comparison of Salty taste among OSMF in stage I, stage II and stage III.



Graph no. 11: Bar diagram of comparison of Bitter taste among OSMF in stage I, stage II and stage III.



Graph no. 12: Bar diagram of comparison of Sour taste among OSMF in stage I, stage II and stage III.

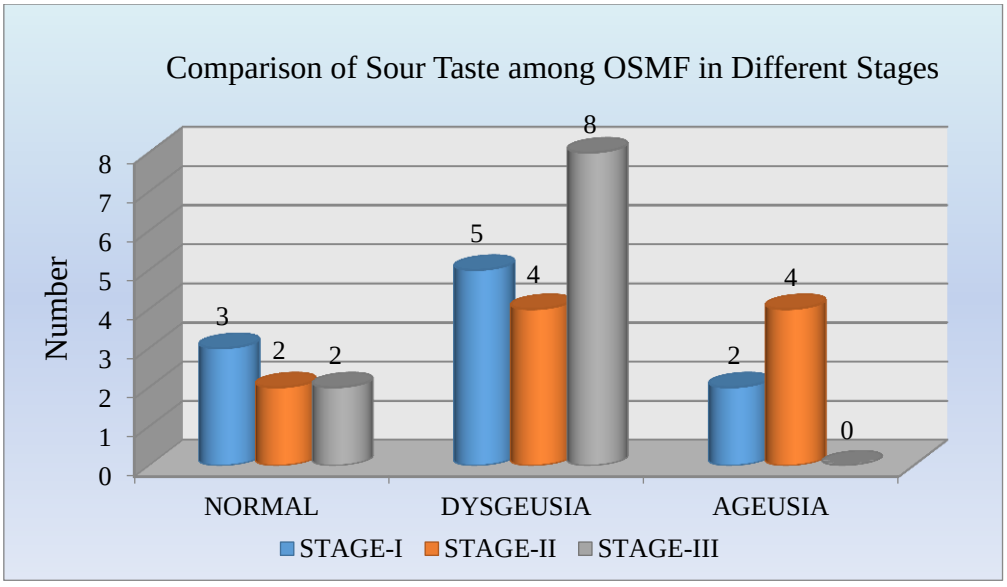


Table no. 11: Candida culture result among OSMF and control groups.

GROUP	NO. OF SAMPLES	TOTAL CANDIDA CULTURE POSITIVE	CANDIDA GROWTH	
			>10 CFU	<10 CFU
OSMF	30	25 (84.33%)	25 (100%)	0
CONTROL	30	3 (10%)	0	3 (100%)
CHI-SQUARE		32.4107	28.0000	
P-VALUE		<0.001, HS	<0.001, HS	

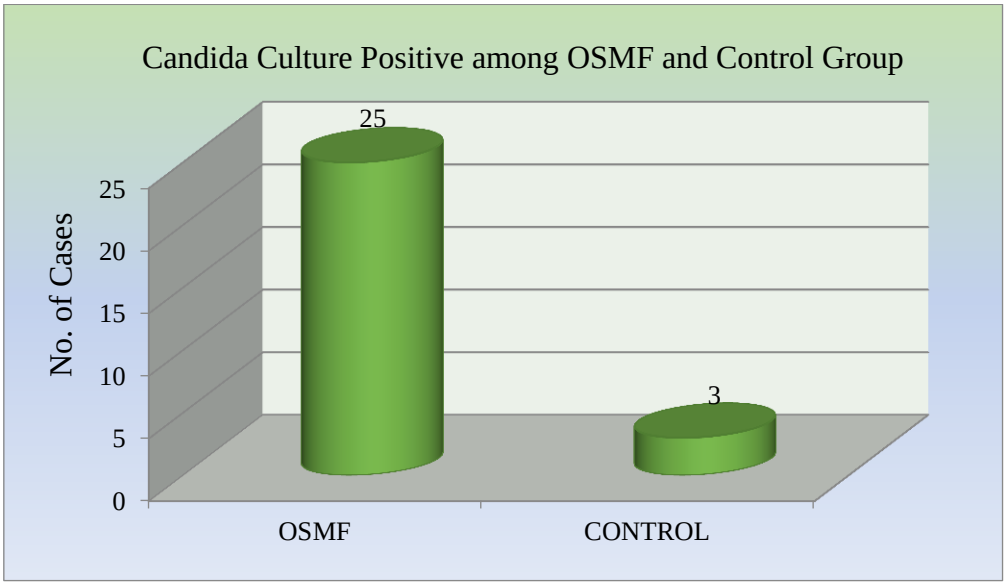
Degree of freedom = 1, P < 0.001, Highly Significant (HS)

Comments (Table no. 11 and Graph no. 13,14)

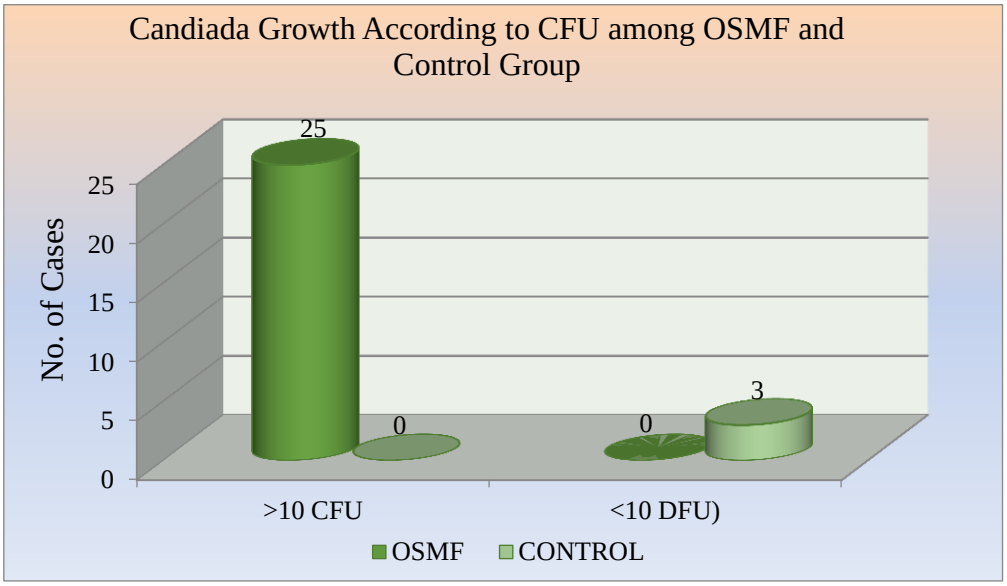
The table depicts that in OSMF group, 25 (84.33%) out of 30 showed culture positivity. All 25 (100%) showed more than 10 Colony forming units (CFU) of Candida on Sabouraud's dextrose agar media. In control group, 3 (10%) out of 30 showed culture positivity. All 3 (100%) samples of control group showed CFU less than 10. This showed a statistically highly significant correlation with P = <0.001 (<0.05) when the Colony forming units were counted in both the groups. Statistical analysis was done using CHI-SQUARE TEST.

Result: This is suggestive of increased Candidal carriage in OSMF group than control group.

Graph no. 13: Bar diagram of Candida culture result among OSMF and control groups.



Graph no. 14: Bar diagram of Candida growth according to CFU among OSMF and control groups.



HYPOTHESIS

Null Hypothesis

Null hypothesis has not been met with no difference between salivary flow rate, Gustatory perception and Candidal carriage among Oral submucous fibrosis patients and normal individuals.

Alternate Hypothesis

Alternate hypothesis has been met with altered Salivary flow rate, Gustatory perception and Candidal carriage in Oral Submucous fibrosis than normal individuals.

Discussion

Oral submucous fibrosis is considered to be the Oral potentially malignant disorder predominantly seen affecting the people of Asian descent.[13] Based on the epidemiological and clinical studies it has been noted that arecanut chewing habit in various forms, frequency and duration is associated with OSMF and its staging. As OSMF is one of the prevalent, preventable and potentially malignant disorder of oral mucosa, it remains to be the prime concern for the dentists and oral pathologists in India. However, most of the oral physicians tend to overlook particularly the changes in SFR or taste perceptions and focus more towards the clinical presentation of OSMF and the management of the disorder.[13]

Saliva is an important factor considering the oral health as it buffers the acids, contains antibodies and immunoglobulins and helps in preventing erosion of gingival

mucosa.[62] During the consumption of habit with arecanut and arecanut containing products, a lot of chemicals and metals like copper, iron etc. are leached out into saliva which then alters its properties and composition.[63]

Moreover, nicotine which is present in tobacco acts on specific cholinergic receptors in the brain, causing neural activation and thereby increasing the SFR for the shorter duration. But, in the cases of long-term usage of tobacco, nicotine enhances epinephrine effect or causes inactivation of taste receptors thereby depressing the salivary reflex or causing degeneration of salivary gland.[47]

In betel quid, tobacco and kharra-gutkha chewer's variations in the SFR and gustatory perception have been reported. Taste stimuli play an important role in salivation, affecting the output qualitatively and quantitatively which ultimately affects the quality of life.[13]

About 3–47% of Candida species are present as a component of normal oral flora in healthy individuals.[61] Overall, seven Candida species are noted of major medical importance; C. albicans, C. tropicalis and C. glabrata which are frequently isolated; while the infrequently isolated forms from the medical specimens are C. parapsilosis, C. stellatoidea, C. guilliermondii, C. krusei and C. pseudotropicalis.[64] C. albicans is the predominant species which under immunocompromised conditions has the potential to infect any tissue within the body. An association between candida and various OPMDs have been reported in the literature.[65] In individuals, candidal carriage varies from 1.0% to 80.6% based on the population surveyed. The potentially damaging effects of candida virulence factors and the nature of the immune response elicited by the host have a delicate balance which ultimately damages the tissue by

resisting host defense and production of hydrolytic enzymes like proteases, phospholipases and haemolysin.[66] Therefore, the frequent changes in the host factors seem to lead changes in candida from a commensal to the pathogenic existence.[67]

OSMF, a chronic oral potentially malignant disorder, is of the multifactorial origin with arecanut and tobacco chewing habits as the predominant etiological factors.[68] The tobacco contents such as nicotine, polonium, polycyclic aromatic hydrocarbons, and nitrosoproline, provides nutrition for candida and stimulate their proliferation.[69] This all together cause an increase in the colonisation of candida by increase in epithelial keratinisation, decrease in leukocyte function and salivary immunoglobulin A, and oral epithelial changes such as atrophy, hyperplasia and dysplasia which disrupts the epithelial architecture.[70]

There is a strong association noted between cancers of the oral cavity and pharynx with the use of these habits (arecanut, tobacco -smoking, chewing and snuff etc.). Some of the investigators have also indicated that the presence of candida infection increases the risk of malignant transformation in OPMDs.[71] In the 1960s, a possible association between candida species and oral neoplasia was reported.[72,73]

The neoplastic potential of candida has been demonstrated from the fact that certain candida species are capable of metabolizing ethanol to carcinogenic acetaldehyde and nitrosamine-N-nitrosobenzylmethylamine and can thus progress oral and upper gastrointestinal tract cancers.[74-76] C. albicans is noted with a higher potential for producing nitrosamines and acetaldehyde than the various other species

of candida. Candidal strains associated with potentially malignant disorder shows more advanced changes with high nitrosation potential of the species.[74,75]

There are certain studies reported with the changes in SFR and candidal carriage among OSMF and its various stages. Considerably, fewer studies have been reported on changes in SFR and gustatory perception in patients with OSMF. Therefore, this study was contemplated for evaluating and comparing the SFR, gustatory perception and candida carriage among the OSMF affected individuals and control group.

This study group was divided into two i.e. 30 OSMF and 30 controls. Out of 30 OSMF group, the individuals were divided equally into 3 stages as 10 of stage I, 10 of stage II and 10 of stage III.

The study showed an overall mean of the SFR among OSMF group as 6.36 mm, 12.46 mm and 17.7 mm at 1,2 and 3 minutes respectively; and among control group as 9.96 mm, 20.8 mm and 29.3 mm at 1, 2 and 3 minutes respectively. This is suggestive of decrease in SFR among OSMF group as observed when compared to control group which is similar to the reports by **Khader et al. (2015)[12]** and **Dyasanoor et al. (2016)[13],** who found overall mean of SFR of 23.4 mm at 3rd minute and concluded hyposalivation/marked decrease in SFR among OSMF patients.

Due to conversion from arecoline to arecadine in presence of lime may the reason behind decrease in SFR among OSMF subjects or due to acinar cell atrophy as disease progresses. A study conducted by **Nyachhon et al. (2011)[77]**, showed various stages of acinar cell degeneration that manifested as small clear cytoplasmic vacuoles

with pyknotic nuclei among OSMF patients. They also noted areas of mucin pooling within the connective tissue stroma.

A study done by **Sangeeth et al. (2014)**[63], showed contradictory findings of an increase in SFR among ghutka chewers and OSMF using spitting method. The difference of results could be because of utilization of modified schirmer test and use of arecanut habit without tobacco. **Qamar et al. (2016)**[48] concluded a significantly negative correlation between RSFR with tobacco chewing, which is in contrast with the present study.

The present study showed a statistically highly significant decrease (P <0.0001) in salivary flow rate in Stage III OSMF, and can be concluded that the salivary flow rate decreases with increase in stages of OSMF. This could be again due to arecoline and arecadine present in areca nut having parasympathomimetic effect.[48] In the present study, OSMF group showed dysgeusia 17 (56.67%) to sour taste followed by ageusia 12 (40%) to salty taste, and normal 17 (56.67%) to sweet and 16 (53.33%) to bitter taste. Among OSMF group, altered or decreased perception may be due to atrophy of the papillae and also due to decrease in SFR. In the present study, the salty and sour tastes were mainly affected followed by bitter and sweet tastes which were similar when compared to a study conducted by **Khader et al. (2015)**[12], who found hypoguesia to salty (62.2%) and dysguesia to sour taste (40%) among OSMF patients when compared to apparently healthy subjects**. Yadav et al. (2017)**[50] found significant taste alteration with salt taste followed by sweet, bitter and sour in OSMF patients, this alteration somehow coincides with the present study. A study done by **Kale et al. (2019)**[57] demonstrated a noticeable decrease in taste perception to

salty taste among tobacco chewers when compared with non-chewers, again somehow coincides with the present study.

The present study also demonstrate dysgeusia to sweet and salty taste among stage III OSMF when compared to stage II and I; dysgeusia to salty taste among stage II when compared to stage III and I; dysgeusia to bitter and sour taste among stage III when compared to stage I and II; normal to sweet, salty and bitter taste among stage I when compared to stage II and III. This showed statistical significance with P = 0.024 (<0.05) for only salty taste on comparison of taste perceptions between stage I, II and III OSMF. A study conducted by **Dyasanoor et al. (2016)**[13], reported hypogeusia to salty and sweet taste and dysgeusia to sour taste among Stage II OSMF when compared to Stage I OSMF subjects, which showed hypergeusia to sweet and salty taste. **Bangi et al. (2019)**[58] found significant changes in sour taste with 33.3% hypoguesia in OSMF subjects and 13.3% hypoguesia to all tastants in gutka chewers and hypoguesia to salt, sour and bitter in stage III compared to stage I and II. A study done by **Gupta et al. (2019)**[56] concluded that the gustation, similar to salivary flow rate, remains unaltered in the initial phase of OSMF. The alterations to taste perception may vary but can be concluded that significant alterations are seen in OSMF group and its stages when compared to controls.

In present study, a statistically highly significant correlation in the Candida carriage rate was seen in OSMF patients when compared with the control group.The results showed a carriage rate of 84.33% and 10% in OSMF and control group respectively. Relatively there were more number of CFUs in stage II and III of OSMF than stage I. The highest number of CFUs in OSMF was 105 in stage III and 6 in

control group. All 25 positive CFUs (100%) showed more than 10 CFUs of Candida on Sabouraud's dextrose agar media in OSMF group and all 3 positive CFUs (100%) of control group showed CFUs less than 10. This was in accordance with the study done by **Nayak et al. (2012)[43],** who found statistically significant CFUs when counted in OSMF as compared to controls.

Salivary flow rate was seen to be decreased in stage II and III OSMF patients, although Candida carriage was seen relatively more in stage II and stage III OSMF than stage I OSMF patients. Results of the present study suggest an inverse relationship between salivary flow rate and candidal colony, which was in accordance with the study of **Torres et al. (2002**).[78]

The results of the present study were also similar to that of **Kamat et al. (2011)[39]**, who found 36.67% cases in OSMF group and 10% in control group yielding candida growth with a statistical significance and suggested that OSMF favors the colonization of Candida. **Shinozaki et al. (2012)[42]** compared OSMF with healthy individual with xerostomia and found significantly reduced salivary flow rate, increased rate of oral mucosal symptoms and higher numbers of Candida speices in OSMF patients than a healthy individuals. Salivary flow rate was negatively correlated with the number of Candida, which is also in agreement with the findings of the present study. A study done by **Chaudhary et al. (2012)[44]** concluded that the rate of oral yeast carriage was significantly higher in OSMF (24.24%) as compared to healthy individuals (18.57%), which is also in agreement with the results of the present study. The present study confirmed the findings of **Gupta et al. (2015)[46],** who compared salivary flow and candida carriage in patients with oral submucous fibrosis

and concluded that salivary flow rate was constantly reduced with different grades of OSMF while candidal carriage was seen in grade II and grade III OSMF patients compared to stage I and stage IV OSMF and control group. They suggested that a higher candidal carriage in grade II and grade III OSMF patients could be related to decreased salivary flow rate. **Panditray et al. (2018)[53]** studied the prevalence of candida species and salivary flow rates in patients diagnosed clinically as stage I to IV OSMF and concluded that the salivary flow rates progressively decreased from stage I to IV, while CFUs were highest in stage III and lowest in stage I followed by stage IV and stage II OSMF, which is also corroborating with the findings of the present study. On the contrary, **Ariyawardana et al. (2007)[36]** showed 63.6% of oral yeast carriage in Sri Lankan patients with OSMF and 50% in control group, however it was not statistically significant, which contradicts the present study. A study done by **Anila et al. (2011)[22]** compared and found the incidence and intensity of Candida (primarily C. albicans) higher in OSMF patients than in healthy controls, but these findings were within the normal limit (3-47%), contradicting the present study. **Samatha et al. (2019)[59]** found 53.3% of OSMF patients and 6.7% of healthy controls having candida growth culture and concluded that the candidal colonies were higher in the OSMF group than healthy controls. However, the candidal carriage in OSMF group was not statistically significant, thus contradicting the present study, which shows highly significant candida carriage in OSMF group compared to healthy individuals.

This finding of increased Candida carriage in OSMF patients implies that the alterations in the overlying epithelium seen in OSMF would breach the physiological barrier rendered in healthy status, thus making a favorable conducive microenvironment which eventually increases the colonization of Candida species.[39]

Saliva is an important factor considering the oral health; therefore along with the routine clinical examination and investigations for OPMDs, the primary care oral physicians can also assess is the salivary flow rate, gustatory/taste perception and candida culture examination to rule out the effects of the underlying habits and assess the role of candidal carriage in malignant transformation. The management including counselling of the patient, quitting of the habits, intake of more water, delivering mouth exercises, pharmacotherapy including antifungals and follow-up, can bring satisfaction with an improved quality of life to the patients.

LIMITATIONS

This study has entailed the changes in saliva, taste parameters and candida carriage among OSMF subjects. The changes in saliva and taste parameters are entailed among all the 3 stages of OSMF. However, there are limited numbers of subjects for stages of OSMF group.

1. Stage IV OSMF were not included in the study.

2. A larger sample size would be helpful in confirming the results.

3. Follow-up of the patient can be done to evaluate the correlation between stages of OSMF and prognosis.

FURTHER RESEARCH

1. Future studies can be conducted including all the four stages of OSMF with larger sample size to evaluate the present parameters.

2. Evaluating and identifying the different species of candida present in OSMF.

3. As there is no information on whether candida is involved in malignant transformation of OSMF or is a secondary colonizer, further confirmation is required and can be done involving a larger pool of OSMF patients with regular follow up, isolation of strains of candida and measuring the rate of production of nitrosamines which may influence the malignant transformation of OSMF.

Conclusion

OSMF is a major global threat to public health with the prevalence increased over the past four decades from 0.03% to 6.42% in India. It has been reported that in India, about 80% of oral cancers are preceded by OPMDs. OSMF is well recognized for its malignant potential rate varied from 7-30%.

The present study showed a decrease in the salivary flow rate among OSMF patients and its different stages from stage III to stage I & stage II; also showed alterations in gustatory perceptions and a higher incidence of candidal growth in the OSMF patients when compared to the healthy individuals. Therefore, we can conclude that there is decrease in SFR with increase in candidal carriage as the stages of OSMF advances.

Alterations in all the above parameters could be an early sign of oral mucosal deterioration. Besides from increasing the risk of cancer development, arecanut and tobacco have negative effects causing alterations in the SFR and gustatory perception among OSMF affected individuals, which can cause cachexia and may often lead to depression, affecting the quality of life.

Awareness towards ill-effects of tobacco has increased with time in a large number of the general population world-wide by the means of advertisements and screening camps etc., however many aspects of their use have not been adequately understood and implemented by the people, resulting in the increase rate of OPMDs and Oral cancer.

Consequently, we arrive again at the first line of the dissertation, with a quote by **Hippocrates**, the father of modern medicine - **"There are in fact two things, science and opinion; the former begets knowledge, the latter ignorance."**

And

"Every human being is the author of his own health and disease."

- Siddhartha Gautam Buddha.

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CASE RECORD FORM

Title: Evaluation of salivary flow rate, Gustatory perception and Candidal carriage in patients with Oral submucous fibrosis – An observational analytical case control study.

Patient's ID:_____

Age:_____Sex:_____

Date	Clinical findings	Stage (for OSMF)	Group (A/B)

1. Determination of salivary flow rate.

Distance covered by saliva on the Schirmer strip will be measured against time.

TIME OF EVALUATION DURING THE DAY (9am to 11 am)	Distance after 1 min	Distance after 2 mins	Distance after 3 mins

2. Determination of taste perception.

Perception of taste as normal, dysgeusia or ageusia will be measured in three categories: Sweet, salty, bitter and Sour.

SWEET			SALTY			BITTER			SOUR		
Normal	Dysgeusia	Ageusia	Normal	Dysgeusia	Ageusia	Normal	Dysgeusia	Ageusia	Normal	Dysgeusia	Ageusia

3. Determination of candida carriage.

Candida growth on Sabouraud's dextrose agar media	
Candida culture positive	**No. of colony forming units (CFU/mL)**

(Confidential)

Informed Consent Form

"Evaluation of salivary Flow Rate, Gustatory Perception and Candida carriage in patients with Oral Submucous fibrosis".

Mr./Mas./Mrs./Ms_____

Resident of:_____

Age : _____ years,

Exercising my free will/choice, without any pressure/lure of incentive in any form, hereby giving my consent for the project to be conducted.

I acknowledge the doctor has informed me about the research project and explained the procedures in the best language known to me that is to be performed suitably and sufficiently to my satisfaction. I agree to let my salivary investigations to be taken as required. I agree to take part in this project and shall report to the dental hospital when called on given appointment dates and time. I am aware of the fact that I can opt out of the study at any time without having to give any reason and this will not affect my future treatment in the hospital. I shall co-operate with the doctors and paramedical staff, in all respect. I permit to publish my results/data in this study. I shall not be given any reimbursement or compensation.

I hereby record my consent for the participation in the said trial.

Patient's Name & Sign	Investigator's Signature	Witness Name & Sign
Date : _____	Date : _____	Date : _____
Time : _____	Time : _____	Time : _____

MASTER SHEET

GROUP A: HEALTHY INDIVIDUALS.

1. SALIVARY FLOW RATE DISTANCE IN MM					
SR. NO.	**AGE**	**SEX**	**1 MIN**	**2 MIN**	**3 MIN**
1	28	Female	7	25	31
2	30	Female	11	24	30
3	24	Male	15	25	30
4	30	Male	9	23	29
5	21	Female	12	25	32
6	38	Female	10	22	30
7	45	Male	9	21	28
8	31	Female	8	23	30
9	27	Female	11	24	31
10	27	Male	12	26	31
11	21	Male	14	24	30
12	20	Male	12	18	29
13	25	Male	9	17	29
14	22	Male	10	18	28
15	35	Male	11	20	27
16	39	Male	11	22	30
17	33	Male	10	17	28
18	35	Male	9	16	27
19	28	Male	11	18	30
20	44	Female	8	22	29
21	18	Female	12	22	31
22	18	Male	11	22	30
23	41	Male	9	23	30
24	23	Male	12	23	31
25	40	Female	7	17	27
26	28	Male	9	18	29
27	36	Male	6	17	27
28	36	Female	7	20	30
29	22	Male	10	17	29
30	40	Male	7	15	26

2. GUSTATORY PERCEPTIONS IN CONTROL GROUP.

Sr no.	Sweet			Salty			Bitter			Sour		
	Normal	Dysgeusia	Ageusia	Normal	Dysgeusia	Ageusia	Normal	Dysgeusia	Ageusia	Normal	Dysgeusia	Ageusia
1	1	0	0	1	0	0	1	0	0	1	0	0
2	1	0	0	1	0	0	0	0	1	1	0	0
3	1	0	0	0	0	1	1	0	0	1	0	0
4	1	0	0	1	0	0	1	0	0	1	0	0
5	1	0	0	1	0	0	1	0	0	1	0	0
6	1	0	0	1	0	0	1	0	0	1	0	0
7	1	0	0	1	0	0	1	0	0	1	0	0
8	1	0	0	1	0	0	1	0	0	1	0	0
9	1	0	0	1	0	0	1	0	0	1	0	0
10	1	0	0	1	0	0	1	0	0	1	0	0
11	1	0	0	1	0	0	1	0	0	1	0	0
12	1	0	0	1	0	0	1	0	0	0	0	1
13	1	0	0	1	0	0	1	0	0	1	0	0
14	1	0	0	1	0	0	1	0	0	1	0	0
15	1	0	0	1	0	0	1	0	0	1	0	0
16	1	0	0	1	0	0	1	0	0	1	0	0
17	1	0	0	1	0	0	1	0	0	1	0	0
18	1	0	0	1	0	0	1	0	0	1	0	0
19	1	0	0	1	0	0	1	0	0	0	1	0
20	1	0	0	0	0	1	1	0	0	1	0	0
21	1	0	0	1	0	0	1	0	0	1	0	0
22	1	0	0	1	0	0	1	0	0	0	0	1
23	1	0	0	1	0	0	0	1	0	1	0	0
24	1	0	0	1	0	0	1	0	0	1	0	0
25	1	0	0	1	0	0	1	0	0	1	0	0
26	1	0	0	1	0	0	1	0	0	1	0	0
27	1	0	0	1	0	0	1	0	0	1	0	0
28	1	0	0	1	0	0	1	0	0	1	0	0
29	1	0	0	1	0	0	1	0	0	0	0	1
30	1	0	0	1	0	0	1	0	0	0	1	0

3. NO. OF COLONY FORMING UNITS IN CONTROL GROUP.

SR NO.	CFU
1	0
2	0
3	0
4	0
5	0
6	0
7	0
8	0
9	0
10	0
11	0
12	0
13	0
14	0
15	0
16	6
17	0
18	0
19	0
20	3
21	0
22	0
23	0
24	1
25	0
26	0
27	0
28	0
29	0
30	0

GROUP B: OSMF PATIENTS

1. SALIVARY FLOW RATE DISTANCE IN MM						
SR NO.	**AGE**	**SEX**	**STAGE**	**1 MIN**	**2 MIN**	**3 MIN**
1	21	Male	I	9	15	19
2	24	Female	I	10	17	22
3	27	Male	I	10	15	17
4	18	Male	I	11	17	22
5	30	Male	I	3	10	14
6	43	Male	I	10	15	20
7	27	Male	I	11	16	25
8	37	Female	I	12	16	22
9	31	Female	I	11	17	23
10	29	Male	I	5	13	20
11	41	Male	II	7	11	16
12	24	Male	II	8	14	24
13	30	Male	II	5	10	15
14	30	Male	II	7	15	21
15	29	Male	II	6	14	21
16	42	Male	II	8	12	20
17	39	Male	II	8	19	25
18	34	Male	II	11	19	23
19	24	Male	II	9	17	22
20	38	Female	II	5	9	19
21	30	Male	III	3	9	15
22	43	Male	III	1	12	15
23	45	Male	III	2	4	8
24	39	Male	III	2	5	11
25	44	Male	III	4	9	12
26	27	Male	III	3	9	14
27	37	Male	III	2	8	9
28	19	Male	III	3	8	12
29	29	Male	III	1	9	12
30	24	Male	III	4	10	13

2. GUSTATORY PERCEPTIONS IN OSMF GROUP.

Sr no.	Stage	Sweet			Salty			Bitter			Sour		
		Normal	Dysgeusia	Ageusia	Normal	Dysgeusia	Ageusia	Normal	Dysgeusia	Ageusia	Normal	Dysgeusia	Ageusia
1	I	1	0	0	1	0	0	1	0	0	0	1	0
2	I	1	0	0	1	0	0	1	0	0	0	0	1
3	I	1	0	0	0	1	0	0	0	1	0	1	0
4	I	1	0	0	1	0	0	1	0	0	1	0	0
5	I	0	0	1	1	0	0	1	0	0	0	1	0
6	I	1	0	0	0	0	1	1	0	0	0	1	0
7	I	1	0	0	0	0	1	1	0	0	0	0	1
8	I	1	0	0	1	0	0	0	1	0	1	0	0
9	I	1	0	0	1	0	0	1	0	0	1	0	0
10	I	1	0	0	1	0	0	0	1	0	0	1	0
11	II	1	0	0	0	1	0	1	0	0	0	1	0
12	II	1	0	0	1	0	0	0	0	1	0	1	0
13	II	0	0	1	0	1	0	0	0	1	0	1	0
14	II	1	0	0	0	0	1	1	0	0	0	0	1
15	II	0	0	1	0	0	1	0	1	0	0	1	0
16	II	0	0	1	0	1	0	0	0	1	0	0	1
17	II	1	0	0	0	0	1	1	0	0	1	0	0
18	II	1	0	0	0	1	0	1	0	0	1	0	0
19	II	0	1	0	0	1	0	1	0	0	0	0	1
20	II	0	1	0	0	0	1	1	0	0	0	0	1
21	III	0	0	1	0	0	1	0	0	1	0	1	0
22	III	0	1	0	0	0	1	0	0	1	0	1	0
23	III	1	0	0	0	1	0	1	0	0	0	1	0
24	III	0	1	0	0	0	1	0	0	1	1	0	0
25	III	0	0	1	0	1	0	1	0	0	1	0	0
26	III	0	0	1	0	0	1	1	0	0	0	1	0
27	III	1	0	0	1	0	0	0	1	0	0	1	0
28	III	1	0	0	0	1	0	0	1	0	0	1	0
29	III	0	0	1	0	0	1	0	1	0	0	1	0
30	III	0	1	0	0	0	1	0	1	0	0	1	0

3. NO. OF COLONY FORMING UNITS IN OSMF GROUP.

SR. NO.	OSMF STAGE	CFU
1	I	0
2	I	0
3	I	22
4	I	0
5	I	19
6	I	24
7	I	0
8	I	15
9	I	0
10	I	12
11	II	42
12	II	31
13	II	35
14	II	38
15	II	43
16	II	44
17	II	32
18	II	32
19	II	36
20	II	51
21	III	71
22	III	83
23	III	105
24	III	74
25	III	50
26	III	34
27	III	40
28	III	52
29	III	57
30	III	43