SERUM LIPID PROFILE AND C-REACTIVE PROTEIN AS A PROGNOSTIC MARKER IN ORAL SUBMUCOUS FIBROSIS

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CERTIFICATE BY THE GUIDE

This is to certify that **Dr. K. JAYANTHISWARI,** Post graduate student (2017-2020) in the Department of Oral Medicine and Radiology (Branch IX**)**, Tamilnadu Government Dental College and Hospital, Chennai 600003, has done this dissertation titled **"SERUM LIPID PROFILE AND C-REACTIVE PROTEIN AS A PROGNOSTIC MARKER IN ORAL SUBMUCOUS FIBROSIS"** under my direct guidance and supervision in partial fulfillment of the M.D.S. degree examination in May 2020 as per the regulations laid down by Tamilnadu Dr. M.G.R. Medical University, Chennai- 600 032 for **M.D.S., Oral Medicine and Radiology (Branch – IX)** degree examination.

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This is to certify that the Dissertation entitled "**SERUM LIPID PROFILE AND C-REACTIVE PROTEIN AS A PROGNOSTIC MARKER IN ORAL SUBMUCOUS FIBROSIS"** is a bonafide work done by **Dr.K.JAYANTHISWARI,** Post Graduate student (2017-2020) in the Department of Oral Medicine and Radiology under the guidance of **Prof.Dr.G.V.MURALI GOPIKA MANOHARAN, MDS.,** Professor, Department of Oral Medicine and Radiology, Tamil Nadu Government Dental College and Hospital, Chennai – 600 003.

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DECLARATION

TITLE OF DISSERTATION	**"SERUM LIPID PROFILE AND C-REACTIVE PROTEIN AS A PROGNOSTIC MARKER IN ORAL SUBMUCOUS FIBROSIS"**
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I, **Dr. K.JAYANTHISWARI,** hereby declare that no part of the dissertation will be utilized for gaining financial assistance/any promotion without obtaining prior permission of the Principal, Tamil Nadu Government Dental College and Hospital, Chennai 600003. In addition, I declare that no part of this work will be published either in print or in electronic media without the guide who has been actively involved in the dissertation. The author reserves the right to publish the work with the prior permission of the Principal and Guide, Tamil Nadu Government Dental College & Hospital, Chennai - 600003.

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LIST OF CONTENTS

LIST OF ABBREVIATIONS

OSF	Oral submucous fibrosis
TC	Total cholesterol
TG	Triglycerides
HDL	High density lipoprotein
LDL	Low density lipoprotein
VLDL	Very low density lipoprotein
CRP	C-reactive protein
WHO	World Health Organization
ESR	Erythrocyte sedimentation rate
ROS	Reactive oxygen species
GTH	Glutathione
HLA	Human leucocyte antigen
MICA	Major histocompatibility class I chain related gene A
MMP3	Matrix metalloproteinase 3
ANA	Antinuclear antibodies
TNF	Tumor necrosis factor
TGF	Tissue growth factor
GST	Glutathione S transferase
OPL	Oral pre-malignant lesion

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Urkund Analysis Result

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ABSTRACT

TITLE: "SERUM LIPID PROFILE AND C-REACTIVE PROTEIN AS A PROGNOSTIC MARKER IN ORAL SUBMUCOUS FIBROSIS"

Background: Oral submucous fibrosis is a chronic, premalignant condition of the oral mucosa, affecting millions of people globally and it is one of the precancerous conditions most prevalent in India. Various biochemical markers are available for detection of potentially malignant disorders. One such important marker is serum lipid profile. Previously published studies have evaluated the serum lipid profile and C-reactive protein in various potentially malignant disorders and oral squamous cell carcinoma, but to our knowledge no studies are available comparing the pre-treatment and post-treatment lipid profile and C-reactive protein values in oral potentially malignant disorders, so that their real prognostic value can be assessed.

Aim: The aim of the present study is to evaluate the serum lipid profile and C - reactive protein as a prognostic marker in OSF patients.

Objectives: To evaluate the serum lipid profile including (i) Total cholesterol, (ii) LDL cholesterol (iii) HDL cholesterol (iv) VLDL cholesterol and (v) triglycerides (vi) C-reactive protein in patients with various stages of OSF. To correlate alterations in serum lipid profile and C-reactive protein pre and post-treatment. To correlate the alterations in serum lipid profile and C-reactive protein with improvement in symptoms post-treatment.

Methods: A total of 30 clinically diagnosed OSF patients of either gender in the age group of 20 – 50 years were selected. A complete history taking followed by a thorough oral examination was done to all patients. Burning sensation of the patient was assessed by using visual analog scale and mouth opening measured using caliper and measuring scale. These 30 patients were subdivided into 4 stages according to Khanna et.al staging system of OSF. 5 ml blood sample was collected before treatment and after 6 weeks of treatment for estimation of serum lipid profile & CRP.

Results: The mean age of the patient was 30.73 ± 7.5, with a male predominance (96.6%). The mean increase in mouth opening post treatment was 6.03 ± 1.7. All patients (100%) in Grade 1 & Grade 2 after completion of the treatment regimen, reported a complete relief from pain/burning sensation. The mean values of serum lipid profile (Total cholesterol, Triglycerides, HDL, LDL, VLDL) were decreased among OSF patients and the difference between pre and post treatment were statistically significant. The comparison of mean values of the pre-treatment and post treatment CRP level of the study subjects among OSF patients were found to be statistically significant (p=0.022). The mean CRP levels decreased by 0.55 ± 1.2 post treatment.

Conclusion: Hence, pre-treatment and post treatment serum lipid profile and CRP levels contributes to be of prognostic value in OSF.

Key words: Oral Submucous Fibrosis, Lipid profile, C - reactive protein.

INTRODUCTION

Oral submucous fibrosis (OSF) is a chronic disease of the oral cavity, which is characterized by an epithelial and subepithelial inflammatory reaction followed by fibroelastic changes in the submucosa which was first described by Schwartz in 1952[1]. Pindborg (1966) defined OSF as, "an insidious, chronic disease affecting any part of the oral cavity and sometimes the pharynx. Although occasionally preceded by and/or associated with vesicle formation, it is always associated with juxta-epithelial inflammatory reaction followed by a fibroelastic change of the lamina propria, with epithelial atrophy leading to stiffness of the oral mucosa and causing trismus and inability to eat."[1] This disease occurs most commonly in South East Asia but cases have been reported worldwide in countries like Kenya, China, UK, Saudi Arabia and other parts of the world. Over the years, the incidence of OSF has increased in various parts of the Indian subcontinent. Its prevalence ranges up to 0.4% in Indian rural population and the malignant transformation rate is 7.6%.[2]

The etiology of OSF is multifactorial. Chewing of areca nut is one of the most important causative factors. Arecanuts contain alkaloids of which arecoline seems to be a primary etiological factor which modulates matrix metalloproteinase, lysyl oxidases and collagenases affecting the metabolism of collagen leading to increased fibrosis[3]. Along with this, ingestion of capsaicin in chillies, genetic and immunologic processes, micronutrient deficiencies of iron, zinc and essential vitamins serves as pre-disposing factors. These factors derange the repair of the inflamed oral mucosa, leading to defective healing and resultant

scarring. OSF is diagnosed clinically, though additional investigations are required for early diagnosis and better prognosis. Various biochemical markers are available for detection of potentially malignant disorders. One such important marker is serum lipid profile. Lipids are major cell membrane components essential for various biological functions, including cell growth and division of normal as well as malignant tissues. The newly forming cells would need many basic components well above the normal limits, used in physiological processes. As the neoplastic disease is related to new growth, there is a greater utilization of lipids, including total cholesterol (TC), lipoproteins and triglycerides (TGs) for new membrane biogenesis. Cells fulfill these requirements either from circulation or from degradation of major lipoprotein fractions such as very low-density lipoprotein (VLDL), low-density lipoprotein (LDL) or high-density lipoprotein (HDL).[4]

It is believed that arecanut carcinogens induce fibrosis leading to generation of free radicals and reactive oxygen species, which are responsible for the high rate of oxidation/peroxidation of polyunsaturated fatty acids. This peroxidation further releases peroxide radicals. This affects the essential constituents of the cell membrane and might be involved in carcinogenesis. C-reactive protein is synthesised by hepatocytes and regulated by pro inflammatory cytokines such as interleukins (IL-1 and 6) and tumour necrosis factor. CRP acts on the cell during inflammation, according to induction hypothesis, acute and chronic inflammation increased CRP levels leads to excessive cellular proliferations and also cause irreversible DNA damage. According to response hypothesis, immune response of the host in cancer results

in increased level of CRP. Studies have shown association between serum CRP and PMD & head and neck cancers.[5] Alterations in the circulatory cholesterol levels have been associated with rapidly dividing cells in malignancy. Studies have shown that glucocorticoid use was associated with a higher serum HDL cholesterol level and a lower ratio of total cholesterol to HDL cholesterol.[6] Hence lipids and CRP can serve as marker in early neoplastic changes, disease prognosis and follow-up cases. Previously published studies have evaluated the serum lipid profile and C-reactive protein in various potentially malignant disorders and oral squamous cell carcinoma, but to our knowledge no studies are available comparing the pre-treatment and post-treatment lipid profile and C-reactive protein values in oral potentially malignant disorders, so that their real prognostic value can be assessed. Hence, I intended to do the present study.

AIM AND OBJECTIVES

AIM:

The aim of the present study is to evaluate the serum lipid profile and C - reactive protein as a prognostic marker in OSF patients.

OBJECTIVES:

1. To evaluate the serum lipid profile including (i) Total cholesterol, (ii) LDL cholesterol (iii) HDL cholesterol (iv) VLDL cholesterol and (v) triglycerides (vi) C-reactive protein in patients with various stages of OSF.
2. To correlate alterations in serum lipid profile and C-reactive protein pre and post-treatment.
3. To correlate the alterations in serum lipid profile and C-reactive protein with improvement in symptoms post-treatment.

REVIEW OF LITERATURE

Oral submucous fibrosis (OSF) is a chronic, premalignant condition of the oral mucosa, which was first described by **Schwartz** in 1952. [7]

Pindborg (1966) defined OSF as "an insidious, chronic disease affecting any part of the oral cavity and sometimes the pharynx. Although occasionally preceded by and/or associated with vesicle formation, it is always associated with juxta‑epithelial inflammatory reaction followed by a fibroelastic change of the lamina propria, with epithelial atrophy leading to stiffness of the oral mucosa and causing trismus and inability to eat." [8]

HISTORY

OSF has been well established in Indian medical literature since the time of Susrutha; a renowned Indian physician who lived in the era 2500-3000 B.C. He described a condition termed "Vidari" under mouth and throat diseases, which was characterized by "a progressive narrowing of mouth, depigmentation of oral mucosa and pain on taking food." [9] In modern literature, OSF was first described by **Schwartz** in 1952, in five Indian women from Kenya. He called the condition "Atrophia Idiopathica (Tropica) Mucosae Oris". In India, **Joshi** described the disease for the first time in 1953 and coined the term "Submucous fibrosis of the palate and pillars". [10] The other names suggested include "Diffuse oral submucous fibrosis" (**Lal** 1953), "Idiopathic scleroderma of the mouth" (**Su** 1954), "Idiopathic palatal fibrosis" (**Rao** 1962), and "Sclerosing stomatitis" (**Behl** 1962).[11]

REGIONAL PREVALENCE OF OSF

It is predominantly seen in Southeast Asia and Indian subcontinent with few cases reported from South Africa, Greece and United Kingdom. The prevalence rate of OSF in India is about 0.2–0.5%.[12] The disease predominantly occurs mostly in India and in South East Asia, but the cases have been reported worldwide like Kenya, China, UK, Saudi Arabia and other parts of the world as Asians are migrating to these parts. Moreover, recent data suggests that prevalence of OSF in India has increased from 0.03% to 6.42%.

Northern Zonal Council: According to an epidemiological survey conducted on the geriatric Indian population Jodhpur, 64% of the patients presented with one or more oral lesions, among which it accounted 30% of oral submucous fibrosis. [13]Whereas according to a cross- sectional study conducted on the younger age group in the rural area of Jaipur,

Rajasthan, the prevalence of OSF in the study population was 231 (3.39%). Majority of subjects were males 188 (81.38%). The prevalence of OSF was maximum in 15 to 24 years of age group 98 (42.42%).[14]

North-Central Zonal Council: According to an etiological and epidemiological study done in Patna, Bihar it was observed that Male:Female ratio was 2.7: 1. A maximum number of cases belonged to 21-40 years of age. Most of the OSF cases used heavy spices and chillies, and gutkha was most commonly used by the OSF cases. [15] In a study conducted to evaluate the prevalence of use of tobacco & its associated products & Oral Sub Mucous

Fibrosis among teenagers, it showed that 34.1% of the study subjects used tobacco and among them, 14.2% of Oral Sub Mucous Fibrosis cases were

identified. [16] Similarly, according to a population-based case control study in rural and urban Lucknow, it was found that patients who use pan masala were at higher risk of developing oral submucous fibrosis. [17] In a study done among habitual gutkha, areca nut and pan chewers of Moradabad, India, between the ages of 11-40 years. The prevalence of OSF was 6.3% (63/1000), and gutkha chewing was the most common abusive habit amongst OSF patients. [18] In a study conducted in a dental institute in Modinagar, Uttar Pradesh, India, the overall prevalence of OML was (16.8%), the most prevalent being smoker's palate (10.44%) followed by leukoplakia (2.83%), oral submucous fibrosis (1.97%), oral candidiasis (1.61%), recurrent aphthous stomatitis (1.53%), oral lichen planus (0.8%) and others (0.78%).[19]

North Eastern Zonal Council: There are no studies done to evaluate the prevalence of OSF in north eastern zone. Whereas a study was done to obtain baseline information about the prevalence of tobacco use among school children in eight states in the north-eastern part of India.

Eastern Zonal Council: In a study done in the villages of two districts of the West Bengal state to detect early oral premalignant, it was found that oral submucous fibrosis showed the highest prevalence (2.7%) among the various OPLs detected. [20]

Western Zonal Council: According to an extensive epidemiologic house-to-house survey conducted in Poona district, Maharashtra it was found that oral submucous fibrosis had a prevalence of 0.03%. It was found that for oral submucous fibrosis prevalence depends on sex if tobacco habits are taken into account. [21] Similarly, a house to house epidemiological survey was conducted in Bhavnagar district, Gujarat state among 5018 men who reported the use of

tobacco or areca nut, 164 were diagnosed as suffering from OSF with a prevalence of 3.2%. A prevalence of 0.16% was noted in a survey carried out in the same district in 1967. It was found that there was a significant increase in the prevalence of OSF. [22]

According to a study conducted in the semi-urban district of Sangli in Western Maharastra, among 623 patients who had significant mucosal lesions, 152 had oral submucous fibrosis. [23]

Southern Zonal Council: According to a case-control study conducted within the framework of an on-going randomized oral cancer screening trial in Kerala, India, an inverse dose response relationship was seen between BMI and risk of OSF. [24] According to a hospital based cross-sectional study oral soft tissue lesions were found in 4.1% of the study subjects. The prevalence of OSF was 0.55%. The prevalence of smoking, drinking alcoholic beverages and chewing was 15.02%, 8.78% and 6.99% respectively.[25] From the study conducted to evaluate the prevalence of oral mucosal lesions in Manipal, Karnataka State, India the result showed the presence of one or more mucosal lesions in (41.2%) of the population. The prevalence of oral submucous fibrosis was 2.01%.[26] According to an epidemiological survey conducted among alcohol misusers attending a rehabilitation center in Chennai, south India. A total of 25% of the study group had at least one OML. The common oral lesions were smoker's melanosis (10.2%), oral submucous fibrosis (8%), and leukoplakia (7.4%).[27]

ETIOLOGY

The etiological factor in the causation of OSF is believed to be multifactorial, Areca nut plays an important role in the disease manifestation. [28, 29]

The chronic irritation caused by consumption of areca nut in the form of pan masala, gutkha causes injury thereby leading to chronic inflammation, cytokine production and development of oxidative stress.

Oxidative stress and subsequent reactive oxygen species (ROS) generation can induce cell proliferation, cell senescence or apoptosis, depending upon the level of ROS production. [30]

1. **Areca nut:** The term areca nut is used to denote the unhusked whole fruit of the areca nut tree and term betel nut is exclusively referred to the inner kernel or seed which is obtained after removing husk. Arecoline, an active alkaloid found in betel nuts stimulate fibroblasts to increase production of collagen by 150%. Areca nuts have also been shown to have high copper content, and chewing areca nuts for 5-30 minutes significantly increases soluble copper levels in oral fluids. This increased level of soluble copper supports the hypothesis as an initiating factor in individuals with OSF. [31]
2. **Chilli:** The suspicion that chilli is an etiological agent arose on the basis of ecological observations and was strengthened by the clinical and histological characteristics of this condition. OSF is found mostly among Indians and other population groups who use chillies (Capsicum annum and Capsicum frutescence) to spice their food.

From the observation of blood eosinophilia, tissue eosinophils in biopsy specimens and subepithelial vesicles among patients with OSF, authors have suggested an allergic nature of this disease possibly due to chilli intake. In an epidemiological and etiological study of OSF in Patna, India, usage of chilli was recorded in 154 of 157 cases, with 64 of the cases reporting heavy consumption. [15]

The hypothesis of chilli as an etiological agent was tested in an animal experimental study where capsaicin, an active principle of chillies was applied topically to the palates of Wistar rats. It was observed that capsaicin was capable of evoking a limited connective tissue response in healthy animals but the reaction was enhanced in protein or vitamin deficient animal systems. In contrast to the response observed by these investigators, other studies failed to reproduce the results in hamster cheek pouch experiments. [32]

There are some ecological arguments against the chilli hypothesis, OSF has not been reported in Mexico or other South American countries where chilli consumption is widespread. Indirect observations from a 10 year prospective study in Ernakulam district, Kerala, India, also did not substantiate the etiologic role of chillies. [33]

3. **Misi:** Misi is a black coloured powder containing various chemical substances like washing soda, borax, charcoal of myrobalan, and fuller's earth in varying proportions. It is widely used by female villagers of Eastern Uttar Pradesh as a cosmetic to keep the teeth shiny and clean. In a study conducted in this population, Misi usage was seen exclusively among women. Misi was used by 21 of 24 female patients. Misi is considered to be a causative factor of OSF among these women. [34]

4. **Immunological disorders:** Raised ESR and globulin levels are indicative of immunological disorders. Serum immunoglobulin levels of IgA, IgG and IgM are raised significantly in oral submucous fibrosis.

5. **Genetic Predisposition:** The possibility of genetic susceptibility for this condition has been probed by Cannif et al. [35] They performed HLA tissue typing and observed that the frequencies of HLA A10, DR3 and DR7 in

their sample of 44 patients were significantly different from the ethnically, regionally, and age-matched control group. These observations were interpreted to imply a possible genetic susceptibility to the action of extrageneous factors such as areca alkaloids and tannins.

Another study conducted on HLA typing in Taiwnese patients with OSF found significantly greater phenotype frequency of HLA-B76 and haplotype frequencies of HLA-B48/Cw7, B51/Cw7 and B62/Cw7 in OSF patients than in healthy control subjects. These findings suggested that some Taiwanese areca quid chewers with specific HLA phenotypes or haplotypes are prone to have OSF. [36]

In a study conducted to analyse the association of polymorphism of the MICA (major histocompatibility class I chain related gene A) and the risk for OSF, significantly higher phenotype frequency of allele A6 of MICA was found in OSF patients than in controls. The results of this study suggested that allele A6 in MICA might confer a risk for OSF. [37]

Another study conducted on the functional polymorphisms of matrix metalloproteinase 3 gene among male OSF patients using areca, reported that the 5A genotype in MMP3 promoter was observed more frequently in OSF patients than in controls. The results indicated that the 5A genotype of MMP3 promoter was associated with the risk of OSF. [38]

Autoimmunity

One of the earliest names by which OSF was identified was “idiopathic scleroderma of the mouth” and in view of the female preponderance of patients, its presentation in middle life and histologic similarities, the analogy seems

reasonable. The well documented findings of clinical, immunologic, and histologic abnormalities in OSF and similar reports in other connective tissue disorders, such as rheumatoid arthritis, progressive systemic sclerosis, systemic lupus erythematosus, and polymyositis, suggest a fundamental autoimmune basis for the disease. The alterations in cellular and humoral immunity seen in OSF are further suggestive of an autoimmune phenomenon. [39]

Caniff et al, has reported a high incidence of autoantibodies including antinuclear (ANA), antismooth muscle (SMA), antigastric parietal cell (GPCA), antithyroid microsomal (TMA) and antireticulin antibodies in patients with OSF. [35]

A high incidence of autoantibodies has also been reported in Taiwanese subjects with OSF. This study demonstrated a significantly higher positive ANA (23.9%), SMA (23.9%), and GPCA (14.7%) in OSF patients compared to healthy controls (9.2%, 7.3%, 5% respectively) which suggests that autoimmunity may play a role in the development of OSF. [40]

Recent investigations have also explored the role of cytotoxic T lymphocyte associated antigen 4 (CTLA-4) in OSF. Patients with OSF have a higher frequency of the G allele at position +49 on exon 1 of CTLA-4 compared with controls. CTLA-4 polymorphism has also been associated with certain autoimmune diseases such as systemic lupus erythematosus, insulin dependent diabetes mellitus, Graves' disease, Hashimoto's thyroiditis, multiple sclerosis and rheumatoid arthritis. [41]

6. **Antioxidant status and cytokines:** Glutathione S transferases (GST) are part of the antioxidant system. GSTT1 and GSTM1 null phenotypes increase the risk of OSF.

7. **Nutritional deficiency:** A subclinical vitamin B complex deficiency has been suspected in cases of OSF with vesiculations and ulcerations of oral cavity. [42] Iron deficiency anemia, vitamin B complex deficiency and malnutrition are promoting factors that derange the repair of the inflamed oral mucosa, leading to defective healing and resultant scarring. [43]
8. **Defective iron metabolism:** Microcytic hypochromic anemia with high serum iron has been reported in submucous fibrosis (**Rajendran**, 1994).[42]

PATHOGENESIS

Areca alkaloids causing fibroblast proliferation

Among the areca alkaloids such as arecoline, arecadine, guvacoline, guvacine, arecoline is the main agent responsible for fibroblast proliferation. Under the influence of slaked lime (Ca(OH)2), arecoline get hydrolyzed to arecadine, which has pronounced effects on fibroblasts. [44] A study by **Harvey et al** showed that exposures to 0.1-10 μg/ml arecoline stimulates fibroblasts and concentrations more than 25 μg/ ml, inhibits fibroblast growth and collagen synthesis. [45] **Jeng et al** found that depletion of cellular glutathione (GTH) levels by arecoline predisposes the oral mucosal fibroblasts to various genotoxic and cytotoxic stimulation. [46]

Clonal selection of OSF fibroblasts by arecoline

Studies have shown that arecoline causes elevated collagen synthesis by OSF fibroblasts compared to normal fibroblast. This could reflect clonal selection of a cell population in altered tissues under the influence of local factors such as IL-1 from inflammatory cells. [47]

Stablization of collagen structure by Tannins and Catechins

Areca flavonoids tannins and catechins can cause increased fibrosis by forming a more stable and non-soluble collagen structure by inhibiting collagenase enzyme activity. [48] Studies have shown that there is 1.5 fold increase in collagen production by OSF fibroblasts and with the progression of disease type 3 collagen is completely replaced by type 1 collagen which is more resistant to degradation. [49] Also there has been an excess of alpha 1 (1) chains relative to alpha 2(1) chains, suggesting an alteration of collagen molecule during the disease progression.[50]Recently a study done on human buccal fibroblasts showed an increased expression of an insoluble cytoskeleton protein (57kDa) called vimentin in OSF patients under arecoline influence. This protein vimentin is primarily expressed by mesenchymal cells, during cell growth, and tumorigenesis. Thus elevated vimentin expression stimulated by arecoline in OSF may be suggestive of transformational changes in buccal fibroblasts of OSF patients. [51]

Inhibition of collagen phagocytosis

According to studies, there is a gross imbalance in the extracellular matrix remodeling in OSF. In fibrotic connective tissue lesions without marked inflammation such as OSF, the main route of collagen degradation is by phagocytosis and not by extracellular digestion.[52] In OSF, the reduction of phagocytic activity is inversely dose dependent to levels of arecoline, safrole and nicotine in saliva. [53] Arecoline causes a suppression of T cell activity which in turn decreases the cell mediated immunity and thus results in decreased phagocytic activity of the cells.[54]

High copper content in areca nut and fibrosis

The average daily intake of copper by adults from diet in developing countries is between 0.6 and 1.6 mg/ day.[55] An adult Indian chewing areca nut

daily consumes over 5 mg of copper /day. [56] The copper released during chewing is brought in direct contact with oral mucosal keratinocytes for prolonged periods of time. Earlier studies have shown that it takes 40 min for raised salivary copper levels to return to its baseline value.[57] The uptake of copper into the epithelial cells is a non energy, non enzyme dependent diffusion. The copper is either bound to protein metallothione or transferred across the basolateral membrane.[58]

At cellular level, there is evidence to support the role of membrane bound copper transporting adenosine triphosphate (Cu-ATPase) in uptake of copper by the cells.[59]

The mechanism of copper accumulation into the cells is explained by the presence of an extracellular tripeptide called as GHL tripeptide (glycy-L- histidyl-L- lysine) which is released within the lamina propria zone of areca nut chewers during initial inflammatory phase of OSF. The first two residues of GHL molecule are involved in bonding with copper, whereas the side chain lysine may be involved in recognition of receptors that function in uptake of copper into the cells.[60] Copper also causes up regulation of lysyl oxidase enzyme which plays a crucial role in cross linking of collagen and elastin molecules. Lysyl oxidase is a copper-dependent enzyme which is also an intrinsic protein of connective tissue. It is induced at detectable levels during fibrogenesis and fibroproliferative process.[61]

Copper may also bind to the protein product of p53 causing p53 aberrations in the oral keratinocytes.[57] In a study it was found that the concentration of copper in saliva, which can cause significant increase in collagen synthesis, was found to be 2-4 μg, whereas the peak effect was noted at 50 μg of copper chloride.[62] Studies done to evaluate serum and tissue copper levels in OSF patients showed raised tissue copper levels in buccal mucosal biopsies of OSF

patients. The tissue copper levels measured by mass absorption spectrometry showed that the tissue copper levels in OSMF patients were 5.5 µg / gm compared to 4 µg/ gm in non areca nut chewers.

Also the concentration gradient of copper was noted in lining mucosa with higher content of copper in epithelium compared to deeper connective tissues and muscle layers. Also raised copper was demonstrated in fibrotic side than non fibrotic side in unilateral OSF patients' biopsies. Thus the site on which patient habitually kept the quid had increased copper levels.

This, along with the length of time quid chewed, consistency of the quid, affects the uptake of copper by the epithelium.[57]

However the serum copper, ceruloplasmin, urinary and fecal copper levels of OSF patients were within the lab reference ranges. Also no fibrosis was found elsewhere in the body suggesting the local effect of copper as the oral cavity is directly exposed to copper challenge.[63]

Copper also affects specific growth characteristics of fibroblasts. The cell doubling time of OSF fibroblast was reported to be 3.2 days as compared to 3.6 days for normal fibroblasts when they were cultured under influence of copper.[64]

Increased expression of fibrogenic cytokines

It has been postulated that external stimuli such as areca nut may induce OSF by increasing the levels of cytokines in lamina propria and also increasing the production of cytokines by the peripheral mononuclear cells.[65] The epidemiology of OSF strongly suggests an individual susceptibility which could be cytokine based, especially as initial feature of OSF in chronic inflammation accompanied by fibrosis. Also up regulation of proinflammatory cytokines ie., IL-

6 and IL-8 has been seen. It may be due to the T-cell activation, which occurs secondary to the chronic inflammation. Also an up regulation of certain fibrogenic cytokines such as TNF-α, TGF-β, platelet derived growth factor, basic fibroblast growth factors is seen in OSF. An under expression of antifibrotic cytokine interferon-gamma may also contribute to increased fibrosis.

The above features are suggestive of an altered immune response in circulating monocytes along with an increase in number of local antigen presenting cells and lymphocytes in OSF patients. This increases the genetic susceptibility of these patients and thus causes the penetration of arecoline and arecadine into the oral mucosa.[66]

Incorporation of copper in areca nut

The incorporation of copper into the areca nut is through the Bordeaux mixture which is sprayed as a fungicide on areca plantations in regions with scheduled monsoons and of which copper sulfate is an important constituent. There is evidence to suggest that the metal matrix binding of copper in plants is associated with lectins and glycoproteins.[56]

The copper content of various constituents of quid are red areca (18.3ppm), white areca (14.9 ppm), betel leaf (18.5 ppm), gutkha (13.2 ppm), flavored areca (12.2 ppm), tobacco (6.3 ppm). The above data shows that betel leaf contains highest amount of copper.[67]

The processed form of betel nut ie., the freeze-dried products (panmasala, gutkha, mawa) contain higher concentration of copper as compared to raw form, this may be because of the copper which is added to it as a preservative.

CLINICAL FEATURES

Pindborg and **Sirsat** surmised that the onset of the disease is insidious and is often of 2 to 5 years duration. The disease seems to affect some areas within the mouth more frequently than others. The retromolar areas and buccal mucosa are commonly involved, as are the soft palate, palatal fauces, uvula, tongue and labial mucosa. Many observers state that the disease originates from the posterior part of the oral cavity with subsequent involvement of the anterior structures. [68]

Symptoms

Early symptoms

The most common initial symptom is burning sensation of the oral mucosa, aggravated by spicy food. Vesiculation, excessive salivation, ulceration, pigmentation changes, recurrent stomatitis, defective gustatory sensation, and dryness of the mouth have also been indicated as early symptoms.[69] Some patients may have an itching sensation as reported by **Bhatt** and **Dholakia**[70] which is probably due to release of histamine from mast cells.

Late symptoms

As the disease progresses, there is a gradual stiffening of the oral mucosa. The mucosal rigidity leads to restricted mouth opening and tongue protrusion. When the fibrosis extends to the pharynx and oesophagus, the patients may experience difficulty in swallowing food. The fibrosis also leads to difficulty in speech, pain in the throat and ears, and a relative loss of auditory acuity due to stenosis of the opening of the eustachian tube. Patients may rarely complain of nasal regurgitation or nasal intonation to their speech.[35] Defective gustatory function has been reported in these patients, which may be due to reduced contact surface of the tongue mucosa while chewing, atrophy of taste buds, or perineural fibrosis.[71]

Signs

Early signs

The earliest clinical sign of the disease is blanching of the oral mucosa. This blanching imparts a marble like appearance to the mucosa, and can be localized, diffuse or in the form of lace-like network. [68] Fibrosis may not be evident, or may be seen arching from the anterior faucial pillars into the soft palate as a delicate reticulum of interlacing white strands which later become confluent. [35] Slight rigidity may be felt in the oral tissues and the bands are diffuse and mild in the early stages. The occurrence of vesicles has been reported in some patients in the early stages of the disease which later form ulcers. [72]

Late signs

As the disease progresses, thick fibrous bands appear in the submucosal layer of the oral soft tissues. One of the main diagnostic clinical criteria for OSF is the presence of these fibrous bands either in the buccal mucosa, the posterior part of the palate or the labial mucosa. The fibrous bands run vertically in the buccal mucosae. Involvement of the lips is characterized by the presence of circular fibrous bands around the rima oris. In severe labial involvement the lips are leathery and there maybe difficulty in everting them. In the palate the bands radiate from the pterygomandibular raphae to the anterior faucial pillars. The faucial pillars become thick and short and the tonsils may be pressed in between the fibrosed pillars. [68] The progressive oral fibrosis is usually bilateral and causes an increasing restriction of mandibular opening. Unilateral involvement of a pterygomandibular raphe may produce mandibular deviation. [72]

The tongue becomes progressively less mobile and there may be an associated atrophy of the dorsal filliform papilla. The floor of the mouth is blanched and leathery. The gingiva is fibrotic, depigmented and devoid of its

normal appearance. [68] The occurrence of petechiae has also been reported.[67] When the soft palate is affected its mobility is affected and the uvula becomes shrunken and bud like. In advanced cases the jaws may be inseparable, and the totally inelastic mucosa is forced against the buccal aspects of the teeth where sharp edges or restorations may cause ulceration which becomes secondarily infected.[35]

DIFFERENT CLASSIFICATION, STAGING AND GRADING SYSTEMS

The different classification systems existing in literature can be broadly categorised as follows:

A: CLASSIFICATIONS BASED ON THE CLINICAL ASPECTS OF THE DISEASE:

1. **Desa J.V.**[73]

divided OSF into 3 stages:

Stage I: Stomatitis and vesiculation

Stage II: Fibrosis

Stage III: As its sequelae

2. **Bhatt A. P. and Dholakia H.M**.[74] clinically grouped the patients into three grades as:

Grade I: Comprised of mild and early cases with a very slight fibrous bands and little closure of the mouth.

Grade II: Moderately pronounced symptoms with fibrous bands extending from the cheek to the palate.

Grade III: Excessive amount of fibrosis involving the cheek, palate, uvula, tongue and the lips with narrow opening of the mouth.

3. **Gupta D.S. and Golhar B.L**.[75] classified into four stages based on the increasing intensity of trismus as:

Very early stage: The patients complain of burning sensation in the mouth or ulceration without difficulty in mouth opening.

Early stage: Along with burning sensation, the patients complain of slight difficulty in opening the mouth.

Moderately advanced stage: The trismus is marked to such an extent that the patient cannot open his/her mouth more than two fingers width therefore experiencing difficulty in mastication.

Advanced stage: Patient is undernourished, anaemic and has a marked degree of trismus.

4. **Pindborg J.J** [76] divided OSF into 3 stages as:

Stage I: Stomatitis includes erythematous mucosa, vesicles, mucosal ulcers, melanotic mucosal pigmentations and mucosal petechiae.

Stage II: Fibrosis occurring in the healing vesicles and ulcers is the hallmark of the stage. Early lesions demonstrate blanching of the oral mucosa. Older lesions include vertical and circular palpable fibrous bands in the buccal mucosa and around the mouth opening or lips resulting in mottled marble like appearance of the mucosa because of the vertical thick fibrous bands in association with blanched mucosa. Specific findings include reduction of mouth opening, stiff and small tongue, blanched and leathery floor of the mouth, fibrotic and depigmented gingiva, rubbery soft palate with decreased mobility, blanched and atrophic tonsils, shrunken bud like uvula and sunken cheeks, not commensurate with age or nutritional status.

Stage III: Sequelae of OSF as follows: Leukoplakia is found in more than 25 %of the individuals with OSF. Speech and hearing defects may occur due to involvement of the tongue and eustachian tubes.

5. **Katharia S.K. et al** [77] described a scoring system based on the mouth opening present between upper and lower central incisors as:

Score 1: Mouth opening between 37 to 40mm

Score 2: Mouth opening between 33 to 36mm

Score 3: Mouth opening between 29 to 32mm

Score 4: Mouth opening between 25 to 28mm

Score 5: Mouth opening between 21 to 24mm

Score 6: Mouth opening between 17 to 20mm

Score 7: Mouth opening between 13 to 16mm

Score 8: Mouth opening between 09 to 12mm

Score 9: Mouth opening between 05 to 08mm

Score 10: Mouth opening between 00 to 04mm

6. **Bailoor D.N.** [78] classified on the basis of diagnosis as:

Stage I: Early OSF Mild blanching.

No restriction in mouth opening (normal distance between central incisor tips:

Males 35 to 45 mm, Females 30 to 42 mm).

No restriction in tongue protrusion (normal mesioincisal angle of the upper central incisor to the tip of the tongue when maximally extended with the mouth wide open:

Males 5 to 6 cm, Females 4.5 to 5.5 cm).

Cheek flexibility: CF= V1-V2where V2 is a point measured between at one-third the distance from the angle of the mouth on a line joining the tragus of the ear to the angle of the mouth. The patient is then asked to blow his cheeks fully and the distance between the two points is marked on the cheek as V1. Mean values for cheek flexibility: Males 1.2 cm and Females 1.08 cm. Burning sensation on taking spicy or hot foods only.

Stage II: Moderate OSF Moderate to severe blanching. Mouth opening reduced by 33%. Cheek flexibility also demonstrably reduced. Burning sensation in absence

of stimuli. Palpable bands felt. Lymphadenopathy either unilateral or bilateral. Demonstrable anaemia on haematological examination.

Stage III: Severe OSF More than 66% reduction in the mouth opening, cheek flexibility and tongue protrusion. Tongue may appear fixed. Severe burning sensation, patient is unable to do day to day work. Ulcerative lesions may appear on the cheek. Thick palpable bands. Bilateral lymphadenopathy.

7. **D.R.Lai** [76] grouped OSF on the basis of interincisal distance as:

A: Interincisal distance greater than 35mm.

B: Interincisal distance 30 to 35 mm.

C: Interincisal distance 20 to 30 mm.

D: Interincisal distance less than 20 mm.

8. **Haider S.M**. [79] classified on the basis of severity of disease taking objective parameters like mouth opening into consideration.

Clinical staging

1. Faucial bands only.

2. Faucial and buccal bands.

3. Faucial, buccal and labial bands.

Functional staging

1. Mouth opening greater than 20 mm.

2. Mouth opening between 11 to 19 mm.

3. Mouth opening less than 10mm.

9. **Ranganathan K. et al** [80] divided OSF based on mouth opening as follows:

Group I: Only symptoms with no demonstrable restriction of mouth opening.

Group II: Limited mouth opening 20 mm and above.

Group III: Mouth opening less than 20 mm.

Group IV: OSF advanced with limited mouth opening. Precancerous or cancerous changes are seen throughout the mucosa.

10. **Rajendran R.** [81] reported the clinical features of OSF as follows:

Early OSF:

Comprises of burning sensation in the mouth, blisters especially on the palate, ulceration or recurrent generalized inflammation of oral mucosa, excessive salivation, defective gustatory sensation and dryness of mouth.

Advanced OSF:

Comprises of blanched and slightly opaque mucosa, fibrous bands in the buccal mucosa running in vertical direction. Palate and faucial pillars are the areas first involved with gradual impairment of tongue movement and difficulty in mouth opening.

11. **Bose T. and Balan A**.[82] classified based on clinical features as:

Group A: Mild cases - Only occasional symptoms, pallor, vesicle formation, presence of one or two solitary palpable bands, loss of elasticity of mucosa, variable tongue involvement with protrusion beyond vermillion border. Mouth opening is greater than 3 cm.

Group B: Moderate cases - Symptoms of soreness of mucosa or increased sensitivity to chillies, diffuse involvement of the mucosa, blanched appearance, buccal mucosa tough and inelastic fibrous bands palpable, considerable restriction of mouth opening (1.5 to 3 cm) and variable tongue movement.

Group C: Severe cases - Symptoms are more severe, broad fibrous bands palpable, blanched opaque mucosa, rigidity of mucosa, very little opening of mouth (less than 1.5 cm), depapillated tongue and protrusion of tongue very much restricted.

12. **More C.B. et al** [83] gave the following classification based on clinical and functional parameters as:

I: Clinical staging:

Stage 1 (S1): Stomatitis and/or blanching of oral mucosa.

Stage 2 (S2): Presence of palpable fibrous bands in buccal mucosa and/or oropharynx, with/without stomatitis.

Stage 3 (S3): Presence of palpable fibrous bands in buccal mucosa and/or oropharynx, and in any other parts of oral cavity, with/without stomatitis.

Stage 4 (S4): A: Any one of the above stage along with other potentially malignant disorders e.g. oral leukoplakia, oral erythroplakia, etc.

B: Any one of the above stage along with oral carcinoma.

II: Functional staging:

M1: Inter-incisal mouth opening up to or greater than 35 mm.

M2: Inter-incisal mouth opening between 25 to 35 mm.

M3: Inter-incisal mouth opening between 15 to 25 mm.

M4: Inter-incisal mouth opening less than 15 mm.

13. **Patil S. and Maheshwari S.** [84]

Suggested a new classification based on cheek flexibility. Here, cheek flexibility was measured as a distance in millimetres, from maxillary incisal midline to the cheek retractor during retraction. Normal cheek flexibility observed was: Males 35 to 45 mm, Females 30 to 40 mm.

Grade 1 (Early): Cheek flexibility of 30 mm and above.

Grade 2 (Mild): Cheek flexibility between 20 to 30 mm.

Grade 3 (Moderate): Cheek flexibility less than 20 mm.

Grade4 (Severe): Any of the above condition without concurrent presence of potential malignant lesions.

Grade 5 (Advanced): Any of the above condition with concurrent presence of oral carcinoma.

B: CLASSIFICATIONS BASED ON HISTOPATHOLOGICAL ASPECTS OF THE DISEASE:

1. **Pindborg J.J. and Sirsat S.M.** [85]

Very early stage: Finely fibrillar collagen dispersed with marked oedema with plump young fibroblasts containing abundant cytoplasm. Blood vessels are dilated and congested. Inflammatory cells, mainly polymorphonuclear leukocytes with occasional eosinophils are found.

Early stage:

Juxta-epithelial area shows early hyalinization. Collagen is still in separate thick bundles. Moderate numbers of plump young fibroblasts are present. With dilated and congested blood vessels. Inflammatory cells are primarily lymphocytes, eosinophils and occasional plasma cells. Moderately advanced stage: Collagen is moderately hyalinised. Thickened collagen bundles are separated by slight residual oedema. Fibroblastic response is less marked. Blood vessels are either normal or compressed. Inflammatory exudate consists of lymphocytes and plasma cells.

Moderately advanced stage:

Collagen is moderately hyalinised. Thickened collagen bundles are separated by slight residual oedema. Fibroblastic response is less marked. Blood vessels are either normal or compressed. Inflammatory exudate consists of lymphocytes and plasma cells.

Advanced stage:

Collagen is completely hyalinised. A smooth sheet with no separate bundles of collagen is seen. Oedema is absent. Hyalinised area is devoid of fibroblasts. Blood vessels are completely obliterated or narrowed. Inflammatory cells are lymphocytes and plasma cells.

2. **Utsonumiya H. et al** [49] divided OSF based on the concept of Pindborg J.J. and Sirsat S.M. and modified it as follows:

Early stage:

Large number of lymphocytes in the sub epithelial and connective tissue zones along with myxedematous changes.

Intermediate stage:

Granulation changes close to the muscle layer and hyalinization appears in sub epithelial zone where blood vessels are compressed by fibrous bundles. Reduced inflammatory cells in sub epithelial layer are seen.

Advanced stage:

Inflammatory cell infiltrate hardly seen. Number of blood vessels dramatically less in the sub epithelial zone. Marked fibrous areas with hyaline changes extending from sub epithelial to superficial muscle layers are seen. Atrophic, degenerative changes start in muscle fibres.

3. **Kumar K. et al** [40] graded OSF as follows:

Grade I: Loose, thick and thin fibres.

Grade II: Loose or thick fibres with partial hyalinisation.

Grade III: Complete hyalinisation.

C: CLASSIFICATIONS BASED ON CLINICAL AND HISTOPATHOLOGICAL ASPECTS OF THE DISEASE:

1. **Khanna J.N. and Andrade N.N.** [86] developed a group classification system to aid in the surgical management of OSF. It is the most accepted classification by the clinicians.

Group I: Very early cases: Clinically: Common symptom is burning sensation in the mouth, acute ulceration and recurrent stomatitis and not associated with mouth opening limitation.

Histology: Fine fibrillar collagen network interspersed with marked oedema, blood vessels dilated and congested, large aggregate of plump young fibroblasts present with abundant cytoplasm, inflammatory cells mainly consist of polymorphonuclear leukocytes with few eosinophils. The epithelium is normal.

Group II: Early cases Clinically: Buccal mucosa appears mottled and marble like, widespread sheets of fibrosis palpable, interincisal distance of 26-35 mm.

Histology: Juxta-epithelial hyalinization present, collagen present as thickened but separate bundles, blood vessels dilated and congested, young fibroblasts seen in moderate number, inflammatory cells mainly consist of polymorphonuclear leukocytes with few eosinophils and occasional plasma cells, flattening or shortening of epithelial rete-pegs evident with varying degree of keratinization.

Group III: Moderately advanced cases

Clinically: Trismus, interincisal distance of to 25 mm, buccal mucosa appeal's pale firmly attached to underlying tissues, atrophy of vermilion border, vertical fibrous bands palpable at the soft palate, pterygomandibular raphe and anterior faucial pillars.

Histology: Juxta-epithelial hyalinization present, thickened collagen bundles, residual edema, constricted blood vessels, mature fibroblasts with scanty cytoplasm and spindle-shaped nuclei, inflammatory exudate which consists of lymphocytes and plasma cells, epithelium markedly atrophic with loss of rete pegs, muscle fibres seen with thickened and dense collagen fibres.

Group IVA: Advanced cases

Clinically: Severe trismus, interincisal distance of less than 15 mm, thickened faucial pillars, shrunken uvula, restricted tongue movement, presence of circular band around the entire lip and mouth.

Group IVB: Advanced cases

Clinically: Presence of hyperkeratotic leukoplakia and/or squamous cell carcinoma.

Histology: Collagen hyalinised smooth sheet, extensive fibrosis, obliterated mucosal blood vessels, eliminated melanocytes, absent fibroblasts within the hyalinised zones, total loss of epithelial rete pegs, presence of mild to moderate atypia and extensive degeneration of muscle fibres. The authors are of the view that patients in group I and group II can be managed by symptomatic treatment, whereas those in group III and group IV definitely require surgical management.

LABORATORY INVESTIGATIONS

There are no characteristic laboratory findings in OSF. The investigations carried out include

- Blood chemistry and haematological investigations
- Cytogenetics
- Immunological studies

Blood chemistry and haematological investigations: increased erythrocyte sedimentation rate [87, 88, 72] anaemia [87, 88, 89] eosinophilia [87, 89] increased gamma globulin [88] decrease in serum iron and an increase in total iron-binding capacity [90]. The percentage saturation of transferrin also decreased and a significant reduction in total serum iron and in albumin was found.

Cytogenetics :

Chromosomal instability has long been associated with the neoplastic process and the quantitative assay of sister chromatid exchange (SCE) provides an easy, rapid and sensitive method for studying chromosome / DNA instability and its subsequent repair processes. Studies conducted to investigate SCE levels in the peripheral blood of patients with OSF, reported increased levels when compared

to normal controls. The increase in frequencies of SCEs observed in patients with OSF may be attributed to the genotoxic effect of the constituents of betel quid.[90] Silver-binding nuclear organizer region proteins (AgNORs) count may be a promising predictor of the biological behaviour of OSF. The pooled mean AgNOR in clinically advanced OSF has been reported to be higher than in moderately advanced cases. [91]

Immunological studies:

Increased serum levels of major immunoglobulins have been reported in patients with OSF. [92] Elevated levels of circulating immune complexes (CIC) have also been reported in OSF and their level may help in predicting its malignant transformation. [93]

TREATMENT MODALITIES FOR ORAL SUBMUCOUS FIBROSIS

OSF is well known for its resistant and chronic nature. Being a premalignant condition with debilitating consequences, no conservative treatment that has given complete resolution of symptoms is identified till date. Various treatment modalities are available to treat this condition which includes medicinal approach, surgical management and physiotherapy. Proper treatment begins with education of the patient regarding the ill effects of arecanut and related chewing products. The patient should be informed about the irreversible nature of the disease despite quitting the habit and possibilities of developing oral cancer.

Behavioural therapy:

Counselling against continuing the habit is an essential step in the treatment of any disease.

Interventions have showed that basic strategies such as short films, personal communication and showing photographs of harmful effects can be

significantly effective in deterring people from starting or continuing the tobacco habit. [94, 95]

Physiotherapy:

Physiotherapy is a useful adjunct in the treatment of OSF. A study among a Nepali population has shown physiotherapy to effectively improve mouth opening values among patients. [96]

Steroids:

The steroids act by inhibiting generation of inflammatory factors and increasing the apoptosis of inflammatory cells.[97, 98, 99] Although steroids are one of the most often used drugs for OSF clinicians should be alert to avoid any complications such as one reported of central serous chorioretinopathy following a 2 month treatment with a combination of triamcinolone, hyaluronidase and placentrix.[100]

Enzymes:

Collagenase – It acts by lysing fibrogen, increasing vascular circulation and epithelial regeneration

Hyaluronidase – It depolymerises hyaluronic acid thereby lowering the viscosity of intercellular cementing substance These enzymes provide good results when combined with steroids.[98, 99]

Nutritional supplements:

Vitamin A plays an important role in maintaining the normal growth and repair of epithelial tissues. Hence a novel treatment modality has been proposed recently by a few authors, which involves supplementation with zinc acetate 50 mg three times daily and Vit A 25,000 IU once daily. [98, 99]

Antioxidants:

Lycopene is a phytochemical, synthesized by plants (tomatoes) and microorganisms. Lycopene is a powerful antioxidant and has a singlet-oxygen-quenching ability twice as high as that of beta-carotene and ten times higher than that of alpha-tocopherol. [99, 101, 102]

Systemic drugs: (Cardiovascular)

Pentoxifylline is a tri-substituted methylxanthine derivative, which leads to decrease in red cell and platelet aggregation, granulocyte adhesion, fibrinogen levels, and lowers whole blood viscosity. But the gastrointestinal tract and central nervous system side effects of pentoxifylline were the major deterrents Buflomedil is a vasoactive agent which claims to exert beneficial effects on the microcirculation Nylidrin, a sympathomimetic agent, chemically related to the epinephrine ephedrine series, produces vasodilation of the arterioles of skeletal muscles. [98,103,104]

Turmeric:

Previous experimental data and the results of extended clinical Phase I trial of turmeric oil for 3 months suggest that Turmeric extract oleoresin has potential as a chemoprotective agent, particularly in patients of OSF. [105,106]

Immune milk:

Immune milk is, milk from cows immunized with human intestinal bacteria. Proposed anti-inflammatory activity is by modulation of cytokines. Only one study showed the effect of oral administration of milk from cows immunized with human intestinal bacteria in OSF. Forty-five grams of immune milk powder twice a day for 3 months resulted in significant improvement in intolerance to spicy foods in 80% and increase in inter incisor distance in 69.2% patients. [99]

Interferon gamma:

IFN gamma is a known anti-fibrotic cytokine. In an open uncontrolled study intra-lesional interferon gamma treatment resulted in reduced burning sensation, increased suppleness of the buccal mucosa and improvement in the mouth opening by 42% in OSF patients. [99]

Ayurvedic therapy:

Oxitard formulation contains the extracts of Magnifera indica, Withania somnifera, Daucus carota, Glycyrrhiza glabra, Vitis vinifera, Syzygium aromaticum, powders of Emblica officinalis and Yashada bhasma; and oils of Triticum sativum. The role of chilies being an important factor in disease progression, this drug does have efficacy to address that issue. One study by Singh et al., used Oxitard capsule in 48 cases, in patients having difficulty in mouth opening and pain a dose of two capsules twice daily for a period of 3 months was standardized; they concluded that there was significant increase in mouth opening along with decrease in pain in the mouth. [107]

Aloe vera:

Recently 5 mg aloe vera was used topically thrice daily for 3 months and was compared to antioxidants twice daily in 20 patients with 10 patients in each group. The results showed that the aloe vera group demonstrated a better treatment response and could be considered as a relatively safer, economical and less invasive treatment option for OSF. [108]

Surgical management:

Various surgical techniques have been proposed such as cutting of the fibrous bands followed by split thickness skin graft, tongue flaps, nasolabial flaps, temoralis myotomy or coronoidotomy. [86,109,110] Surgical techniques are sometimes

fraught with disadvantages like relapse, facial scars, repeated surgeries for debulking of flaps etc., Hence an adjunct like oral stent [111] or a graft stabilizing clip [112] has been proposed to prevent post-surgical relapse which helps to maintain the interincisal opening.

LIPID PROFILE:

Lipids are defined as a very heterogenous group of biomolecules that are generally insoluble in water but which readily dissolve in nonpolar solvents, such as ether and chloroform. Lipids may also be defined as hydrophobic or amphiphilic small molecules; the amphiphilic nature of some lipids allows them to form structures such as vesicles, liposomes, or membranes in an aqueous environment. Lipids can be classified based on their composition and the function they perform. [113] On the basis of their composition, lipids are broadly classified in to simple lipids (esters of fatty acids with alcohol; these include fats, waxes), complex lipids (esters of fattyacids with alcohols containing additional groups such as phosphate, nitrogenousbase, carbohydrate, protein etc.; these include phospholipids, nonphosphorylated lipids, lipoproteins, sulfolipids), and derived lipids (derivatives obtained on the hydrolysis of simple and complex lipids which possess the characteristics of lipids; these include eicosanoids, isoprenoids, fat soluble vitamins, steroids, ketone bodies, fatty acids).On the basis of their function, lipids are broadly classified as storage lipids (fats, oils), structural lipids (phospholipids, non-phosphorylated lipids),and lipids as signals, cofactors and pigments (phosphatidylinositol, eicosanoids, steroid hormones, fat soluble vitamins, lipid quinines, dolichols). [113]

Cholesterol is an amphipathic lipid and, as such, is an essential structural component of all the cell membranes and the outer layer of plasma lipoproteins. It

is present in the tissues and in plasma lipoprotein either as free cholesterol or combined with a long-chain fatty acid.

Cholesterol cannot dissolve in the blood. It has to be transported to and from the cells by carriers called lipoproteins. [114-119]

There are three main categories of lipoproteins:

(i) VLDL: These are lipoproteins that carry cholesterol from the liver to organs and tissues in the body. They are formed by a combination of cholesterol and TG. VLDL is heavier than LDL, and is associated with atherosclerosis and heart disease. The normal range for VLDL is 10–35 mg/dl. [120]

(ii) LDL: It is also known as "bad cholesterol." LDLs are produced by the liver and carry cholesterol and other lipids (fats) from the liver to different areas of the body like muscles, tissues, organs, and the heart. High levels of LDL indicate increase in the risk of heart disease. LDL levels less than 100 mg/dl are considered optimal. [120]

(iii) HDL: HDL is considered the "good" cholesterol. HDL is produced by the liver to carry cholesterol and other lipids (fats) from the tissues and organs back to the liver. Normal HDL level ranges between 40 and 60 mg/dl. [120]

C-REACTIVE PROTEIN:

C-reactive protein, a member of the pentaxin protein family was first identified by **Tilet & Francis**(1930) in the plasma of patient with pneumonia. It is an alpha globulin with a molecular weight of 110,000 to 140,000 Daltons. CRP, named for its capacity to precipitate the somatic C-polysaccharide of *Streptococcus pneumonia* [121]. Liver is the site of its synthesis & normally present as trace constituent of serum of plasma at levels less than 0.3 mg/dl. The synthesis of CRP in the hepatocytes may be regulated by pro-inflammatory cytokines like

interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor-necrosis factor (TNF), which have been linked with inflammatory disorders. Therefore, these pro-inflammatory cytokines are currently the subject of intense studies as influencing factors in various types of inflammatory disorders [122].

CRP and other acute-phase molecules are usually present at relatively low levels in plasma, but may be raised dramatically within 72 h of tissue injury, or with infection. The advantages of CRP over other acute phase proteins is levels appear after the onset of disease and levels increase within 4-6 h after an acute tissue injury, whereas serum levels of all the other acute phase reactants increase 12-24 h from injury. CRP is consistently found in bacterial infection, acute rheumatic fever, and malignant diseases, viral infections, tuberculosis, and also in patients following surgical operations and blood transfusions [123]

Few studies have demonstrated that elevated CRP levels are associated with an increased risk of malignancy and have been described as a prognostic factor [124]. Raised CRP concentrations have been demonstrated to be an indicator of a poor prognosis for SCC of the esophagus [124]. Nevertheless, few studies are available on oral cancers and premalignant lesions [124].

Serum C-reactive protein (CRP) is a very sensitive indicator of current disease activity for inflammation. It has been most widely used for the clinical diagnosis of acute or chronic inflammation. The introduction of a high sensitivity technique (hs CRP) enables identification of the group of patients with chronic inflammation that manifested by a minor elevation of CRP.[125]

LIPID PROFILE & CRP IN ORAL SUBMUCOUS FIBROSIS:

Rawson K *et al.,* [3] in their study, investigated the alterations and clinical significance of serum lipid profiles in 40 clinically diagnosed OSF subjects and 10

healthy subjects and reported that there is a decrease in the serum lipid variables with the progression of disease.

Ajai *et al*., [126] conducted a study for estimation of serum lipid profile in 45 OSF patients with the age range of 20–60 years. They compared the serum lipid profiles in OSF and control groups and also in different stages of OSF and reported a significant reduction in serum lipid levels as the disease progressed.

Lohe *et al*., [127] conducted a study on 70 oral cancer and 70 precancerous condition patients for the evaluation of serum lipid profile levels and found that there was a significant decrease in TC, HDL, VLDL, and TG in oral cancer group and a significant decrease in TC and HDL in oral precancer group, as compared to the controls.

Patel *et al*., [113] carried out a similar study on 184 head and neck cancer patients, 153 patients with oral precancerous condition, and 52 controls and observed a significant decrease in plasma TC and HDL in cancer patients as well as in patients with oral premalignant condition as compared to the controls.

Kanthem RK et al., [128] in their study showed a significant reduction in the levels of TC, HDL and LDL whereas no statistically significant difference was found in the values of TG and VLDL between OSF and control group.

Sharma *et al*., [129] observed a significant decrease in serum cholesterol, LDL in OSF patients.

Mehrotra *et al*., [130] conducted a study on 65 OSMF subjects with the age range of 20–60 years and compared them with normal subjects and found a

significant decrease in plasma TC, HDL with no significant change in the levels of LDL and TGs in OSF patients as compared to controls.

Kumar *et al*., [117] in their study showed a significant decrease in TC, HDL and LDL in patients with OSF as compared to the controls. Their study also showed no significant changes in the levels of TGs and VLDL.

Chalko *et al*., [131] observed a significant decrease in serum cholesterol and LDL in OSF patients.

Anusha et al., [132] observed significant decrease in serum lipid profiles in OSF patients than controls.

Kalpajyoti Bhattacharjee et al., [133] investigated pre-treatment serum C-reactive protein (CRP) levels in oral potentially malignant disorders (oral leukoplakia and Oral submucous fibrosis), and reported an increase in the level of CRP in both leukoplakia and OSF.

Kumar and Bhateja et al., [121] studied CRP levels in oral precancer and cancer in which they observed that CRP level was elevated in PMD.

Kaja, et al., [5] Included 20 cases of oral potentially malignant disorders (10 each of Leukoplakia, Oral Sub mucous fibrosis) to assess the serum C reactive protein levels in potentially malignant disorders and observed that OSF patient showed prominent CRP elevation.

Most of the published studies mentioned above, have evaluated the serum lipid profile and C-reactive protein in various potentially malignant disorders and oral squamous cell carcinoma, but to our knowledge no studies are available

comparing the pre-treatment and post-treatment lipid profile and C-reactive protein values in oral potentially malignant disorders, so that their real prognostic value can be assessed. Hence, I intended to do the present study.

MATERIALS AND METHODS

This is a prospective study involving 30 clinically diagnosed OSF patients of either gender in the age group of 20 – 50 years was selected from the patients reporting to Department of Oral Medicine and Radiology, Tamil Nadu Government Dental College and Hospital. Informed consent was obtained from all patients. A complete history taking followed by a thorough oral examination was done to all patients. Burning sensation of the patient was assessed by using visual analog scale and mouth opening measured using caliper and measuring scale. These 30 patients were subdivided into 4 stages according to **Khanna et.al** staging system of OSF. 5mL of intravenous blood sample was drawn from the prominent vein in the cubital fossa The blood samples were centrifuged at 2500 RPM for 5 minutes and serum separated and stored at $4^{o}C$ and pre-treatment estimation of lipid profile & CRP were done using autoanalyser. At the end of the treatment blood sample was collected and serum lipid profile & CRP estimated using autoanalyser.

STUDY CENTRE:

1. Department of Oral Medicine and Radiology, Tamil Nadu Government Dental College and Hospital, Chennai-600003.

DESIGN OF THE STUDY:

A prospective study

STUDY POPULATION & SOURCE:

In this study, 30 clinically diagnosed OSF patients of either sex in the age group of 20 – 50. Years were selected from patients who reported to the

Department of Oral Medicine and Radiology, Tamil Nadu Government Dental College and Hospital.

INFORMED CONSENT:

An informed consent was obtained from all the patients participating in the study.

SAMPLE DESIGN:

Total number of 30 patients were selected, these 30 patients were subdivided into 4 stages according to the staging system given by Khanna et.al :

- Stage I: Patients with burning sensation, excessive salivation, normal mouth opening
- Stage II: Burning sensation, buccal mucosa – marble like, blanched, and mouth opening – 26–35 mm
- Stage III: Burning sensation, buccal mucosa pale blanched, vertical fibrous bands palpable, mouth opening – 15–25 mm
- Stage IVa: Burning sensation, tongue movements are restricted, shrunken uvula, and mouth opening 2–15 mm
- Stage IVb: Presence of Hyperkeratotic leukoplakia and/or Squamous cell carcinoma.

All the OSF patients were subjected to complete haemogram, Lipid profile, C-reactive protein, urine routine investigations, and were given tobacco cessation counselling.

INCLUSION CRITERIA:

- Clinically diagnosed OSF patients (upto Stage IVa)
- Both gender of age 20 - 50 years old
- Healthy, with no systemic disease (diabetes) and immunodeficiency state

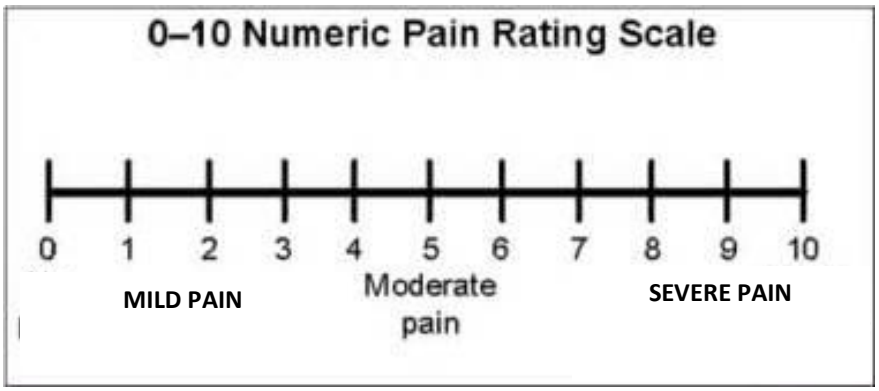
EXCLUSION CRITERIA:

- Stage IVb OSF
- Patients with systemic disease like :
- Obesity
- Diabetes mellitus,
- Thyroid disorder,
- Liver dysfunction,
- Malabsorption syndrome
- Cardiac patients
- Autoimmune collagen diseases
- Osteomyelitis,
- Inflammatory bowel disorder,
- Tuberculosis,
- Malignancy,
- Pneumonia,
- Severe infection,
- Pregnancy
- Patient under oral contraceptives
- Patients who are already undergoing treatment for OSF.

CLINICAL PARAMETER:

Symptoms like burning sensation or pain were assessed using visual analog scale and mouth opening was assessed by measuring inter incisal distance and transferred to scale in mm

1. Pain or Burning Sensation (VISUAL ANALOG SCALE)



METHOD OF DRUG ADMINISTRATION

After the serum lipid and C-reactive protein evaluation, Grade I OSF patients were given supplemental medication of capsule antioxidant twice daily, tablet vitamin B complex twice daily and topical Benzocaine application before food for 6weeks where as patient in Grade II, Grade III, Grade IV a were administered intralesional injection of 2ml of Dexamethasone with 0.5ml of Lignocaine twice weekly and supplemental Medication as above for 6weeks. Patient in Grade 1 were recalled once in a week whereas patients in Grade II, Grade III and Grade IVa were recalled twice in a week and evaluated for improvement in signs and symptoms such as burning sensation and mouth opening. Final changes in burning sensation and mouth opening were recorded after the complete course of treatment.

DATA ANALYSIS:

The data collected were entered into Microsoft Excel sheet and data analysis was done using statistical package for social sciences (SPSS) version 17 with descriptive statistics, ANOVA and paired student “t” test. p value < 0.05 was considered as statistical significance.

FIGURES

FIGURE 1: DIAGNOSTIC INSTRUMENTS

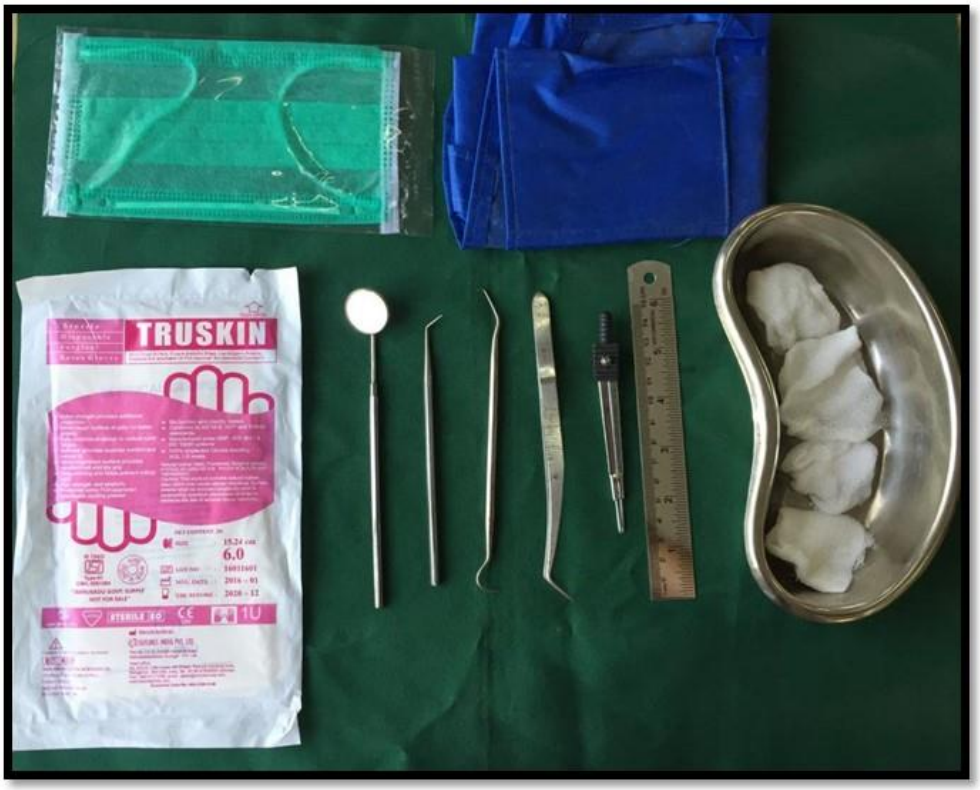


FIGURE 2: BLOOD COLLECTION INSTRUMENTS

FIGURE 3: CENTRIFUGE MACHINE

FIGURE 4: AUTOANALYSER MACHINE

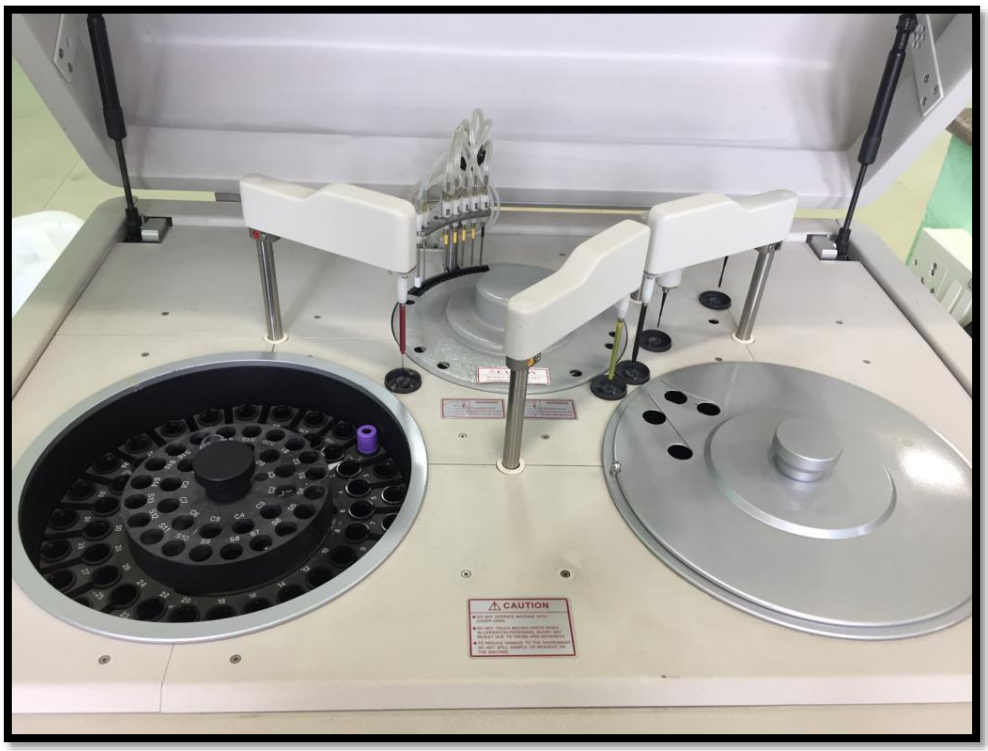


FIGURE 5: BLANCHING OF RIGHT BUCCAL MUCOSA

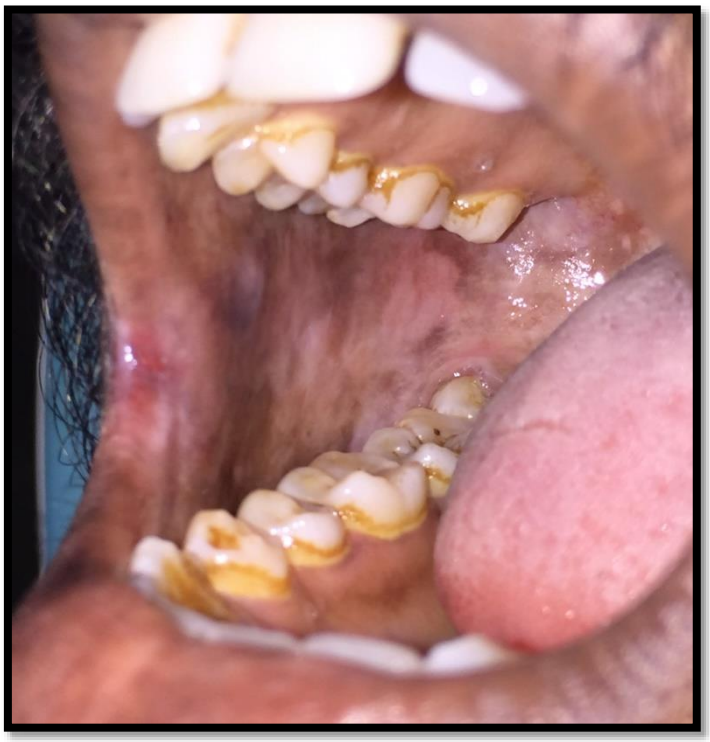


FIGURE 6: BLANCHING OF LEFT BUCCAL MUCOSA

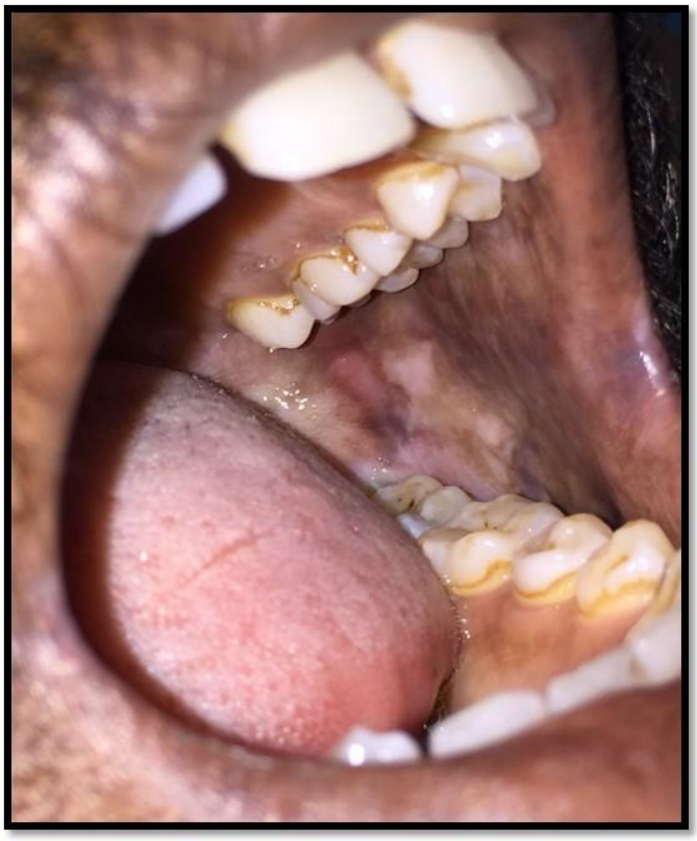


FIGURE 7: DRUGS USED IN THE STUDY

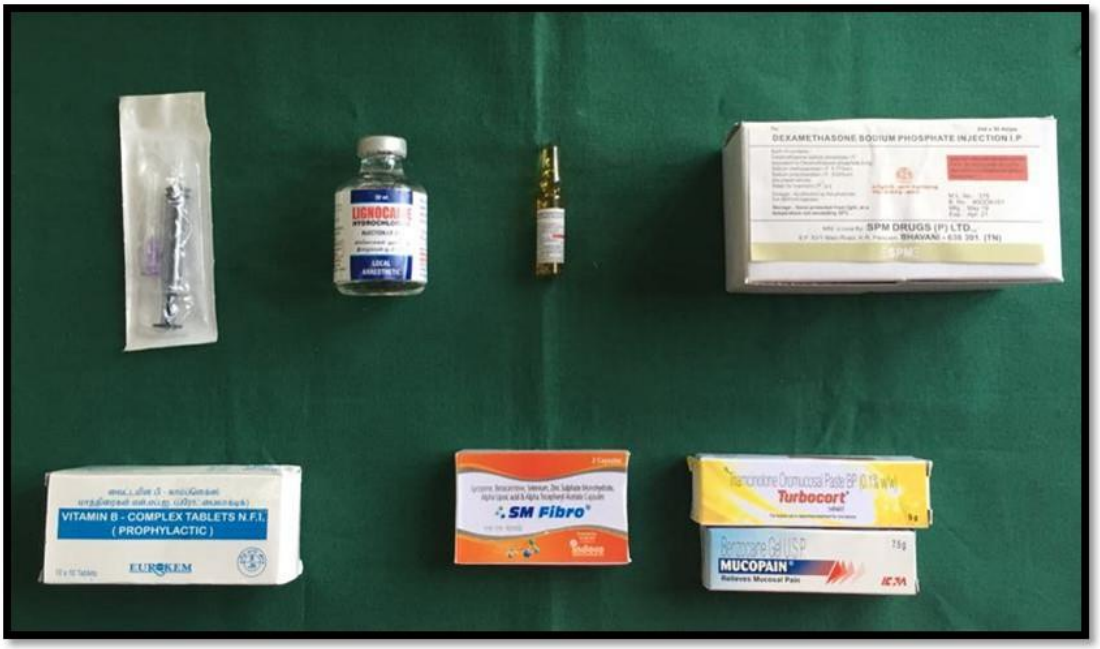


FIGURE 8: INTRALESIONAL INJECTION

FIGURE 9: MOUTH OPENING MEASUREMENT (PRE-TREATMENT)

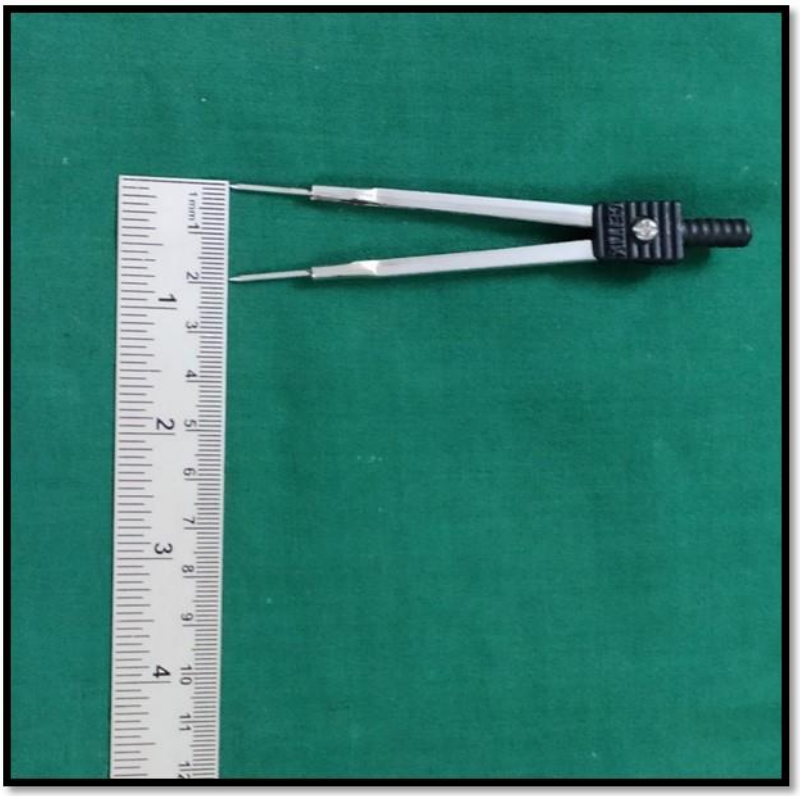
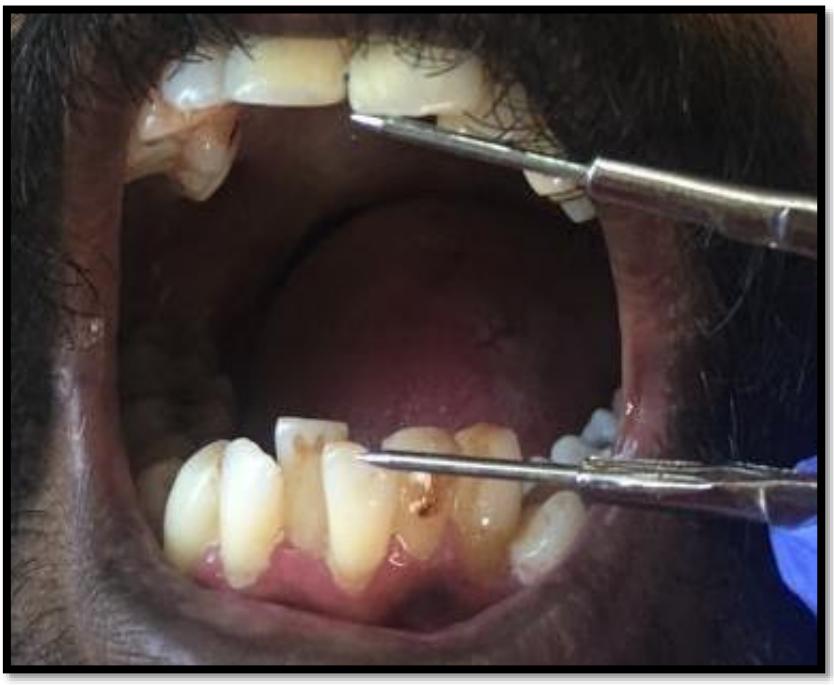
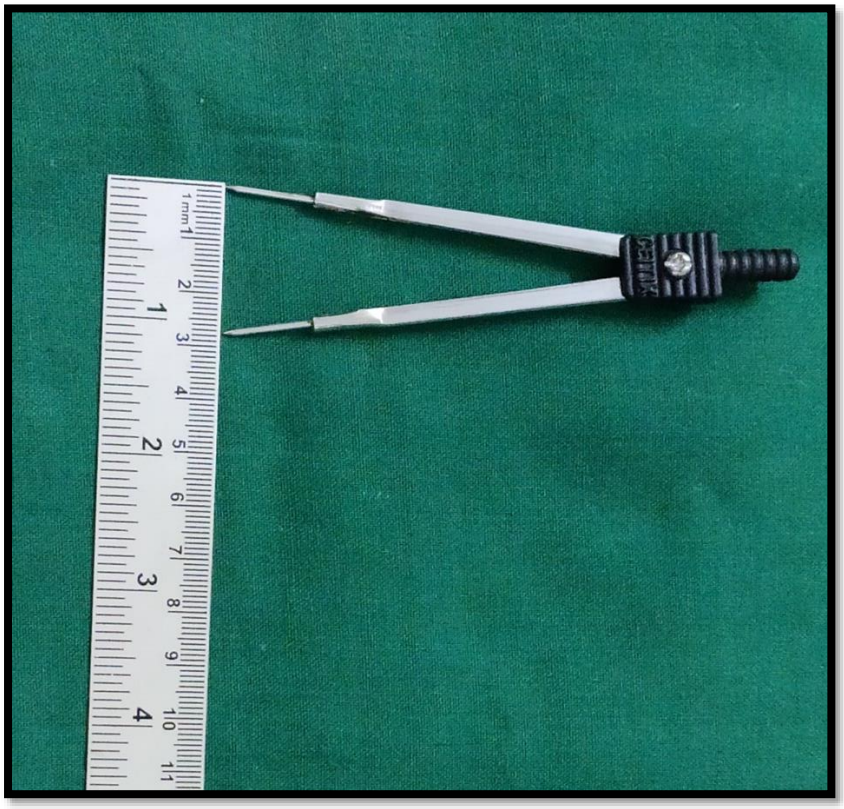


FIGURE 10: MOUTH OPENING MEASUREMENT (POST TREATMENT)





RESULTS

A total of 30 study participants who were clinically diagnosed as Oral Submucous Fibrosis (OSF) patients were included in this study. The OSF patients were classified according to **Khanna et al** and the frequency of distribution was 13.33% of Grade 1 OSF (n = 4), 16.66% of Grade 2 OSF (n = 5), 63.33% of Grade 3 OSF (n = 19) and 6.66% of Grade 4a OSF (n = 2).

AGE AND GENDER DISTRIBUTION:

As per the inclusion criteria, the study included OSF patients of both genders in the age ranging from 20 years to 50 years and the mean age was 30.73 ± 7.5. Out of the total study participants (n = 30), 96.6% (n = 29) were male patients and 3.33% (n = 1) were female patients [Table 1]

The frequency of distribution of age in years among different stages of OSF were 31.25 ± 10.7 (Grade 1), 28.80 ± 6.7 (Grade 2), 31.16 ± 7.7 (Grade 3) and 30.50 ± 4.9 (Grade 4a). There is no statistical significance in the relationship between the age of the patient and the staging of the disease. [Table 2, Chart 1]

MOUTH OPENING:

The mean value of the pre-treatment mouth opening in mm was 23.70 ± 6.1 and the mean values in mm among different stages of OSF were 36.50 ± 0.5 in Grade 1, 26.60 ± 0.5 in Grade 2, 21.32 ± 2.3 in Grade 3 and 13.50 ± 0.7 in Grade 4a. The relationship between mouth opening and the staging of the disease is found to be statistically significant (p=0.000) [Table 3, Chart 2]

The mean value of the post-treatment mouth opening in mm was 29.73 ± 5.8 and the mean values in mm among different stages of OSF were 42.75 ± 0.9 in Grade 1, 31.20 ± 0.8 in Grade 2, 27.53 ± 1.9 in Grade 3 and 21.00 ± 1.4 in Grade

4a. The relationship between mouth opening and the staging of the disease is found to be statistically significant (p=0.000) [Table 4, Chart 3]

In this study, the comparison of mean values of the pre-treatment and post treatment mouth opening levels of the study subjects among different stages of OSF were found to be statistically significant (p=0.000) and the mean increase in mouth opening in mm was 6.03 ± 1.7 [Table 5, Chart 10]

VISUAL ANALOG SCALE SCORE:

The mean value of the pre-treatment visual analog scale score was 4.50 ± 1.6 and the mean values among different stages of OSF were 2.50 ± 1.0 in Grade 1, 2.80 ± 1.0 in Grade 2, 5.11 ± 1.1 in Grade 3 and 7.00 ± 0.0 in Grade 4a. The relationship between visual analog scale score and the staging of the disease is found to be statistically significant (p=0.000) [Table 6, Chart 4]

The mean value of the post-treatment visual analog scale score was 1.20 ± 1.1 and the mean values among different stages of OSF were 0.00 ± 0.0 in Grade 1, 0.00 ± 0.0 in Grade 2, 1.58 ± 1.0 in Grade 3 and 3.00 ± 0.0 in Grade 4a. The relationship between visual analog scale score and the staging of the disease is found to be statistically significant (p=0.000) [Table 7, Chart 5]

In this study, the comparison of mean values of the pre-treatment and post treatment visual analog scale score of the study subjects among different stages of OSF were found to be statistically significant (p=0.000) and the difference in mean values of visual analog scale score was 3.30 ± 1.1[Table 8, Chart 9]. In this study, all patients (100%) in Grade 1 & Grade 2 after completion of the treatment regimen, reported a complete relief from pain/burning sensation and patients in Grade 3 and Grade 4a reported a significant reduction in their symptoms.

SERUM LIPID PROFILE:

TOTAL CHOLESTEROL:

The mean value of the pre-treatment total cholesterol level was 166.89 ± 31.7 and the mean values among different stages of OSF were 178.75 ± 42.5 in Grade 1, 165.60 ± 29.7 in Grade 2, 168.82 ± 30.3 in Grade 3 and 128.00 ± 1.4 in Grade 4a. There is no statistical significance (p = 0.314) in the relationship between total cholesterol values and the staging of the disease. [Table 9]

The mean value of the post-treatment total cholesterol level was 179.80 ± 34.8 and the mean values among different stages of OSF were 184.75 ± 35.1 in Grade 1, 177.80 ± 25.9 in Grade 2, 184.31 ± 36.2 in Grade 3 and 132.00 ± 9.8 in Grade 4a. There is no statistical significance (p = 0.249) in the relationship between total cholesterol values and the staging of the disease. [Table 10]

In this study, the comparison of mean values of the pre-treatment and post treatment total cholesterol level of the study subjects among different stages of OSF were found to be statistically significant (p=0.000) [Table 22, Chart 6]. The mean total cholesterol levels increased by 12.91± 16.8 post treatment.

TRIGLYCERIDES:

The mean value of the pre-treatment triglyceride level was 114.50 ± 80.9 and the mean values among different stages of OSF were 156.00 ± 139.3 in Grade 1, 88.20 ± 38.5 in Grade 2, 119.52 ± 76.9 in Grade 3 and 49.50 ± 6.3 in Grade 4a. There is no statistical significance (p = 0.418) in the relationship between triglyceride values and the staging of the disease. [Table 11]

The mean value of the post-treatment triglyceride level was 145.26 ± 90.1 and the mean values among different stages of OSF were 156.75 ± 110.1 in Grade 1, 89.00 ± 49.0 in Grade 2, 156.10 ± 88.3 in Grade 3 and 160.00 ± 173.9 in Grade

4a. There is no statistical significance (p = 0.526) in the relationship between triglyceride values and the staging of the disease. [Table 12]

In this study, the comparison of mean values of the pre-treatment and post treatment triglyceride level of the study subjects among different stages of OSF were found to be statistically significant (p=0.007) [Table 22, Chart 6]. The mean triglyceride levels increased by 30.76 ± 57.6 post treatment.

HIGH DENSITY LIPOPROTEIN (HDL):

The mean value of the pre-treatment HDL level was 41.02 ± 10.7 and the mean values among different stages of OSF were 42.00 ± 18.1 in Grade 1, 40.80 ± 7.6 in Grade 2, 40.56 ± 10.6 in Grade 3 and 44.00 ± 8.4 in Grade 4a. There is no statistical significance (p = 0.977) in the relationship between the HDL values and the staging of the disease. [Table 13]

The mean value of the post-treatment HDL level was 47.57 ± 10.1 and the mean values among different stages of OSF were 44.75 ± 16.0 in Grade 1, 49.20 ± 5.5 in Grade 2, 47.90 ± 10.1 in Grade 3 and 45.95 ± 12.7 in Grade 4a. There is no statistical significance (p = 0.925) in the relationship between HDL values and the staging of the disease. [Table 14]

In this study, the comparison of mean values of the pre-treatment and post treatment HDL level of the study subjects among different stages of OSF were found to be statistically significant (p=0.000) [Table 22, Chart 6]. The mean HDL levels increased by 6.54 ± 7.6 post treatment.

LOW DENSITY LIPOPROTEIN (LDL):

The mean value of the pre-treatment LDL level was 109.82 ± 25.0 and the mean values among different stages of OSF were 119.00 ± 21.4 in Grade 1, 112.40 ± 26.8 in Grade 2, 110.50 ± 25.3 in Grade 3 and 78.50 ± 3.5 in Grade 4a. There is

no statistical significance (p = 0.303) in the relationship between the LDL values and the staging of the disease. [Table 15]

The mean value of the post-treatment LDL level was 117.89 ± 34.6 and the mean values among different stages of OSF were 129.75 ± 15.1 in Grade 1, 122.80 ± 25.0 in Grade 2, 120.68 ± 35.5 in Grade 3 and 55.35 ± 13.6 in Grade 4a. There is no statistical significance (p = 0.056) in the relationship between LDL values and the staging of the disease. [Table 16]

In this study, the comparison of mean values of the pre-treatment and post treatment LDL level of the study subjects among different stages of OSF were found to be statistically significant (p=0.020) [Table 22, Chart 6]. The mean LDL levels increased by 8.07 ± 18.0 post treatment.

VERY LOW DENSITY LIPOPROTEIN (VLDL):

The mean value of the pre-treatment VLDL level was 22.79 ± 16.1 and the mean values among different stages of OSF were 31.06 ± 27.9 in Grade 1, 17.96 ± 7.5 in Grade 2, 23.72 ± 15.4 in Grade 3 and 9.50 ± 0.7 in Grade 4a. There is no statistical significance (p = 0.425) in the relationship between the VLDL values and the staging of the disease. [Table 17]

The mean value of the post-treatment VLDL level was 28.87 ± 18.4 and the mean values among different stages of OSF were 30.65 ± 22.4 in Grade 1, 18.16 ± 9.4 in Grade 2, 30.98 ± 18.3 in Grade 3 and 32.05 ± 34.8 in Grade 4a. There is no statistical significance (p = 0.587) in the relationship between VLDL values and the staging of the disease. [Table 18]

In this study, the comparison of mean values of the pre-treatment and post treatment VLDL level of the study subjects among different stages of OSF were

found to be statistically significant (p=0.007) [Table 22, Chart 6]. The mean VLDL levels increased by 6.07 ± 11.5 post treatment.

C – REACTIVE PROTEIN (CRP):

The mean value of the pre-treatment CRP level was 2.13 ± 1.8 and the mean values among different stages of OSF were 1.52 ± 0.6 in Grade 1, 1.62 ± 1.6 in Grade 2, 2.54 ± 2.0 in Grade 3 and 0.79 ± 0.1 in Grade 4a. There is no statistical significance (p = 0.444) in the relationship between the CRP values and the staging of the disease. [Table 19]

The mean value of the post-treatment CRP level was 1.58 ± 1.0 and the mean values among different stages of OSF were 1.47 ± 0.8 in Grade 1, 1.38 ± 0.9 in Grade 2, 1.74 ± 1.1 in Grade 3 and 0.80 ± 0.2 in Grade 4a. There is no statistical significance (p = 0.622) in the relationship between CRP values and the staging of the disease. [Table 20]

In this study, the comparison of mean values of the pre-treatment and post treatment CRP level of the study subjects among different stages of OSF were found to be statistically significant (p=0.022) [Table 21, Chart 7]. The mean CRP levels decreased by 0.55 ± 1.2 post treatment.

As per the objective of the study, the post treatment symptom of the OSF patients measured using the visual analog scale score were compared with all parameters like the lipid profile, CRP and mouth opening levels. The relationship between the mean values of lipid profile parameters (TC, TG, HDL, LDL, and VLDL) and the VAS scores were not statistically significant [Table 23]. There was no statistical significance (p = 0.559) in the relationship between the post treatment CRP levels and the VAS scores [Table 24] whereas the relationship between mean values of mouth opening scores and the mean values of VAS scores [Table 25] were statistically significant (p = 0.000).

TABLES

TABLE 1: AGE AND GENDER DISTRIBUTION OF THE STUDY SUBJECTS

Age group (years)	No. of Male subjects (%)	No. of Female subjects (%)
≤ 30	18 (60)	0
31 - 40	8 (26.6)	0
≥ 40	3 (10)	1 (3.3)
Total (N=30)	29 (96.6)	1 (3.3)

TABLE 2: AGE DESCRIPTIVE OF THE STUDY SUBJECTS IN DIFFERENT STAGING OF OSF

Stage	No of subjects (%)	Mean ± S.D	p value
1	4 (13.33)	31.25 ± 10.7	0.945
2	5 (16.66)	28.80 ± 6.7	
3	19 (63.33)	31.16 ± 7.7	
4a	2 (6.66)	30.50 ± 4.9	
Total	30	30.73 ± 7.5	

TABLE 3: PRE-TREATMENT MOUTH OPENING OF THE STUDY SUBJECTS IN DIFFERENT STAGING OF OSF

Variable	Staging	Mean ± S.D	p value
Mouth Opening (Pre)	1	36.50 ± 0.5	0.000*
	2	26.60 ± 0.5	
	3	21.32 ± 2.3	
	4a	13.50 ± 0.7	

TABLE 4: POST TREATMENT MOUTH OPENING OF THE STUDY SUBJECTS IN DIFFERENT STAGING OF OSF

Variable	Staging	Mean ± S.D	p value
Mouth Opening (Post)	1	42.75 ± 0.9	0.000*
	2	31.20 ± 0.8	
	3	27.53 ± 1.9	
	4a	21.00 ± 1.4	

TABLE 5: COMPARISON OF PRE TREATMENT AND POST TREATMENT MOUTH OPENING OF THE STUDY SUBJECTS

Variable	Pre treatment	Post treatment	Significance (p value)
Mouth opening	23.70 ± 6.1	29.73 ± 5.8	.000*

TABLE 6: PRE TREATMENT VISUAL ANALOG SCALE SCORES OF THE STUDY SUBJECTS IN DIFFERENT STAGING OF OSF

Variable	Staging	Mean ± S.D	p value
Visual Analog Scale Score (Pre)	1	2.50 ± 1.0	0.000*
	2	2.80 ± 1.0	
	3	5.11 ± 1.1	
	4a	7.00 ± 0.0	

TABLE 7: POST TREATMENT VISUAL ANALOG SCALE SCORES OF THE STUDY SUBJECTS IN DIFFERENT STAGING OF OSF

Variable	Staging	Mean ± S.D	p value
Visual Analog Scale Score (Post)	1	0.00 ± 0.0	0.000*
	2	0.00 ± 0.0	
	3	1.58 ± 1.0	
	4a	3.00 ± 0.0	

TABLE 8: COMPARISON OF PRE TREATMENT AND POST TREATMENT VISUAL ANALOG SCALE SCORE OF THE STUDY SUBJECTS

Variable	Pre value	Post value	Significance (p value)
Visual Analog Scale Score	4.50 ± 1.6	1.20 ± 1.1	.000*

TABLE 9: PRE TREATMENT TOTAL CHOLESTEROL OF THE STUDY SUBJECTS IN DIFFERENT STAGING OF OSF

Variable	Staging	Mean ± S.D	p value
Total Cholesterol (Pre)	1	178.75 ± 42.5	0.314
	2	165.60 ± 29.7	
	3	168.82 ± 30.3	
	4a	128.00 ± 1.4	

TABLE 10: POST TREATMENT TOTAL CHOLESTEROL OF THE STUDY SUBJECTS IN DIFFERENT STAGING OF OSF

Variable	Staging	Mean ± S.D	p value
Total Cholesterol (Post)	1	184.75 ± 35.1	0.249
	2	177.80 ± 25.9	
	3	184.31 ± 36.2	
	4a	132.00 ± 9.8	

TABLE 11: PRE TREATMENT TRIGLYCERIDES OF THE STUDY SUBJECTS IN DIFFERENT STAGING OF OSF

Variable	Staging	Mean ± S.D	p value
Triglycerides (Pre)	1	156.00 ± 139.3	0.418
	2	88.20 ± 38.5	
	3	119.52 ± 76.9	
	4a	49.50 ± 6.3	

TABLE 12: POST TREATMENT TRIGLYCERIDES OF THE STUDY SUBJECTS IN DIFFERENT STAGING OF OSF

Variable	Staging	Mean ± S.D	p value
Triglycerides (Post)	1	156.75 ± 110.1	0.526
	2	89.00 ± 49.0	
	3	156.10 ± 88.3	
	4a	160.00 ± 173.9	

TABLE 13: PRE TREATMENT HIGH DENSITY LIPOPROTEIN OF THE STUDY SUBJECTS IN DIFFERENT STAGING OF OSF

Variable	Staging	Mean ± S.D	p value
High Density Lipoproteins (Pre)	1	42.00 ± 18.1	0.977
	2	40.80 ± 7.6	
	3	40.56 ± 10.6	
	4a	44.00 ± 8.4	

TABLE 14: POST TREATMENT HIGH DENSITY LIPOPROTEIN OF THE STUDY SUBJECTS IN DIFFERENT STAGING OF OSF

Variable	Staging	Mean ± S.D	p value
High Density Lipoproteins (Post)	1	44.75 ± 16.0	0.925
	2	49.20 ± 5.5	
	3	47.90 ± 10.1	
	4a	45.95 ± 12.7	

TABLE 15: PRE TREATMENT LOW DENSITY LIPOPROTEIN OF THE STUDY SUBJECTS IN DIFFERENT STAGING OF OSF

Variable	Staging	Mean ± S.D	p value
Low Density Lipoproteins (Pre)	1	119.00 ± 21.4	0.303
	2	112.40 ± 26.8	
	3	110.50 ± 25.3	
	4a	78.50 ± 3.5	

TABLE 16: POST TREATMENT LOW DENSITY LIPOPROTEIN OF THE STUDY SUBJECTS IN DIFFERENT STAGING OF OSF

Variable	Staging	Mean ± S.D	p value
Low Density Lipoproteins (Post)	1	129.75 ± 15.1	0.056
	2	122.80 ± 25.0	
	3	120.68 ± 35.5	
	4a	55.35 ± 13.6	

TABLE 17: PRE TREATMENT VERY LOW DENSITY LIPOPROTEIN OF THE STUDY SUBJECTS IN DIFFERENT STAGING OF OSF

Variable	Staging	Mean ± S.D	p value
Very Low Density Lipoproteins (Pre)	1	31.06 ± 27.9	0.425
	2	17.96 ± 7.5	
	3	23.72 ± 15.4	
	4a	9.50 ± 0.7	

TABLE 18: POST TREATMENT VERY LOW DENSITY LIPOPROTEIN OF THE STUDY SUBJECTS IN DIFFERENT STAGING OF OSF

Variable	Staging	Mean ± S.D	p value
Very Low Density Lipoproteins (Post)	1	30.65 ± 22.4	0.587
	2	18.16 ± 9.4	
	3	30.98 ± 18.3	
	4a	32.05 ± 34.8	

TABLE 19: PRE TREATMENT C-REACTIVE PROTEIN OF THE STUDY SUBJECTS IN DIFFERENT STAGING OF OSF

Variable	Staging	Mean ± S.D	p value
C Reactive Protein (Pre)	1	1.52 ± 0.6	0.444
	2	1.62 ± 1.6	
	3	2.54 ± 2.0	
	4a	0.79 ± 0.1	

TABLE 20: POST TREATMENT C-REACTIVE PROTEIN OF THE STUDY SUBJECTS IN DIFFERENT STAGING OF OSF

Variable	Staging	Mean ± S.D	p value
C-Reactive Protein (Post)	1	1.47 ± 0.8	0.622
	2	1.38 ± 0.9	
	3	1.74 ± 1.1	
	4a	0.80 ± 0.2	

TABLE 21: COMPARISON OF PRE TREATMENT AND POST TREATMENT C-REACTIVE PROTEIN OF THE STUDY SUBJECTS

Variable	Pre-treatment	Post treatment	p value
C-reactive protein	2.13 ± 1.8	1.58 ± 1.0	.022*

TABLE 22: COMPARISON OF PRE TREATMENT AND POST TREATMENT OF LIPID PROFILE OF THE STUDY SUBJECTS

Variable	Pre-treatment	Post treatment	Significance (p value)
Total Cholesterol	166.89 ± 31.7	179.80 ± 34.8	.000*
Triglycerides	114.50 ± 80.9	145.26 ± 90.1	.007*
High Density Lipoprotein	41.02 ± 10.7	47.57 ± 10.1	.000*
Low Density Lipoprotein	109.82 ± 25.0	117.89 ± 34.6	.020*
Very Low Density Lipoprotein	22.79 ± 16.1	28.87 ± 18.4	.007*

TABLE 23: RELATIONSHIP BETWEEN VISUAL ANALOG SCALE SCORES AND POST TREATMENT LIPID PROFILE

Variable	VAS score	Mean ± S.D	p value
Total Cholesterol	0	180.00 ± 27.0	0.383
	1	186.80 ± 31.6	
	2	184.83 ± 52.7	
	3	132.00 ± 9.8	
	4	176.50 ± 20.5	
Triglycerides	0	123.50 ± 80.2	0.924
	1	161.20 ± 78.7	
	2	145.66 ± 122.8	
	3	160.00 ± 173.9	
	4	158.50 ± 91.2	
High Density Lipoprotein	0	46.70 ± 10.38	0.906
	1	46.70 ± 10.7	
	2	48.83 ± 11.7	
	3	45.95 ± 12.7	
	4	54.10 ± 2.6	
Low Density Lipoprotein	0	123.80 ± 20.2	0.103
	1	126.40 ± 43.4	
	2	116.33 ± 31.5	
	3	55.35 ± 13.6	
	4	113.00 ± 4.2	
Very Low Density Lipoprotein	0	24.70 ± 15.9	0.920
	1	32.71 ± 16.4	
	2	28.06 ± 24.8	
	3	32.05 ± 34.8	
	4	29.77 ± 20.8	

TABLE 24: RELATIONSHIP BETWEEN VISUAL ANALOG SCALE SCORES AND POST TREATMENT CRP

Variable	VAS score	Mean ± S.D	p value
CRP	0	1.41 ± 0.7	0.559
	1	1.96 ± 0.87	
	2	1.66 ± 1.7	
	3	0.80 ± 0.2	
	4	1.15 ± 0.6	

TABLE 25: RELATIONSHIP BETWEEN VISUAL ANALOG SCALE SCORES AND POST TREATMENT MOUTH OPENING

Variable	VAS score	Mean ± S.D	p value
Mouth Opening	0	35.70 ± 6.1	0.000*
	1	28.00 ± 1.8	
	2	27.00 ± 0.8	
	3	21.00 ± 1.4	
	4	25.50 ± 3.5	

CHARTS

CHART 1: MEAN AGE IN YEARS OF THE STUDY SUBJECTS IN DIFFERENT STAGING OF OSF

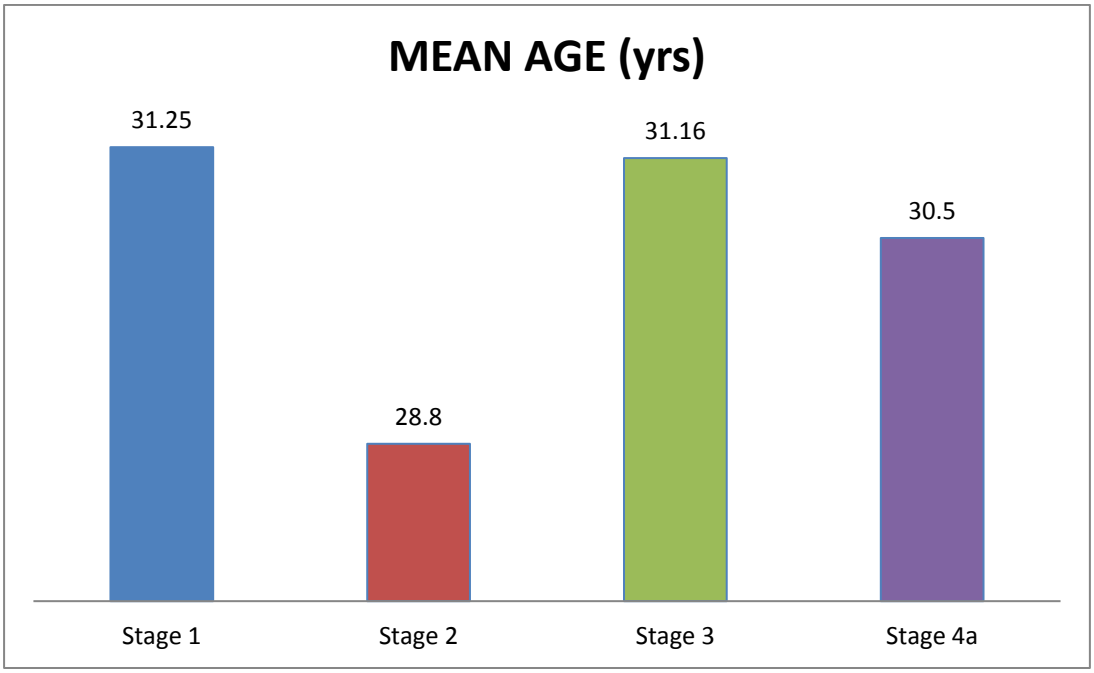


CHART 2: PRE TREATMENT MOUTH OPENING OF THE STUDY SUBJECTS IN DIFFERENT STAGING OF OSF

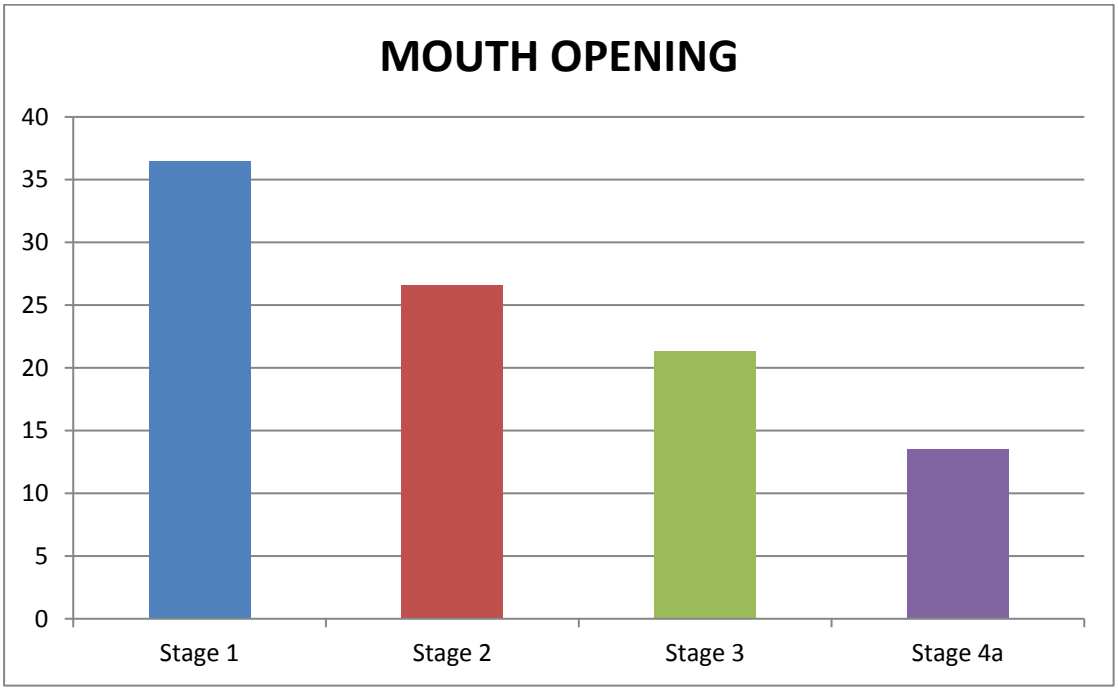


CHART 3: POST TREATMENT MOUTH OPENING OF THE STUDY SUBJECTS IN DIFFERENT STAGING OF OSF

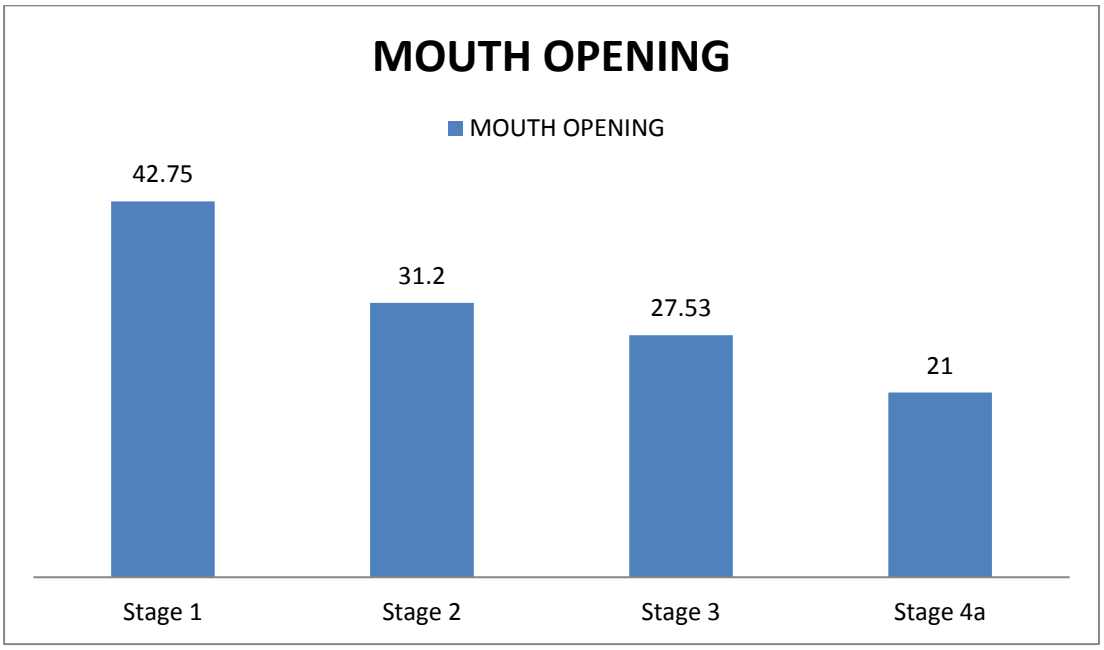


CHART 4: PRE TREATMENT VISUAL ANALOG SCALE SCORES OF THE STUDY SUBJECTS IN DIFFERENT STAGING OF OSF

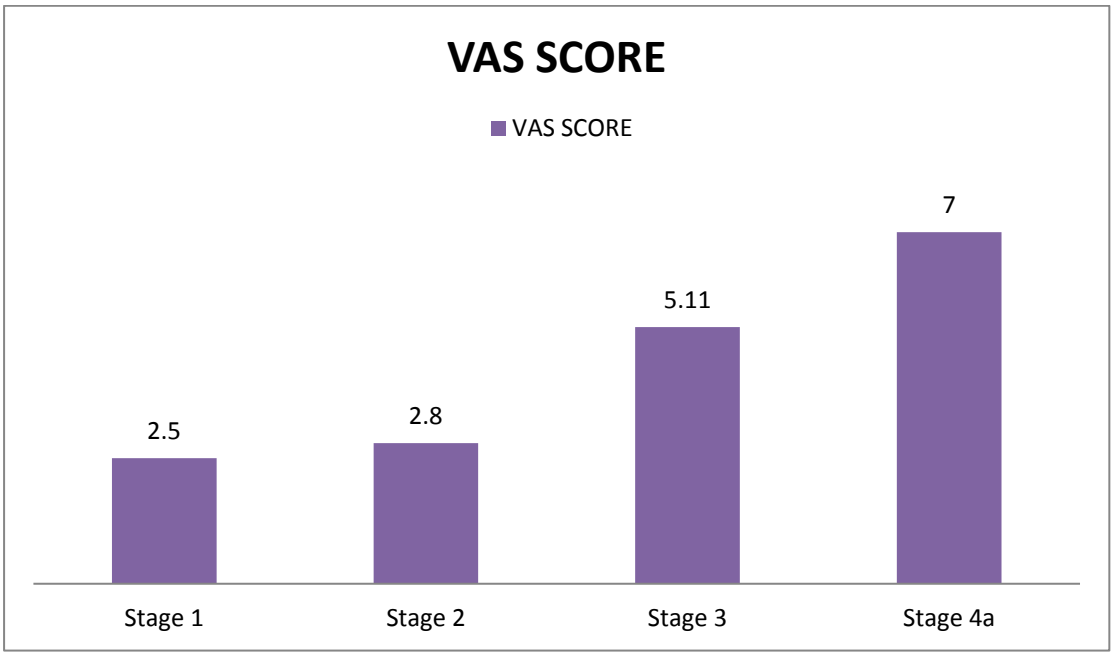


CHART 5: POST TREATMENT VISUAL ANALOG SCALE SCORES OF THE STUDY SUBJECTS IN DIFFERENT STAGING OF OSF

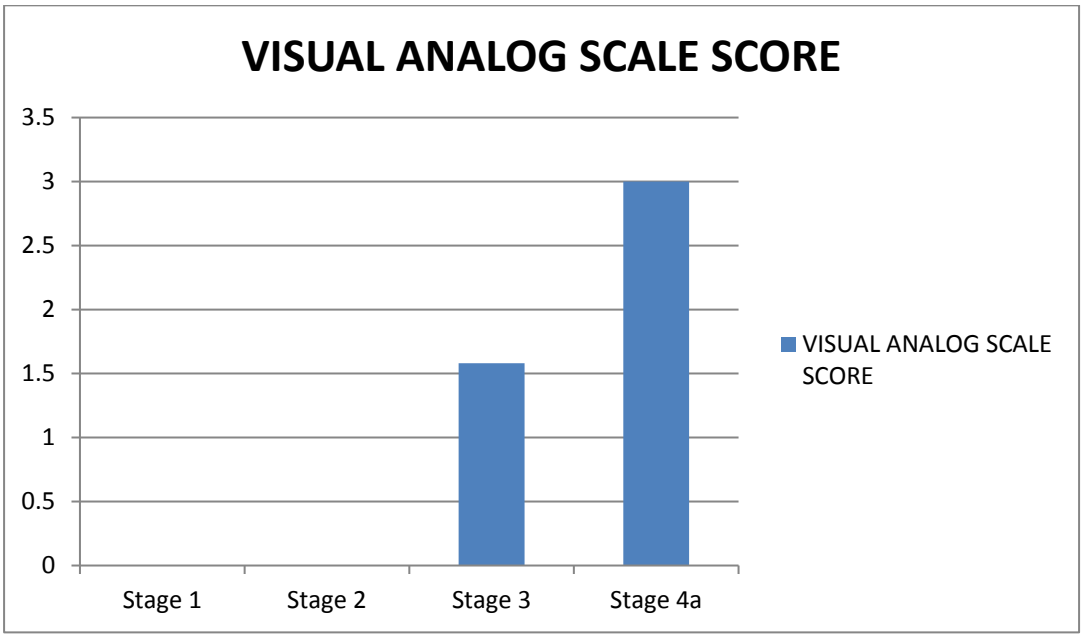


CHART: 6 COMPARISON OF PRE TREATMENT AND POST TREATMENT LIPID PROFILE OF THE STUDY SUBJECTS

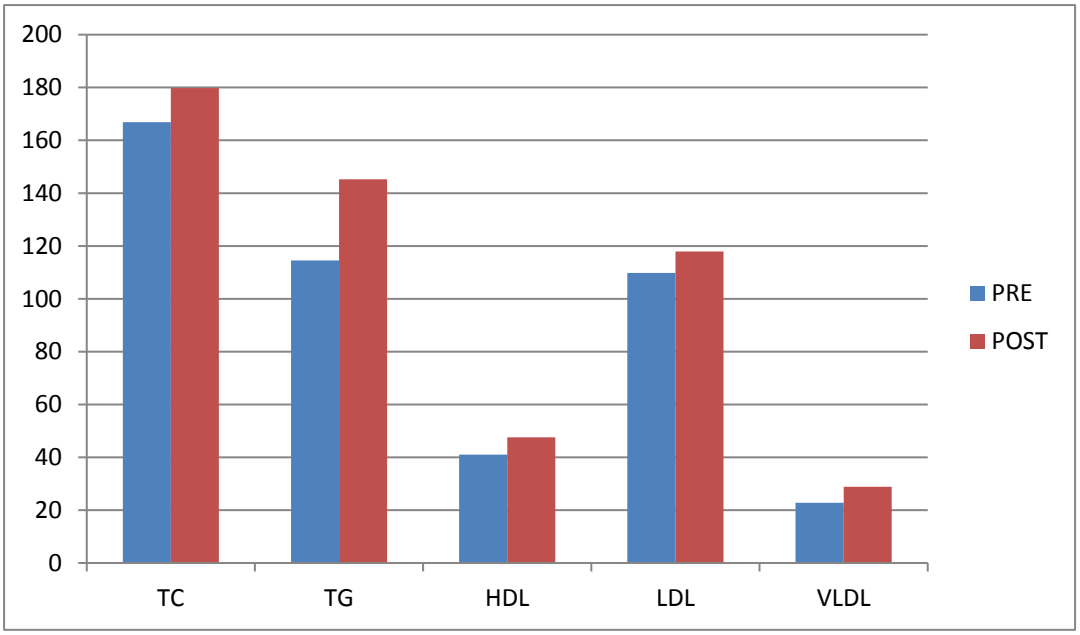


CHART: 7 COMPARISON OF PRE TREATMENT AND POST TREATMENT CRP OF THE STUDY SUBJECTS

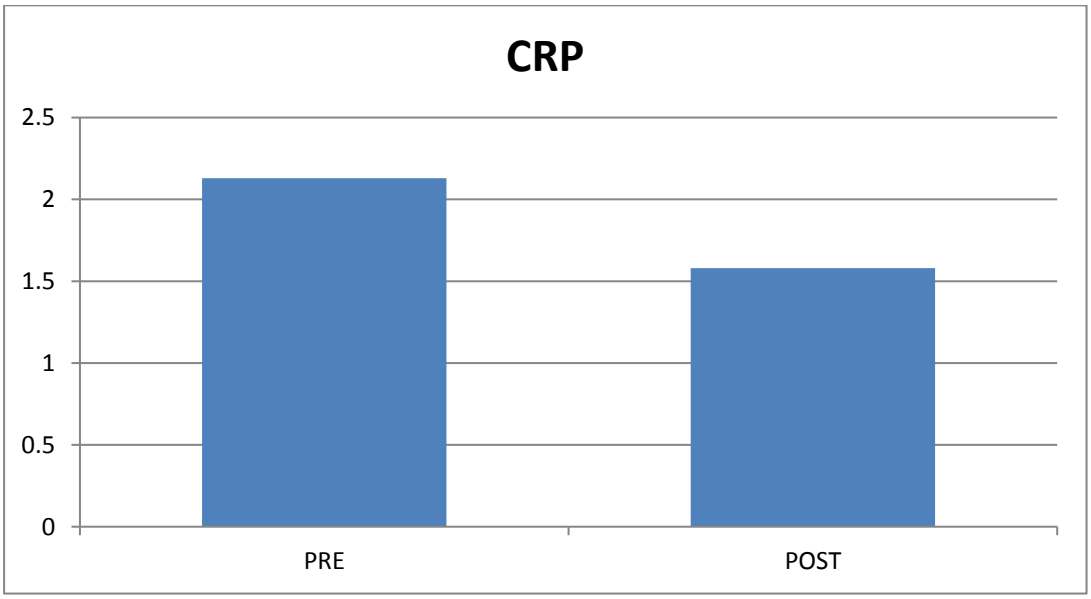


CHART 8: COMPARISON OF PRE TREATMENT AND POST TREATMENT MOUTH OPENING OF THE STUDY SUBJECTS

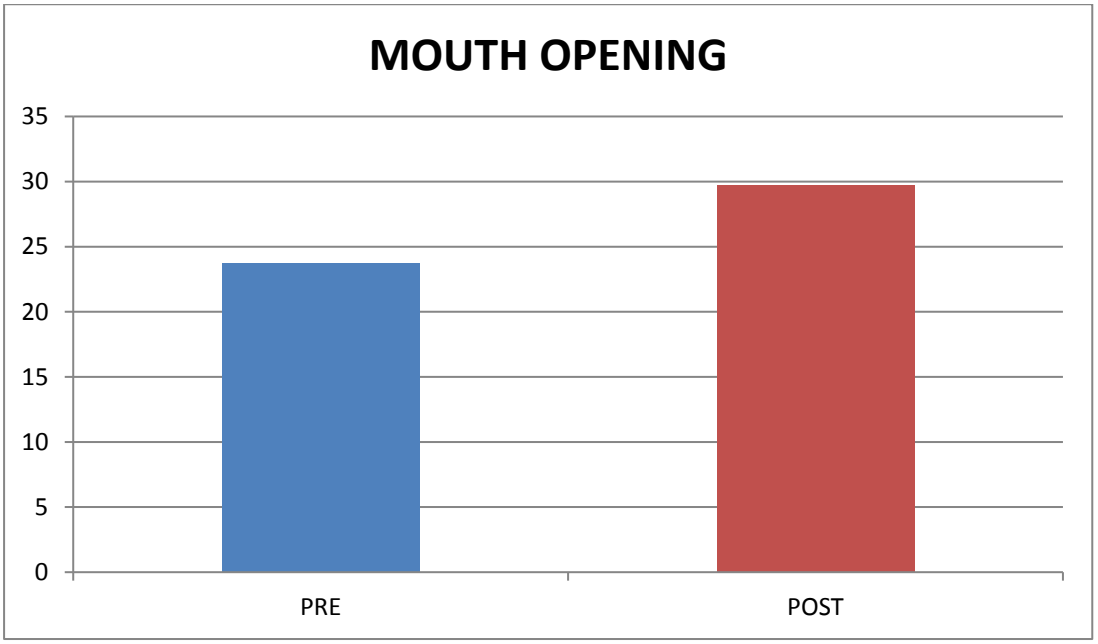


CHART 9: COMPARISON OF PRE TREATMENT AND POST TREATMENT VISUAL ANALOG SCALE SCORE OF THE STUDY SUBJECTS

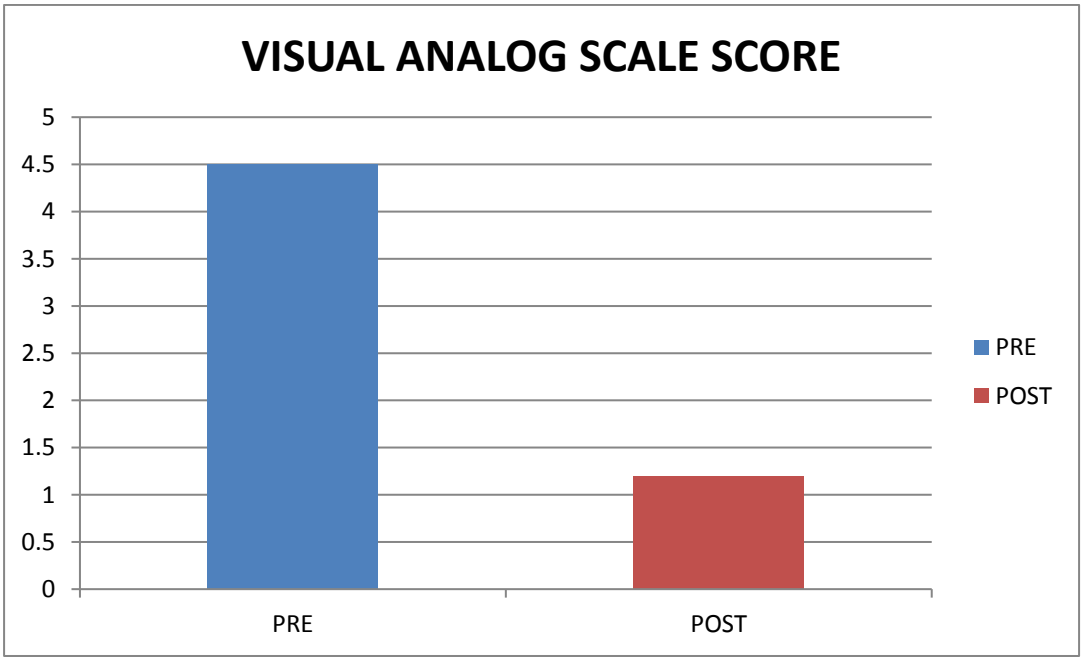
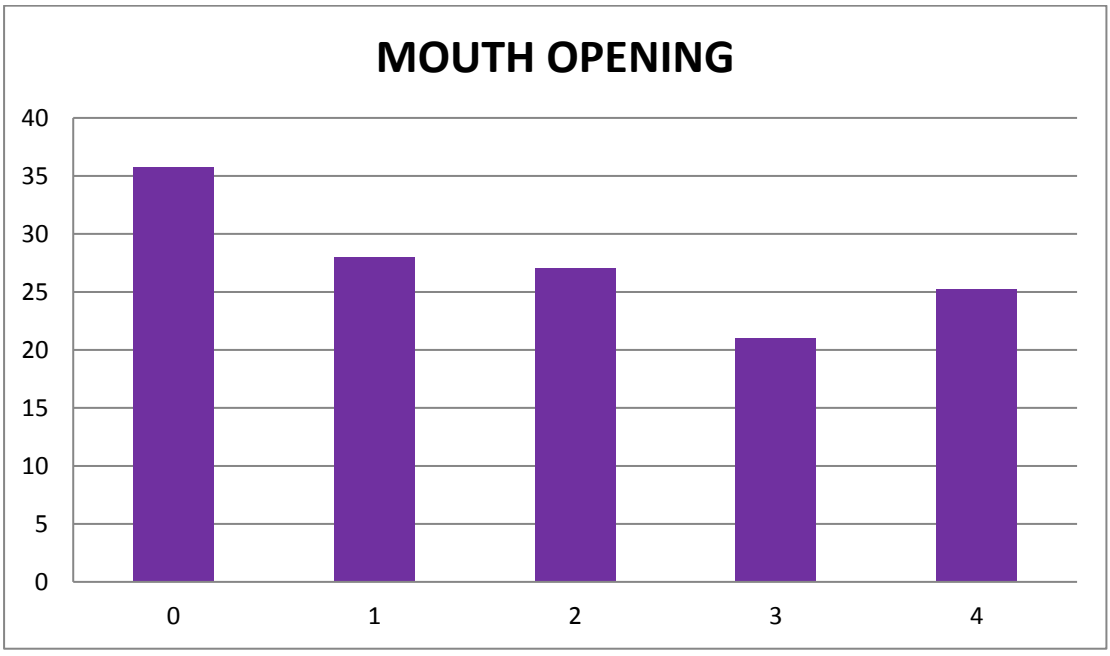


CHART 10: RELATIONSHIP BETWEEN VISUAL ANALOG SCALE SCORES AND POST TREATMENT MOUTH OPENING



DISCUSSION

Oral submucous fibrosis is a chronic, premalignant condition of the oral mucosa, affecting millions of people globally and it is one of the precancerous conditions most prevalent in India. [22]

Though the etiology of OSF is multifactorial, Areca nut plays an important role in the disease manifestation. WHO establishes that the use of smokeless tobacco (betel quid, mishri, gutka) is rampant in South East Asia and it leads to OSF, a debilitating disease. The chronic irritation caused by consumption of areca nut in the form of pan masala, gutka causes injury thereby leading to chronic inflammation, cytokine production and development of oxidative stress. Carcinogens in these substances generate ROS and lipid peroxides thereby leading to tissue injury as a result of elevated lipid peroxidation, further damaging the cellular structural block, namely lipids. [114]

A total of 30 clinically diagnosed Oral Submucous Fibrosis (OSF) patients were included in this study. The age group of the study subjects ranged from 20 years to 50 years and the mean age was 30.73 ± 7.5.

In our study, out of the total study participants 96.6% were male patients and 3.33% were female patients. Such male predominance was also reported in earlier studies by **Rawson et al.** [3] who reported a 80% male patients and **Apala Baduni et al** [134] has reported a 95% male predominance.

In our study, the relationship between mouth opening and the staging of the disease is found to be statistically significant both pre and post treatment and the comparison of mean values of the pre-treatment and post treatment mouth opening levels of the study subjects among OSF patients were found to be

statistically significant. The mean increase in mouth opening post treatment was 6.03 ± 1.7.

According to **Kumar et al** [101] a significant improvement in mouth opening was observed post treatment. Similar results of significant improvement in mouth opening post treatment were also established in studies by **Gupta et al**.[135] and **Maher et al** [136].

In our study, the comparison of mean values of the pre-treatment and post treatment visual analog scale score of the study subjects among different stages of OSF were found to be statistically significant (p=0.000) and the difference in mean values of visual analog scale score was 3.30 ± 1.1. In this study, all patients (100%) in Grade 1 & Grade 2 after completion of the treatment regimen, reported a complete relief from pain/burning sensation and patients in Grade 3 and Grade 4a reported a significant reduction in their symptoms.

Gupta et al; in his study has reported similar results of reduction in burning sensation with biweekly injection of intralesional steroids.

SERUM LIPID PROFILE

TOTAL CHOLESTEROL:

The mean value of the pre-treatment total cholesterol level was 166.89 ± 31.7. There is no statistical significance (p = 0.314) in the relationship between total cholesterol values and the staging of the disease. The mean value of the post-treatment total cholesterol level was 179.80 ± 34.8. There is no statistical significance (p = 0.249) in the relationship between total cholesterol values and the staging of the disease.

In our study, the comparison of mean values of the pre-treatment and post treatment total cholesterol level of the study subjects among OSF patients were found to be statistically significant (p=0.000). The mean total cholesterol levels increased by 12.91 ± 16.8 post treatment. The lower levels of plasma cholesterol and other lipid constituents in patients before treatment might be due to their increased utilization by neoplastic cells for new membrane biogenesis.

Mehrotra *et al.* [130] in his study reported that serum total cholesterol levels were significantly decreased in OSF patients in comparison to normal healthy subjects.

Rawson et al. [3] in his study also observed similar correlations between OSF and serum total cholesterol among OSF patients in comparison to normal healthy subjects.

Ajai *et al.* [126] conducted a study in serum lipid among control and in different stages of OSMF, Which showed significant reduction in serum total cholesterol levels as the disease progressed.

Patel *et al.* [113] carried out a similar study on 184 head and neck cancer patients, 153 patients with oral precancerous condition, and 52 controls. A significant decrease in plasma TC in patients with oral premalignant condition as compared to the controls.

Lohe *et al.* [127] conducted a study on 70 oral cancer and 70 precancerous condition patients for the evaluation of serum lipid profile levels. They found that there was a significant decrease in TC in oral precancer group, as compared to the controls. They concluded that there is an inverse relationship between serum lipid profile and oral cancer and oral precancer.

A study by **Sharma *et al.*** [129] observed a significant decrease in serum cholesterol, LDL in OSF patients.

Kumar *et al.* [117] showed a significant decrease in TC, HDL and LDL in patients with OSF as compared to the controls. The results of our study was in accordance with the results of the above studies.

TRIGLYCERIDES:

The mean value of the pre-treatment triglyceride level was 114.50 ± 80.9. There is no statistical significance (p = 0.418) in the relationship between triglyceride values and the staging of the disease. The mean value of the post-treatment triglyceride level was 145.26 ± 90.1. There is no statistical significance (p = 0.526) in the relationship between triglyceride values and the staging of the disease.

In our study, the comparison of mean values of the pre-treatment and post treatment triglyceride level of the study subjects among different stages of OSF were found to be statistically significant (p=0.007). The mean triglyceride levels increased by 30.76 ± 57.6 post treatment.

Studies by **Rawson *et al.*** [3] **Ajai *et al.*** [126] also observed a decrease in serum triglycerides among OSF patients in comparison to normal healthy subjects. These findings were similar to our present study.

Whereas, **Chalko *et al.*** [131] in contrast, observed a significantly increased TG levels in OSF as compared to controls and **Goyal *et al.*** [4] & **Alexopoulos *et al.*** [140] found no statistically significant changes in TGs of oral precancerous lesions and conditions as compared to controls. **Anusha *et al***. [132] also observed significant decrease in TG level in OSF patients.

HIGH DENSITY LIPOPROTEIN (HDL):

The mean value of the pre-treatment HDL level was 41.02 ± 10.7. There is no statistical significance (p = 0.977) in the relationship between the HDL values and the staging of the disease. The mean value of the post-treatment HDL level was 47.57 ± 10.1. There is no statistical significance (p = 0.925) in the relationship between HDL values and the staging of the disease.

In this study, the comparison of mean values of the pre-treatment and post treatment HDL level of the study subjects among different stages of OSF were found to be statistically significant (p=0.000). The mean HDL levels increased by 6.54 ± 7.6 post treatment.

Findings similar to our present study were observed by **Ajai *et al.*** [126] **Mehrotra *et al.*** [130].

Lohe *et al.* [127] who have reported a significant decrease in HDL levels among OSF patients in comparison to control group. Also studies by **Anusha *et al.*** [132] & **Patel *et al.*** [133] reported that HDL was significantly decreased in all the groups of OSF. Thus, HDL starts decreasing with disease onset and continues to do so till the advanced stages of the disease. It shows highest decrease in OSF group 4.

In contrast to our findings, studies by **Sharma *et al.*** [129] and **Mayeesh R *et al***. [139] observed an increased level of HDL among OSF patients when compared to normal subjects.

LOW DENSITY LIPOPROTEIN (LDL):

The mean value of the pre-treatment LDL level was 109.82 ± 25.0. There is no statistical significance (p = 0.303) in the relationship between the LDL values and the staging of the disease. The mean value of the post-treatment LDL

level was 117.89 ± 34.6. There is no statistical significance (p = 0.056) in the relationship between LDL values and the staging of the disease. In this study, the comparison of mean values of the pre-treatment and post treatment LDL level of the study subjects among different stages of OSF were found to be statistically significant (p=0.020) The mean LDL levels increased by 8.07 ± 18.0 post treatment.

Deepanshu Garg *et al.* [138] in his study have observed a decreased LDL levels among oral precancer patients and cancer patients compared to controls. This finding is in similarity to our present study. **Rawson *et al.*** [3] and **Mayeesh R *et al.*** [139] has compared OSF patients with control group and observed a decrease in LDL levels among OSF patients.

VERY LOW DENSITY LIPOPROTEIN (VLDL):

The mean value of the pre-treatment VLDL level was 22.79 ± 16.1. There is no statistical significance (p = 0.425) in the relationship between the VLDL values and the staging of the disease. The mean value of the post-treatment VLDL level was 28.87 ± 18.4. There is no statistical significance (p = 0.587) in the relationship between VLDL values and the staging of the disease. In this study, the comparison of mean values of the pre-treatment and post treatment VLDL level of the study subjects among different stages of OSF were found to be statistically significant (p=0.007) The mean VLDL levels increased by 6.07 ± 11.5 post treatment.

Similar to our findings, **Rawson *et al.*** [3] and **Anusha *et al.*** [132] also observed significant decrease in VLDL level in OSF patients in comparison with control group.

In contrast to our findings, **Kumar *et al.*** [117] showed no significant changes in the levels of VLDL among OSF patients in comparison with normal subjects.

Goyal *et al.* [4] also found no statistically significant changes in VLDL in cases of oral precancerous lesions and conditions as compared to controls.

C – REACTIVE PROTEIN (CRP):

The mean value of the pre-treatment CRP level was 2.13 ± 1.8 and the mean value of the post-treatment CRP level was 1.58 ± 1.0. There is no statistical significance in the relationship between CRP values and the staging of the disease. In this study, the comparison of mean values of the pre-treatment and post treatment CRP level of the study subjects among OSF patients were found to be statistically significant (p=0.022). The mean CRP levels decreased by 0.55 ± 1.2 post treatment.

Kalpajyoti B *et al.* [133] shows increase in the level of CRP in both leukoplakia and OSF patients. **Kumar and Bhateja *et al.*** [121] studied CRP levels in oral precancer and cancer patients in which they observed that CRP level was elevated in PMD.

In a study by **Vankadara *et al.*** [137] CRP concentrations were found to be significantly higher in patients with OSCC (group III) than in healthy controls (group I). **Kaja *et al.*** [5] compared between OSF and leukoplakia and OSMF patients showed prominent elevation of CRP which could be because of risk of high malignant transformation of OSF.

A thorough review of literature revealed most of the published studies have compared the serum lipid profile and C-reactive protein in various potentially malignant disorders and normal healthy controls, but to our knowledge no studies are available comparing the pre-treatment and post-treatment lipid

profile and C-reactive protein values in the same patient with oral potentially malignant disorders, so that their real prognostic value can be assessed. Hence, the present study was conducted.

Our study findings show the evidence of an inverse relationship between serum lipid profile levels in the potentially malignant condition OSF before treatment and a significant increase in serum lipid profile levels post treatment. The CRP levels were increased in OSF patients before treatment and a significant decrease was observed post treatment. The alterations in lipid profile & CRP levels play a prognostic role in the detection of the disease in early stages.

SUMMARY

Oral submucous fibrosis is one of the precancerous conditions affecting the oral mucosa and is most prevalent in India. Early detection of this condition is the mainstay in the effective management of the disease and reduced levels of serum lipid profile and CRP among OSF patients have been established by many studies in the past. However, all studies compared the serum lipid profile and CRP levels of OSF patients with normal healthy subjects.

To the best of our knowledge after a thorough review of literature, there were no studies so far estimating the serum lipid profile and CRP levels of OSF patients pre and post treatment. Hence this study was proposed to estimate the serum lipid profile and CRP levels of clinically diagnosed OSF patients before and after the treatment and establish their prognostic values.

This study involved 30 clinically diagnosed OSF patients of either gender in the age group of 20 – 50 years from the patients reporting to Department of Oral Medicine and Radiology, Tamil Nadu Government Dental College and Hospital. Informed consent was obtained from all patients. A complete history taking followed by a thorough oral examination was done to all patients. Burning sensation of the patient was assessed by using visual analog scale and mouth opening measured using caliper and measuring scale and based on these observations, all patients were subdivided into 4 stages according to Khanna et.al staging system of OSF. 5mL of intravenous blood sample was drawn from the prominent vein in the cubital fossa. The blood samples were centrifuged at 2500

RPM for 5 minutes and serum separated and stored at 4^{o}C and pre-treatment estimation of lipid profile & CRP were done using Roche cobas III autoanalyser. Grade I OSF patients were given supplemental medication of capsule antioxidant twice daily, tablet vitamin B complex twice daily and topical Benzocaine application before food for 6weeks whereas patient in Grade II, Grade III, Grade IVa were administered intralesional injection of 2ml of Dexamethasone with 0.5ml of Lignocaine twice weekly and supplemental Medication as above for 6weeks. Patient in Grade 1 were recalled once in a week whereas patients in Grade II, Grade III and Grade IVa were recalled twice in a week and evaluated for improvement in signs and symptoms such as burning sensation and mouth opening. Final changes in burning sensation and mouth opening were recorded after the complete course of treatment. At the end of the treatment blood sample was collected and serum lipid profile & CRP estimated and all the values analysed statistically.

In this study, the relationship between mouth opening and the staging of the disease is found to be statistically significant both pre and post treatment and the comparison of mean values of the pre-treatment and post treatment mouth opening levels of the study subjects among different stages of OSF were found to be statistically significant.

The relationship between visual analog scale score and the staging of the disease is found to be statistically significant both pre and post treatment and the comparison of mean values of the pre-treatment and post treatment visual analog

scale score of the study subjects among different stages of OSF were found to be statistically significant.

There is no statistical significance in the relationship between lipid profile values and the staging of the disease. However, the comparison of mean values of the pre-treatment and post treatment lipid profile level of the study subjects among different stages of OSF were found to be statistically significant. There is no statistical significance in the relationship between the CRP values and the staging of the disease. However, the comparison of mean values of the pre-treatment and post treatment CRP level of the study subjects among different stages of OSF were found to be statistically significant.

CONCLUSION

The present study was conducted to estimate the pre-treatment and post treatment lipid profile and CRP levels in patients with oral submucous fibrosis in different stages so that their real prognostic value can be assessed.

In our study, a statistical significance was established between the mouth opening levels, visual analog scale score and different stages of OSF. An inverse relationship was established between the various parameters of lipid profile such as total cholesterol, serum triglycerides, high density lipoprotein, low density lipoprotein and very low density lipoprotein levels and oral submucous fibrosis before treatment and on comparing the pre-treatment and post treatment mean values of lipid profile, it was found to be statistically significant.

The pre-treatment CRP levels were elevated among OSF patients and was found to be statistically significant among different stages and on comparing the pre-treatment and post treatment mean values of CRP, it was found to be statistically significant.

Hence, the pre-treatment and post treatment serum lipid profile and CRP levels in the OSF patients in our study contributes to the prognostic value of serum lipid profile and CRP levels in OSF.

Still, studies involving equal number of large samples among different stages of OSF are required to establish statistically significant results among different stages of the disease. Inclusion of stage IV b OSF in future studies may explore the role of serum lipid profile & CRP as a prognostic marker in all stages of OSF.

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APPENDIX

TAMILNADU GOVERNMENT DENTAL COLLEGE AND HOSPITAL,
CHENNAI-600003

AFFLIATED TO THE TAMILNADU DR.M.G.R.MEDICAL UNIVERSITY,CHENNAI

INSTITUTIONAL REVIEW BOARD-PROVISIONAL CLEARANCE CERTIFICATE

CHAIR PERSON Prof .Dr.B.Saravanan, MDS., PhD., **MEMBER SECRETARY** Prof .Dr.M.B.Aswath Narayanan, BSc.,MDS., **MEMBERS** Prof. Dr.Maheaswari Rajendran, MDS., Prof. Dr.S.Jayachandran, MDS., PhD., Prof. Dr.M.Kavitha, MDS., Prof. Dr.C.Sabarigirinathan, MDS., Prof. Dr.S.Geetha, MD., (General Medicine) Prof. Dr.R.Vanaja, MD., (Microbiology) Prof. Dr.K.M.Sudha, MD., (Pharmacology) Prof. Dr.S.Siva, MD., (Biochemistry) Prof. Dr.Bharathi N Jayanthi,MD., (Gen.Pathology) Prof. Dr.M.Chandrasekar, MVSc, PhD., Mr.G.K.Muthukumar, B.Com., LLB., Mr.Shantaram	**IRB Reference No: 7/IRB/2017** **Project Title:** Serum lipid profile and C-reactive protein as a prognostic marker in Oral submucous fibrosis **Principal Investigator: Dr.K.Jayanthiswari** **Review:** New/Revised/Expedited **Date of review: 13.11.17** **Date of previous review, if revised application: Not Applicable** **Decision of the IRB: Approved** **Recommended time period:** One Year INSTITUTIONAL REVIEW BOARD 30.11.17 TNGDCH-CH3 **Member Secretary** **Chair Person**

The Investigator / Investigating team is advised to adhere to the guide lines given below:

- Should be carried out under the direct supervision of the Guide
- Get detailed informed consent from the patients / participants and maintain confidentiality.
- Carry out the work without affecting regular work and without extra expenditure to the Institution or the Government.
- Inform the IRB in case of any change of study procedure, site, Investigator and Guide.
- Not deviate from the area of work for which applied for clearance.
- Inform the IRB immediately in case of any adverse events or serious adverse reactions. Should abide to the rules and regulations of the institution(s) .
- Complete the work within specific period and if any extension of time is required, should apply for permission again to do the work.
- Submit the summary of the work to the IRB : Students-every 3 months;
  Faculty-every 6 months.
- Should not claim any kind of funds from the institution for doing the work or on completion/ or for any kind of compensations.
- The members of the IRB have the right to monitor the work without prior intimation.
- The investigator and Guide should each declare that no plagiarism is involved in this whole study and enclose the undertaking in dissertation/ thesis.

PATIENT INFORMATION SHEET

I, **Dr.K.Jayanthiswari,** I –MDS student,Department of Oral Medicine and Radiology, primary investigator under the guidance of Prof.Dr.G.V.MuraliGopikaManoharan, MDS, Professor, Department of Oral Medicine and Radiology, Tamilnadu Government Dental College and Hospital, have planned to conduct a study titled**"Serum Lipid profile and C-reactive proteinas a prognostic marker in Oral submucous fibrosis"**in Tamilnadu Government Dental College and Hospital, Chennai-3.

Purpose of the study

To evaluate the serum lipidprofile and CRP as a prognostic marker in OSF patients.

Procedures.

Patients with OSF will be prescribed antioxidants capsules,tablet,vitaminB-complex ,topical 0.1% triamcinolone acetonateoromucosal paste and/or benzocaine gel and givenintralesional 2ml dexamethasone+0.5lignocaine injection according to the Clinical staging of OSF.5mL of intravenous blood sample will be drawn for pre and post treatment estimation of lipid profile and CRP.

Benefits of participation

Patients will be given treatment for OSF and their improvement post treatment is assessed using serum lipid profile and CRP and additionally this study helps to know the cardiac risk in these participants.

Participant's rights

Taking part in this study is voluntary. Patients are free to decidewhether to participate in the study or to withdraw at any time; patient'sdecision will not result in any loss of benefits to which you areotherwise entitled.The results of this special study may be intimated to patient at the endof the study period.

Risk of participation

Nil

Confidentiality

The identity of the patients participating in the research will bekept confidential throughout the study.In the event of anypublication or presentation resulting from the research, nopersonally identifiable information will be shared.

Compensation

Nil

Contactsfor queries related to the study,

Dr.K.Jayanthiswari

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Chennai - 600003.

Phone no: 9944414880

Queries related to patients Rights,

Dr.B. Saravanan. M.D.S, Ph.D

The chairman, institutional ethical committee,

Tamilnadu Government Dental College and Hospital,

Chennai - 600003

Name of the Patient **Signature /Thumb impression**

Name of the investigator **Signature**

Date

ஆராய்ச்சி பற்றிய தகவல் படிவம்

ஆராய்ச்சி தலைப்பு

கு.ஜெயந்திஸ்வரி ஆகிய நான் மரு.க.வெ.முரளி கோபிகா மனோகரன், MDS அவர்களின் வழிநடத்துதலின் கீழ் ஊணீல் உள்ள கொழுப்பு அளவுகளின் விவரம் மற்றும் சி-ரியாக்டிவ் புரதம் அளவு மூலம் வாய் இறுக்க நோய் குணமடைதலை முன்கணிப்பு செய்தல் பற்றிய ஆய்வு.

ஆய்வின் நோக்கம்

இந்த ஆய்வின் நோக்கமானது, வாய் இறுக்க நோயாளிகளின் ஊணீரில் உள்ள கொழுப்பு மற்றும் சி-ரியாக்டிவ் புரதத்தின் அளவுகளை சிகிச்சைக்கு முன் மற்றும் சிகிச்சைக்குப்பின் மதிப்பிட்டு கண்டறிந்து அதன் மூலம் குணமடைதலை முன்கணிப்பு செய்தல்.

செய்முறை

ஆய்வில் பங்கேற்கும் வாய் இறுக்க நோயாளிகளுக்கு முழுமையான மருத்துவ வரலாறு அறியப்பட்டு, முழு வாய் பரிசோதனை செய்யப்படும். முந்திய விலாவின் உட்பகுதியில் தமனியிலிருந்து ஊசி மூலம் 5மி.லி இரத்தம் எடுக்கப்பட்டு, பின்பு ஆய்வுக்கூடத்திற்கு ஊணீரில் உள்ள கொழுப்பு மற்றும் சி-ரியாக்டிவ் புரதம் மதிப்பீட்டிற்கு அனுப்பிவைக்கப்படும்.

ஆய்வில் பங்கேற்கும் வாய் இறுக்கு நோயாளிகளுக்கு வாரத்திற்கு இரு முறை வாயின் உட்புறத்தில் 2மி.லி டெக்ஸாமீதாசோன் மற்றும் 0.5மி.லி லிக்னோகெயின் கலவை ஊசி போடப்படும். கூடுதலாக ஆண்டி ஆக்ஸிடன்டுகள் அடங்கிய கூட்டு மருந்து மற்றும் விட்டமின் பி காம்ப்ளக்ஸ் கொடுக்கப்படும். ஆறு வாரங்கள் சிகிச்சைக்கு பின் மீண்டும் ஊணீரிலுள்ள கொழுப்பு மற்றும் சி-ரியாக்டிவ் புரத மதிப்பீட்டிற்கு இரத்தம் எடுக்கப்பட்டு ஆய்வு கூடத்திற்கு அனுப்பி வைக்கப்படும்.

நன்மைகள்

ஊணீரிலுள்ள கொழுப்பு அளவைக் கொண்டு வாய் இறுக்க நோயுள்ளவர்களின் நோய், சிகிச்சைக்குப் பின் நோய் குணமடைந்து உள்ளதா என்பதை அறிய உதவியாக இருக்கும். கூடுதலாக மேற்கண்டவர்களின் இதய நோய் வருவதற்கான அபாயமும் அறிந்து கொள்ள முடியும்.

இரகசிய தன்மை

நோயாளிகள் பற்றிய குறிப்புகள் ஆராய்ச்சியை வெளியிடும் போது நோயாளிகளின் தனிப்பட்ட விவரங்கள் எதுவும் பதிக்கப்படமாட்டாது.

பங்குபெறுவோரின் உரிமை

இந்த ஆராய்ச்சியில் பங்கு பெறுவது நோயாளிகளின் தனிப்பட்ட விருப்பம். மேலும் நோயாளிகள் இந்த ஆய்வில் இருந்து எப்போது வேண்டுமானாலும் விலகிக் கொள்ளலாம். நோயாளிகளின் இந்த முடிவினால் அவருக்கோ அல்லது ஆராய்ச்சியாளருக்கோ எந்தவித பாதிப்பும் கிடையாது. இந்த ஆராய்ச்சியின் முடிவுகள் நோயாளிகளுக்கு ஆராய்ச்சியின் இடையிலோ அல்லது முடிவிலோ தெரிவிக்கப்படும். இதில் ஏதேனும் பின் விளைவுகள் ஏற்பட்டால் அதை சரிசெய்ய சிகிச்சை அளிக்க தகுந்த உதவிகள் செய்யப்படும்.

இழப்பீடு

ஏதுமில்லை

ஆய்வு பற்றிய தகவல் பெற

கு.ஜெயந்திஸ்வரி,
முதலாம் ஆண்டு MDS, முதுநிலை மாணவி,
வாய் நோய் அறிதல் மற்றும் ஊடுகதிர் துறை,
தமிழ்நாடு பல் மருத்துவக் கல்லூரி மற்றும் மருத்துவமனை,
சென்னை-600 003.
தொலைபேசி: 9944414880

.....	.....
நோயாளியின் பெயர்	கையொப்பம்/கைரேகை
.....	.....
ஆராய்ச்சியாளர் பெயர்	கையொப்பம்

தேதி

INFORMED CONSENT FORM

STUDY TITLE:

"Serum Lipid profile and C-reactive protein as a prognostic marker in Oral submucous fibrosis"

Name: O.P. No:

Address:

Serial No:

Tel. no: Age / Sex :

I,_____ age_____years Exercising my free power of choice, hereby give my consent to be included as a participant in the study "Serum Lipid profile and CRP as a prognostic marker in Oral sub mucous fibrosis" I agree to the following:

- I have been informed to my satisfaction about the purpose of the study and study procedures including investigations to monitor and safeguard my body function.
- I agree to give my full participation of the study.
- I agree to cooperate fully and to inform my doctor immediately if I suffer any unusual symptom.
- I agree to report to the doctor for a regular follow–up as and when required for the research.
- I hereby give permission to use my medical records for research purpose. I am told that the investigating doctor and institution will keep my identity confidential.

Name of the patient Signature / Thumb impression

Name of the investigator Signature

Date

ஒப்புதல் படிவம்

ஆராயச்சியின் தலைப்பு

ஊணீரில் உள்ள கொழுப்பு அளவுகளின் விவரம் மற்றும் சி-ரியாக்டிவ் புரதம் அளவு மூலம் வாய் இறுக்க நோய் குணமடைதலை முன்கணிப்பு செய்தல் பற்றிய ஆய்வு.

பெயர்: புறநோயாளி எண்:

வயது/பால்: ஆராய்ச்சி சேர்க்கை எண்:

முகவரி

தொலைபேசி:

நான்..... வயது ..... என்னுடைய சுய நினைவுடனும் மற்றும் முழு சுதந்திரத்துடனும் இந்த மருத்துவ ஆராய்ச்சியில் என்னை சேர்த்துக் கொள்ள ஒப்புதல் அளிக்கிறேன்.

கீழ்காணப்படும் நிபந்தனைகளுக்குட்பட்டு நான் சம்மதிக்கிறேன்.

- நான் இந்த ஆராய்ச்சியின் நோக்கம் மற்றும் செயல்முறைகள் பற்றி முழுமையாக தெரிவிக்கப்பட்டுள்ளேன்.
- என் உடல்நலம் பாதிக்கப்பட்டாலோ அல்லது எதிர்பாராத வழக்கத்திற்கு மாறான நோய் அறிகுறிகள் தென்பட்டாலோ அதனை விலக்குவதற்கும் முழு உரிமை உள்ளதாக அறிகிறேன்.
- நான் ஏற்கனவே உட்கொண்ட மற்றும் உட்கொள்கின்ற மருந்துகளின் விவரங்களை ஆராய்ச்சியாளரிடம் தெரிவித்துள்ளேன்.
- என் மருத்துவ குறிப்பேடுகள் இந்த ஆராய்ச்சியில் பயன்படுத்திக் கொள்ள சம்மதிக்கிறேன்.
- இந்த ஆராய்ச்சி மையமும் ஆராய்ச்சியாளரும் என்னுடைய விவரங்கள் அனைத்தையும் இரகசியமாக வைப்பதாக அறிகிறேன்.

.....	.....	.....
நோயாளியின் பெயர்	கையொப்பம்	தேதி
.....	.....	.....
ஆராய்ச்சியாளர் பெயர்	கையொப்பம்	தேதி

CASE PROFORMA

DEPARTMENT OF ORAL MEDICINE AND RADIOLOGY

TAMIL NADU GOVERNMENT DENTAL COLLEGE AND HOSPITAL,

CHENNAI-3

.

Date: Serial No:

Name: O.P. No:

Age/Sex:

Address:

Phone No:

Occupation: Income:

Religion:

Centre: Department of Oral Medicine & Radiology
Tamilnadu Govt. Dental College & Hospital, Chennai – 600003.

Presenting complaint with duration:

Past Medical History:

Past Surgical History:

Past Dental History:Personal History:

A) Diet:

B) Tooth cleansing habits:

- Cleaning aids used:
- Frequency:

C) Smoking habit:

- Materials used:
- Frequency:
- Duration of the habit:

D) Chewing habit:

- Materials used:
- Frequency:
- Duration of the habit:

E) Other habits (alcohol, snuff):

Marital Status:

Menstrual History:

Family History:

GENERAL EXAMINATION

Appearance:

Stature:

Build:

Nutritional Status:

Structural alterations:

Deformities:

CONSTITUTIONAL SIGNS

Signs of Anemia:

Cyanosis:

Jaundice:

Clubbing:

Pedal edema:

VITAL SIGNS: Pulse rate:

Respiratory rate:

Blood pressure:

Temperature:

Height:

Weight:

BMI:

Lymph node examination:

CLINICAL EXAMINATION

Extraoral examination:

Facial symmetry:

Temparomandibular joint

INTRA-ORAL EXAMINATION:

Mouth opening (Interincisal distance):

Size and shape of the mouth:

Jaw movements:

- Teeth:
- Gingiva:
- Alveolar mucosa:
- Labial and Buccal mucosa:
- Hard palate
- Soft palate
- Pillar of fauces and Tonsils
- Tongue
- Floor of the mouth
- Retromolartrigone

Provisional Diagnosis

Investigations:

1) Laboratory investigations:

A) Blood:

RBC count:

Total WBC count:

Differential count: P L E

Haemoglobin%:

Peripheral smear:

Erythrocyte sedimentation rate:

Bleeding time:

Clotting time:

B) Urine:

Glucose:

Clinical Diagnosis:

Treatment Plan:

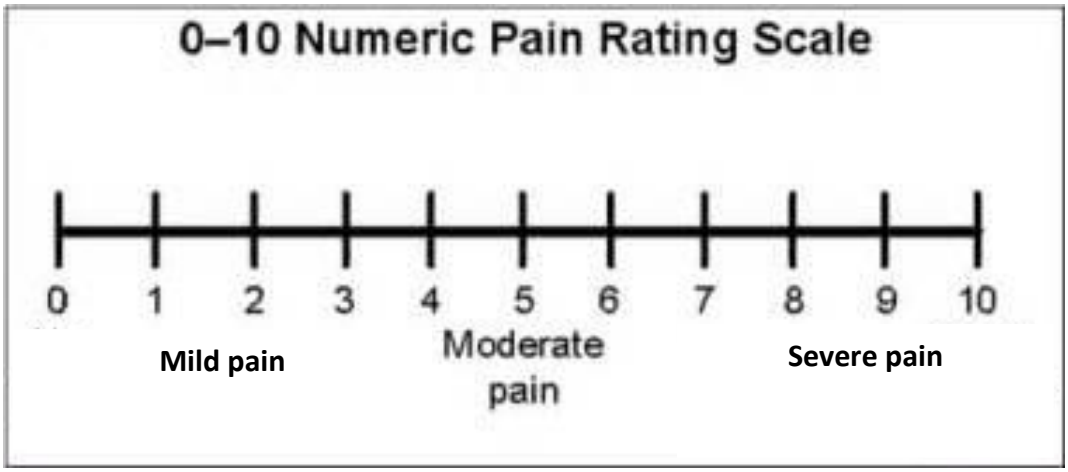
ASSESSMENT FORM

Name: Age/Sex: OP.No:
Serial No:

Diagnosis: Grade:

CLINICAL PARAMETER:

1. Pain or Burning Sensation (VISUAL ANALOG SCALE)



2. Mouth Opening (Interincisal Distance)

Before Treatment	After Treatment
……mm	…….mm

SERUM LIPID PROFILE & CRP EVALUATION:

Variable	Before Treatment	After Treatment
TC mg/dl		
TG mg/dl		
LDL mg/dl		
HDL mg/dl		
VLDL mg/dl		
CRP mg/L		

TRIPARTITE AGREEMENT

This agreement herein after the "Agreement" is entered into on this day……. between the Tamil Nadu Government Dental College and Hospital represented by its **Principal** having address at Tamil Nadu Government Dental College and Hospital, Chennai- 600 003, (hereinafter referred to as, 'the college') And **Prof. Dr.G.V.MURALI GOPIKA MANOHARAN MDS.,** aged 52 years working as **Professor** in Department of Oral medicine and Radiology at the college, (herein after referred to as the 'Principal Investigator') And **Dr. K. Jayanthiswari,** aged 43 years currently studying as final year **Postgraduate student** in the Department of Oral Medicine and Radiology, Tamil Nadu Government Dental College and Hospital, Chennai -3 (hereafter referred to as the 'PG and co- investigator')

Whereas the PG student as part of his curriculum undertakes to research on for which purpose the Principal investigator shall act as Principal investigator and the College shall provide the requisite infrastructure based on availability and also provide facility to the PG student as to the extent possible as a Co-investigator.

Whereas the parties, by this agreement have mutually agreed to the various issues including in particular the copyright and confidentiality issues that arise in this regard

Now this agreement witnessed as follows:

1. The parties agree that all the Research material and ownership therein shall become the vested right of the college, including in particular all the copyright in the literature including the study, research and all other related papers.

2. To the extent that the college has legal right to do go, shall grant to licence or assign the copyright so vested with it for medical and/or commercial usage of interested persons/entities subject to a reasonable terms/conditions including royalty as deemed by the college. The Royalty so received by the college shall be shared equally by all the three parties.
3. The PG/Research student and PG/Principal Investigator shall under no circumstances deal with the copyright, Confidential information and know- how-generated during the course of research/study in any manner whatsoever, while shall sole west with the college.
4. The PG student and Principal Investigator undertake not to divulge (or) cause to be divulged any of the confidential information or, know-how to anyone in any manner whatsoever and for any purpose without the express written consent of the college.
5. All expenses pertaining to the research shall be decided upon by the principal investigator/Co-investigator or borne sole by the PG student.(co-investigator)
6. The college shall provide all infrastructure and access facilities within and in other institutes to the extent possible. This includes patient interactions, introductory letters, recommendation letters and such other acts required in this regard.
7. The Principal Investigator shall suitably guide the Student Research right from selection of the Research Topic and Area till its completion. However the selection and conduct of research, topic and area research by the Student Researcher under guidance from the Principal Investigator

shall be subject to the prior approval, recommendations and comments of the Ethical Committee of the College constituted for this purpose.

8. It is agreed that as regards other aspects not covered under this agreement, but which pertain to the research undertaken by the PG student, under guidance from the Principal Investigator, the decision of the College shall be binding and final.
9. If any dispute arises as to the matters related or connected to this agreement herein, it shall be referred to arbitration in accordance with the provisions of the Arbitration and Conciliation Act, 1996.
10. In witness where of the parties herein above mentioned have on this the day month and year herein above mentioned set their hands to this agreement in the presence of the following two witnesses.

College represented by its

Principal

PG Student

Student Guide

Witnesses

1.

2.

MASTER CHART

S.No	Age (Yrs)	Sex	OSF Grade	Serum total Cholesterol mg/dl		Triglycerides mg/dl		HDL mg/dl		LDL mg/dl		VLDL mg/dl		CRP mg/l		Mouth opening mm		VAS score (Pain/burning)	
				PRE	POST	PRE	POST	PRE	POST	PRE	POST	PRE	POST	PRE	POST	PRE	POST	PRE	POST
1	26	M	III	184	191	67	223	60	56	121	116	13.4	44.54	1.2	1.6	20	28	6	4
2	27	M	IV a	129	139	54	283	38	36.9	81	45.7	10	56.7	0.78	1	13	22	7	3
3	29	M	III	188	225	202	231	45	65	108	140	40.44	46.26	5.9	3.6	22	31	5	1
4	28	M	I	165	168	91	118	24	33	116	136	18.1	23.6	0.6	0.6	36	42	3	0
5	29	M	III	195	236	268	287	32	37	132	158	53.6	57.4	3.4	2.4	19	26	6	1
6	32	M	III	155	162	62	94	48.8	52.2	83.8	110	12.5	15	1	0.7	19	23	7	4
7	29	M	III	189.7	198	255	270	28.9	32	109.8	120	51	58	3.2	2.1	24	30	6	1
8	35	M	III	149	154	62	91	30	35	97	105	12.4	18	5	4.8	22	26	5	2
9	22	M	III	180	170	85	109	49	56	119	90	16.9	19.2	2.9	2.3	20	27	5	1
10	26	M	III	167	172	155	163	28	42	117	105	30.9	33.6	0.7	1.3	24	30	2	0
11	33	M	III	147	140	188	139	29	35	87	99	37.8	30.6	0.8	1	24	28	6	1
12	47	M	I	198	210	365	320	51	35	149	148	72.9	64	1.7	1.3	37	42	3	0
13	22	M	II	139	168	68	62	40	52	91	111	13.6	12.3	0.4	0.2	27	32	3	0
14	31	M	III	169	175	49	48	54	50	113	116	9.7	9.6	1	1.9	16	27	6	1

15	26	M	II	198	205	86	44	32	40	146	147	17.4	8.7	0.6	0.8	27	31	3	0
16	51	F	III	197	216	93	176	38	42	175	234	18.5	35.2	6.7	1.9	20	26	5	1
17	23	M	III	162	169	58	124	50	52	96	93	11.6	24.8	0.1	0.3	19	26	6	1
18	23	M	I	180	184	86	110	63	68	113	120	17.24	19.2	2.2	2.6	36	43	3	0
19	28	M	III	198	266	247	387	31	36	150	165	49.4	77.4	6.2	2.5	20	28	5	2
20	24	M	II	148	152	50	65	47	54	81	96	10.5	16	0.8	1.9	26	31	3	0
21	34	M	IV a	127	125	45	37	50	55	76	65	9	7.4	0.8	0.6	14	20	7	3
22	43	M	III	165	187	126	138	38	42	102	116	22	27.5	3.2	2.3	24	29	5	1
23	27	M	I	172	177	82	79	30	43	98	115	16	15.8	1.6	1.4	37	44	1	0
24	23	M	III	130	142	57	68	44	54	73	84	11.3	13.4	1	0.9	22	27	4	2
25	41	M	III	196	230	135	154	51	60	141	144	26.9	29	0.8	0.4	23	28	3	2
26	36	M	II	198	206	152	166	35	48	130	152	30.3	33	4.3	2.6	27	32	1	0
27	23	M	III	168	182	44	62	22	46	95	92	9	13	0.5	0.6	23	27	6	2
28	39	M	III	126	135	58	112	44	62	94	108	12	17.6	1.8	0.8	20	26	5	2
29	36	M	II	145	158	85	108	50	52	114	108	18	20.8	2	1.4	26	30	4	0
30	29	M	III	142	152	60	90	48	56	86	98	11.5	18.6	3	1.8	24	30	4	1