STUDY OF ALTERATIONS OF LIPID PROFILE IN PATIENTS SUFFERING FROM ORAL SUB MUCOUS FIBROSIS

A Thesis Submitted to
Babu Banarasi Das University
for the Degree of

Doctor of Philosophy

in

Dental Sciences

by

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May, 2017

DECLARATION

I, hereby, declare that the work presented in this thesis, entitled **"Study of Alterations of Lipid Profile in Patients Suffering from Oral Submucous Fibrosis"** in fulfillment of the requirements for the award of Degree of Doctor of Philosophy of Babu Banarasi Das University, Lucknow is an authentic record of my own research work carried out under the supervision of **Dr. Anuj Maheshwari**.

I also declare that the work embodied in the present thesis is my original work and has not been submitted by me for any other Degree or Diploma of any university or institution.

Date: **Dr. Neeta Misra**

CERTIFICATE OF THE SUPERVISOR

This is to certify that the thesis, entitled **"Study of Alterations of Lipid Profile in Patients Suffering from Oral Submucous Fibrosis"** submitted by **Dr. Neeta Misra** for the award of Degree of Doctor Philosophy by Babu Banarasi Das University, Lucknow is a record of authentic work carried out by him/her under my/our supervision. To the best of my/our knowledge, the matter embodied in this thesis is the original work of the candidate and has not been submitted elsewhere for the award of any other degree or diploma.

Signature
Dr. Anuj Maheshwari

Signature
Dr. Shivani Pandey

ACKNOWLEDGEMENT

"The price of success is hard work, dedication to the job at hand, and the determination that whether we win or lose, we have applied the best of ourselves to the task at hand."

At the very outset, I bow my head to the Almighty God, who blessed me with his worthy blessings, bestowed me with his kind grace, provided me necessary strength, courage and good health to reach this stage and made it possible for me to bring out this manuscript.

Carrying out this mammoth task had been neither easy nor ephemeral. No good work can be done bereft of helping hands.

Now as this excursion halts to its final destination, the mind envisions, the heart getting nostalgic at the same time with fond remembrance of all those moments and matters have structured the ebullient path leading to its ultimate goal.

At this pleasure filled juncture, I find myself overwhelmed and unable to distance myself from the temptation of acknowledging all those who paved the path for my success.

I would like to thank Dr A.K Mittal, Vice-chancellor, BBD University, Lucknow and Dr. S. Ahmad Ali, PhD co-ordinator, BBD University, Lucknow, as without their streamlined guidance, constant and vigilant supervision, keen and diligent interest, and immutable encouragement, this imposing work would not have seen the light of the day. The erudition and critical judgment with which they guided my thesis was not only inevitable but vital also. Some debts can never be repaid and one has to remain obliged to the last day.

It is my proud privilege, profound luck and immense delectation to express my heartfelt desire of everlasting gratitude, soaring high within, for my esteemed teachers and guide Dr. Anuj Maheshwari, Professor & Head, Dept of Internal Medicine, BBDCODS, BBD University, Faizabad Road, Lucknow, co-guide Dr. Shivani Pandey, Additional Professor, Department of Biochemistry, K.G.M.U., Lucknow,

I express my gratitude to Dr. Vivek Govila, Principal, Babu Banarasi Das College of Dental Sciences, Lucknow for giving me co-operation and allowing me to use the facilities of the college during this work.

I am deeply indebted to Dr. Deepak U, Dr. Shiva Kumar G.C., Dr. Priya Singh, Dr. Saurabh Srivastava, Dr. Sumalatha, Dr. Puja Rai, Dr Akansha Yadav, Dr. Arpita Mohan, Dr. Amanpreet Kaur & Dr. Gargi Saran from Department of Oral Medicine and Radiology, BBDCODS, BBD University, Lucknow for their priceless suggestions, constant counseling and affectionate attitude that helped me during the preparation of this thesis.

I would like to thank My Parents, My In-Laws, My husband Dr. Pradyumna Misra & sons Ashwin & Abhinav who supported me to remain occupied while completing the project; they extended required support and strength even at the cost

of their sufferings. Thank you for all that you did for me, without your support I could not have reached here. I thank my family for their unbounded love and affection, from the bottom of my heart.

I would like to thank and appreciate the efforts general pathology technicians and all the non-teaching staff in the department of Oral Medicine and Radiology, for helping me out in this study.

As a lot burns without flame, I would be failing in my duty as a kith, if I don't recognize the magnanimous contributions made by I thank them for providing me all kind of support and encouragement during my study and this project. I thank my family for their unbounded love and affection, from the bottom of my abyssal heart.

I beg forgiveness from those, whose names I have inadvertently missed but they should find solace that their knowledge has been extended to others by way of this thesis.

Humbly and honestly all the words of knowledge and wisdom belong to others and mistakes are mine and mine alone.

Date: ***Dr. Neeta Misra***

PREFACE

It is a matter of immense pleasure and gratification that I am presenting my work study of alteration of serum lipid profile in oral sub mucous fibrosis patients.

I, as oral physician, over the years have been encountering oral sub mucous fibrosis patients and their likely risk of future malignancies. Early detection of the potentially malignant disorders and also preventing them from malignant transformation seems to be the best possible treatment outcome and overall prognosis.

21st century has seen tobacco, gutkha abuse at its peak and we know in oral cancer development, tobacco plays an etiological role. Tobacco usage leads to the uptake of many hazardous compounds and their metabolites.

In recent years, emphasis has been placed on detecting molecular markers from body fluids such as saliva, urine and other for detecting & predicting oral cancer progression. Biochemical studies in evaluation of cancer have shown that various substances alter quantitatively in the serum during tumour development and are referred to as tumour marker.

Altered lipid profile patterns have been associated with oral pre-malignancies and malignancies because lipids play a vital role in the maintenance of cell integrity. Tobacco carcinogens induce generation of free radicals and reactive oxygen species, which cause lipid peroxidation. Because of the lipid peroxidation, there is a greater utilization of lipids for new membrane biogenesis.

Present study was aimed To assess the variation of serum lipid profile values, between the oral tobacco habit group and oral submucous fibrosis study was conducted in Department of Oral Medicine and Radiology of Babu Banarasi Das College of Dental Sciences, Lucknow

100 subjects out of with clinically diagnosed Oral Submucous Fibrosis were enrolled and 100 controls with no apparent lesions of the oral mucosa having tobacco habit. Serum lipid, including (i) Total cholesterol, (ii) LDL cholesterol (LDLC), (iii) HDL cholesterol (HDLC), (iv) VLDL cholesterol (VLDLC) and (v) Triglycerides, (vi) TC/HDLC ratio, (vii) LDL/HDLC ratio were analyzed using spectrophotometry kits. The result revealed that decrease in plasma total cholesterol, triglycerides, HDL, LDL and VLDL in the subjects with the Oral potentially malignant disorders as compared to the controls was statistically significant ip between serum lipid profile and OSMF This study supports the evidence of an inverse relationship between serum lipid profile and OSMF lipid status may be used as a useful indicator for early changes occurring in neoplastic cells and serve as one of the early diagnostic aids in oral potentially malignant disorders.

I have tried to summarize and concise the text without missing important features of oral sub mucous fibrosis. Various terminologies suggested for the disease have been compiled in chapter two. Pathogenesis has been described in detail in same chapter.

A unique feature of this book is that all classifications suggested for this disease has been collected and described in one chapter.

All the factors related to lipid profile and carcinogenesis are discussed in chapter five in detail in easy language.

In the present study, I have summarized the role of lipids and thrown light on possible association between the serum lipid profile and OSMF. Lipid profile can be added on to the other test as an additional indicator and can serve as another evaluating parameter to observe early changes.

Although every attempt has been made to avoid any error or controversy shortcomings are inevitable.

-Neeta Misra

ABBREVIATIONS

DNA	::	Deoxyribonucleic acid
ACETYL-CoA	::	Acetyl coenzyme A
LDL-C	::	Low density lipoprotein cholesterol
HDL-C	::	High density lipoprotein cholesterol
TC	::	Total cholesterol
VLDL	::	Very low density lipoprotein
BMF	::	Buccal Mucosal Fibroblasts
CD	::	Cluster of differentiation
PGs	::	Prostaglandins
ANE	::	Areca nut extract
BQ	::	Betel Quid
CTGF	::	Connective tissue growth factor
DM	::	Diabetes Mellitus
ECM	::	Extracellular Matrix
HLA	::	Human leukocyte antigen
INF-γ	::	Interferon Gamma
IU	::	International unit
LOX	::	Lysyl oxidase activity
MCH	::	Major Histocompatibility Complex
MMPs	::	Matrix Metalloproteinases
OSMF	::	Oral Submucous Fibrosis
PAI	::	Plasminogen activator inhibitor

PCP	::	Procollagen Proteinases
Plg	::	Plasminogen
RCT	::	Randomized Control Trial
ROS	::	Reactive Oxygen Species
TGF	::	Tumor growth factor
TIMP	::	Tissue inhibitor of matrix metalloproteinase
tPA	::	Tissue plasminogen activator
uPA	::	Urokinase plasminogen activator
WHO	::	World Health Organisation
HPV	::	Human papilloma viruse
LoH	::	Loss of heterozygosity
Rb	::	Retinoblastoma
mRNA	::	Messenger ribonucleic acid
OL	::	Oral leukoplakia
GA	::	General anaesthesia
LA	::	Local anaesthesia
CO	::	Carbon dioxide

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CHAPTER-1

INTRODUCTION

In recent years, head and neck cancer has become one of the leading cause of morbidity and mortality.[1] Oral carcinoma is one of the most prevalent cancers and is one of the 10 most common causes of death. Oral cancer is most commonly preceded by clinically definable potentially malignant disorders. A variety of potentially malignant disorders mainly includes Oral submucous fibrosis, leukoplakia, erythroplakia, oral lichen planus.[2] They are the indicators of risk of likely future malignancies. Early detection of the potentially malignant disorders and preventing them from malignant transformation seems to be the best tool that can improve the treatment outcome and prognosis.[3]

In recent years, emphasis has been placed on detecting molecular markers from body fluids such as saliva, urine and others, for detecting, predicting the prognosis, and monitoring the oral cancer progression.[4] The idea of screening the patients by blood based tests is appealing in various aspects including its ease, economic advantage and amendable to repeated sampling.

Biochemical studies in evaluation of cancer have shown that various substances alter quantitatively in the serum during tumor development and are referred to as tumor markers. So if the biochemical changes occur even before frank

cancer has occurred, we can predict even in oral potentially malignant disorders whether a particular is at risk or not.[5]

In oral cancer development tobacco plays an etiological role. The recent decades have seen a massive global increase in tobacco use. Tobacco usage leads to the uptake of many hazardous compounds and their metabolites extracted from burnt tobacco. Usage of only tobacco/ tobacco related products is responsible for causing various potentially malignant disorders.[6]

Potentially malignant disorders is a term used to refer to all clinical presentations that carry a risk of cancer.[7] Malignant transformation rate of Oral Leukoplakia, a premalignant lesion is 1% to 2 % over 5 years and oral submucous fibrosis (OSMF) have malignant transformation rate 2.3% - 7.6% in 10-17 years.[8]

The change in lipid profile has long been associated with malignancies, as neoplastic disease is related to new growth, there is greater utilization of lipids for new membrane biogenesis. Lipids are major cell membrane components essential for various biological functions including cell growth and division of normal and malignant tissues, maintenance of the structural and functional integrity of all biological membrane activity of membrane-bound enzymes and stabilization of DNA helix.[1]

Cholesterol is an amphipathic lipid and is an essential component of all cell membranes and of the outer layer of plasma lipoproteins. It is present in tissues and in plasma lipoproteins either as free cholesterol or combined with a long-chain fatty acid, as cholesterol ester. It is synthesized in many tissues from acetyl-CoA and is ultimately eliminated from the body in the bile as cholesterol or bile salts.

Lipoproteins transports free cholesterol in the circulation, where it readily equilibrates cholesterol in other lipoproteins and in membranes.[5]

Cholesterol ester is a stored form of cholesterol found in most tissues. It is transported as cargo in the hydrophobic core of lipoproteins. Receptor-CK is a cell surface cholesterol sensor present in various human organs. It regulates various genes invoved in cholesterol homeostasis, cell growth and cell death through a 47 kDa transcription factor, as well as through other transcription factors. Of the lipoprotein fractions, LDL-C most clearly reflects the decrease in total cholesterol. The role of HDL-C and triglycerides in explaining the overall pattern of total cholesterol change is less clear.[5] Fundamentally the development of a malignancy requires the uncontrolled and excessive proliferation of cells. These newly forming cells would need many basic components well above the normal limits, used in physiologic process.

One such component is lipid which form major cell membrane components essential for various biological functions including cell growth and division of normal and malignant tissues. The increased requirements of lipids to fulfill the need of these new cells would be expected to diminish the lipid stores.[9] Regulation of cholesterol is mediated by lipoproteins receptors. Plasma triglycerides and cholesterol are packed into lipoproteins for transport. There are two main categories: high density lipoprotein (HDL) being associated with carrying "cholesterol" out of the blood system and low density lipoprotein (LDL) which transport 75% of plasma cholesterol. Cell receptors metabolize circulating LDL and clear nearly 80% of it from the body, while the rest of is associated with deposition of cholesterol on the

walls of arteries.[10] The excessive use of tobacco products has been associated with various lesions in the oral cavity.

It is believed that tobacco carcinogens induce generation of free radicals and reactive oxygen species responsible for the high rate of oxidation / peroxidation of polyunsaturated fatty acids. This peroxidation further releases peroxide radicals. This affects essential constituent of cell membrane and are thus involved in carcinogenesis. Because of the lipid peroxidation, there is greater utilization of lipids including TC, lipoproteins, and triglycerides for new membrane biogenesis.[3] Cells fulfill these requirements from circulation either by synthesis through the metabolism or from degradation of major lipoprotein fraction such as VLDL, LDL, or HDL.[5]

Changes in the circulatory cholesterol level have been associated with etiology of breast cancer and colorectal cancer,[7] however only few studies are reported on serum lipid profile alteration in potentially malignant disorders.

Therefore, a need was felt to conduct a study to evaluate the variation in serum lipid profile in subjects with oral tobacco habits and in oral potentially malignant disorders which may be helpful in understanding pathogenenesis, early identification and their conversion into malignancy and thus improving the prognosis and to evaluate whether alterations in serum lipid profile levels have a diagnostic or prognostic role in early diagnosis of oral potentially malignant disorders.

CHAPTER-2

REVIEW OF LITERATURE

2.1 Definition

The NIH's National Cancer Institute (NCI) (http://www.cancer.gov /dictionary/?CdrID=45618), describes biomarkers in its dictionary of cancer termed it as "A biological molecule found in blood, other body fluids, or tissues that is a sign of a normal or abnormal process, or of a condition or disease. A biomarker may be used to see how well the body responds to a treatment for a disease or condition. Biomarkers are also called molecular marker and signature molecules."

2.2 Biomarkers of OSCC

Lipids are major cell membrane components essential for various biological functions including cell growth and division of normal and malignant tissues. Altered circulatory cholesterol levels have been implicated in the etiology of various cancers like breast cancer and colorectal cancer.[1] It has been proposed that lower levels of blood cholesterol are associated with an increased risk of cancer occurrence.[4] However, the reports on altered lipid levels in oral malignancies and potentially malignant disorders (PMDs) are few and conflicting.[6,9]

In oral malignancies, the blood cholesterol undergoes significant and early changes. Because of rapidly proliferating tumor cells, there is reduction in blood cholesterol levels due to increased demand. There are many theories put forth explaining the association of altered lipid profile and potentially malignant or malignant disorders.[10]

2.2.1 Diagnostic utility of Biomarkers

The term precancer, precancerous lesion, premalignant, intraepithelial neoplasia and potentially malignant have been used in the international literature to broadly describe clinical presentations that may have potential to become cancer. They all convey the concept of two step or multistep process of cancer development.[7]

Biochemical studies in evaluation of cancer have shown that various substances alter quantitatively in the serum during tumor development and are referred to as tumor markers. So, if the biochemical changes occur even before frank cancer has occurred, we can predict even in oral precancerous lesions and conditions whether a particular individual is at risk or not.[2]

Fundamentally the development of malignancy requires the uncontrolled and excessive proliferation of cells. These newly forming cells would need many basic components well above the normal limits, used in physiological process. One such component is lipid which is one of the major cell membrane components essential for various biological functions including cell division and growth of normal and malignant tissues. The increased requirements of lipids to fulfill the need of these new cells would be expected to diminish the lipid stores.[9]

Cells fulfill these requirements from circulation either by synthesis through the metabolism or from degradation of major lipoprotein fraction such as VLDL, LDL or HDL.[5]

In recent years, emphasis has been placed on detecting molecular markers from body fluid such as saliva, urine and others for detecting cancer, predicting prognosis and monitoring disease progression. The idea of screening and following patients with malignancy by blood tests is appealing from several points of view including its ease, economic advantage, non invasiveness and possibility of repeated sampling.[10]

2.3 ORAL SUBMUCOUS FIBROSIS

2.3.1 Introduction

A condition resembling OSMF was described as early as "600 BC'' by Sushruta and it was named as "VIDARI" having features of progressive narrowing of the mouth, depigmentation of oral mucosa and pain on taking food.[11]

OSMF was first described by Schwartz in 1952 while examining five Indian women from Kenya, to which he ascribed the descriptive term "atrophia idiopathica (tropica) mucosae oris". Later in 1953, Joshi from Bombay (Mumbai) coined the term "submucous fibrosis of palate and pillars".[12]

Different authors have used different terms for OSMF like; idiopathic scleroderma of the mouth (Su *et al.* in 1954), idiopathic palatal fibrosis (Rao *et al.* in 1962), sclerosing stomatitis (Behl *et al.* in 1962), and Juxta epithelial fibrosis (Pindborgh *et al.* in 1966).[13]

In 1966, Pindborgh defined OSMF 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 a juxta-epithelial inflammatory reaction followed by a fibro elastic change of the lamina propria, with epithelial atrophy leading to stiffness of the oral mucosa and causing trismus and inability to eat."[13]

Since then several authors coined various terminologies for this condition. These terminologies are summarized by Abrol BM[14] (1977) which are as follows:

- Lal (1953) – diffuse oral submucous fibrosis

- Su (1954) – idiopathic scleroderma of mouth
- Desa (1957) – submucous fibrosis of palate and cheek
- George (1958) – submucous fibrosis of palate and mucous membrane.
- Rao (1962) – idiopathic palatal fibrosis
- Behl (1962) – sclerosing stomatitis
- Pindborg and Sirsat (1964) – oral submucous fibrosis.
- Goleria (1970) – subepithelial fibrosis
- Abrol *et al.* (1972) – idiopathic oral fibrosis.

Of all the terminologies in the literature the term "Oral Submucous Fibrosis" is currently widely used and it implies the nature of the condition in a simplified form.

2.3.2 Epidemiology

Numerous published reports on OSMF suggest that the disease predominantly affects people of South East Asian origin. It affects between 0.2% and 1.2% of urban population in India. The cases have also been reported among Indians living in Kenya, Malaysia, Uganda, South Africa, Fiji Islands, and UK. Sporadic cases have been reported in other ethnic groups from countries such Taiwan, Nepal, Thailand, South Vietnam and Srilanka.[15]

An epidemiological assessment of OSMF among Indian villagers, based on baseline data, recorded a prevalence of 0.2% (n=10071) in Gujarat, 0.4% (n=1027) in Kerala, 0.04% (n=10169) in Andhra Pradesh, and 0.07% (n=20388) in Bihar.[7] The

prevalence among 101,761 villagers in the state of Maharashtra (central India) was 0.03%. In a 10-year follow up study of oral precancer, Gupta et al. in 1980 observed that the incidence of OSMF in Ernakulum, Kerala was 8 for men and 19 for women per 100,000.[16]

There is a wide variation in the prevalence figure between different studies, and may probably be due to differences in the clinical criteria for diagnosis, differences in abusive habits, differences in frequency of habit, differences in geographic distribution and differences in races and other causes. While some investigators adhere to the initial signs and symptoms, other looked for fibrous bands as the diagnostic criterion.[17]

Worldwide estimate in 1996 indicate that 2.5 million people are affected by the disease. In 2002, the statistics for OSMF from Indian subcontinent alone was about 5 million people.[18]

Age

Most of the patients with OSMF are in the 20 to 40 year age group. This condition exhibits some regional variations in the age distribution: for instance in Pune, the mean age (37 years) of 24 patients was lower compared to mean age (52 years) of 64 patients in Ernakulam.[19] In a study in Delhi the authors have observed that men were more affected in the younger age group compared to women, in the ratio of 2.2:1, whereas females were more susceptible in the older age group.[20] In Bhavnagar, Gujarat, the mean age was 21.9 year (age range 15-24 yr).[21] In Aligarh on 82 cases of OSMF, the mean age was 41.07 years (age range 15-74 years).[22] In a cross-sectional study on total of 1000 OSMF cases in Central India the mean age of

all cases affected with OSMF was 28.8.[23] In Lucknow, Bombay, and Trivandrum, the average age of the men was 50.5 years (range 22 to 79); that of the women was 43.5 years (range, 25 to 70).[24]

Sex

Oral submucous fibrosis affects both sexes. Male predominance has been observed in several studies. In Lucknow, Bombay, and Trivandrum among 115 patients 81 were men, and 37 were women.[24] In Bhavnagar, Gujarat, 58 out of 60 OSMF subjects were men.[21] In Aligarh of 82 cases of OSMF, the male to female ratio was 1.34:1.[22] In another study in South Africa out of 122 patients of OSMF 116 were females and 6 were males with ratio of 19.3:1.[25] In another study on Oral submucous fibrosis in Patna, out of 157 cases, male: female ratio was 2.7:1.[26] In a hospital based cross-sectional study in South India on 2017 patients ,63.75% were males and 36.25% were females.[27]

2.3.3 Aetiology

Although various etiological agents are proposed, the exact etiology of oral submucous fibrosis has not yet been identified. Various etiological agents and predisposing factors have been studied and current evidence suggests that arecoline in areca nut plays a major role in initiating the disease process. However role of different etiological agents and factors so far studied are:

- Chillies (capsaicin)
- Genetic predisposition (association with specific HLA antigens)
- Carcinogenicity of tobacco and areca nut

- Immunologic factors
- Nutritional factors (deficiencies of iron, zinc and essential vitamins.)
- Autoimmune process

and more recently collagen related genes are implicated in the susceptibility and pathogenesis of oral submucous fibrosis.

Thus etiology of OSMF still remains obscure. In past, many authors have proposed various hypotheses with a multifactorial origin for this particular condition.[17]

Areca nut chewing

OSMF is thought to be a disease of collagen metabolism secondary to betel nut usage. The betel quid is placed in the buccal vestibule for about 15 minutes to an hour and repeated several times a day, which leads to constant contact between the mixture and oral mucosa. The alkaloids from the quid are absorbed into the mucosa and undergoes metabolism. Micro trauma produced by the friction of coarse fibers of areca nut also facilitates diffusion of the alkaloids into the sub epithelial connective tissue resulting in juxtaepithelial inflammatory cell infiltration.[28]

Betel nut contains alkaloids, flavonoids, and copper, all of which in turn are thought to affect collagen synthesis and breakdown. Four alkaloids: arecoline, arecaidine, guvacine, and guvacoline, are all involved in stimulating fibroblasts to produce collagen. In addition, flavonoids (tannins and catechins) are found to inhibit collagenase, decreasing collagen breakdown. Betel quid also causes localized mucosal inflammation, causing a recruitment of activated T cells and macrophages locally, resulting in an increase in cytokines and tumor growth factor beta (TGF-β).

TGF-beta is found to significantly increase collagen production by activating procollagen genes, elevating procollagen proteinase levels, and upregulating lysyl oxidase activity (LOX). TGF-beta also inhibits collagen degradation by activating the tissue inhibitor of matrix metalloproteinase (TIMP) gene and activating the plasminogen activator inhibitor (PAI). Activation of TIMPs and PAI genes in turn result in a decrease in collagenase activity and thuss in turn resulting in a decrease in collagen degradation. Areca nut has a high copper content and copper has been found to stimulate LOX, an enzyme essential to the final cross linking of collagen fibers. All of this results in a significant increase in collagen production and decrease in collagen breakdown.[29]

Sinor P.N. *et al.* (1990) conducted a study on OSMF subjects and equal number of controls in Bhavnagar, Gujarat to elucidate the etiology of OSMF and observed that in the study group 98% chewed areca nut regularly in one form or the other whereas among controls 35% chewed areca nut, giving an overall relative risk of 109.6. Areca nut chewing was practiced most commonly in the form of mawa: a mixture containing mainly areca nut (over 90% by weight), some tobacco, and a few drops of lime. Mawa chewers and those who chewed mawa along with other chewing habits showed very high relative risks. The relative risks increased with increase in the frequency as well as the duration of chewing habits.[21]

Maher *et al.* (1991) conducted a case-control study on relationship of chewing and smoking habits and oral submucous fibrosis (OSMF) in Karachi in 1989/90, and found that areca nut had the greatest role in the etiology of OSMF followed by pan and then pan with tobacco. They also noted that the frequency and

quantity of areca nut consumed was a more important factor than the duration of the habit.[30]

Shah N. and Sharma P.P. (1998) in their study on 236 consecutive cases of OSMF found that chewing of areca nut/quid or pan masala (a commercial preparation of areca nuts, lime, catechu and undisclosed colouring, flavoring and sweetening agents) was directly related to OSMF.[20]

Saraswathi T.R. *et al.* (2006) in a cross-sectional study in South India in 2006 observed that OSMF was most prevalent lesion among those who chewed panmasala or gutkha or betel quid with or without tobacco.[27]

Chilies

The predominant occurrence of oral submucous fibrosis among Indians who season their food with chilies, the frequent intolerance to spicy food, and the vesiculation following the intake of food laced with chilies have suggested the role of chilies in OSMF. Chilies can damage the cells of the mucosa and it probably causes chronic inflammation, which leads to the formation of excessive fibrosis.[26]

Cox S.C. and Walker D.M. (1996) in their study observed that along with nutritional deficiencies, the use of chilies, betel nut chewing and immunological processes have a major etiological role in OSMF.[11]

Sami A.M, K.K. Chaubey *et al.*[26] (2006) stated that chilies have an indirect effect on the pathogenesis of OSMF, as hypersensitivity to chilies is the common factor in the development of OSMF as it causes chronic inflammation, which leads to the formation of excessive fibrosis.

Nutritional deficiency

Nutritional deficiencies have been mentioned as possible aetiological factors. Mucosal changes similar to those in vitamin B and iron deficiency are seen in oral submucous fibrosis and seem analogous to sideropenic dysphagia.[15] The normal maturation of the epithelium is dependent upon an iron containing enzyme cytochrome oxidase. In iron deficiency anemia, level of this enzyme is very low and so there is atrophy of epithelium and lack of maturation. Due to initial burning sensation, vesiculation, fibrosis and trismus there is lack of consumption of normal diet which initiates anemia. Thus, the relationship of iron deficiency anemia with OSMF is a vicious cycle.[31]

PR Murte *et al.*[32] (1995) hypothesized that this condition was an Asian version of sideropenic dysphagia wherein the chronic nutritional deficiency leads to mucosal susceptibility to irritants such as chilli and areca nut use.

Akansha yadav et al [33] (2015) conducted a study with 100 subjects ,50 each case and control and concluded that serum zinc , copper, and iron levels could be used as a potential prognostic and diagnostic markers in OSMF patients.

Immunologic and genetic predisposition

Immunologic process is believed to play a role in the pathogenesis of OSMF. Auto antibodies to gastric and parietal cells, as well as thyroid microsomal, antinuclear, reticulin, and anti-smooth muscle antibodies have been found in 65% of patients with the disease.[15]

Haque MF *et al.* (1997) investigated the presence and distribution of inflammatory cells and MHC class II antigen expression by epithelial and

immunocompetent cells using a three-stage immunoperoxidase method on frozen sections. Thirty OSMF tissue specimens and ten normal buccal mucosa were studied and compared. The predominant cell populations detected in normal tissues were CD3, CD4 and HLA-DR-positive cells. The increase in CD4 and cells with HLA-DR were observed in OSMF tissues and this suggests that most lymphocytes are activated and that the number of Langerhans cells is increased and suggest an ongoing cellular immune response that results in an imbalance of immunoregulation and an alteration in local tissue architecture.[34]

Haque M.F. *et al.*[35] (2001) have investigated the effect of intralesional INF-γ on the fibrosis of OSMF patients and the immunohistochemical analysis of pre and post treatment inflammatory cell infiltrates and cytokine levels in the lesional tissue. They noted that the increased collagen synthesis in response to arecolines was inhibited in the presence of INF-γ.

Chen H.M. *et al.*[36] (2004) in their study on 135 Taiwanese patients with OSMF, concluded that some areca quid chewers with particular HLA phenotypes and haplotypes are prone to have OSMF. In addition, some particular HLA haplotypes may play important roles than the individual HLA phenotypes for the genetic susceptibility to OSMF.

2.3.4 Pathogenesis

The role of the constituents of areca nut in the pathogenesis of OSMF has been studied in detail over last two decades. It is apparent that fibrosis and hyalinization of subepithelial tissues account for most of the clinical features encountered in this condition. Moreover, substantial amount of research on elucidating the etiology and pathogenesis appear to have been focused on changes in

the extracellular matrix (ECM). It is logical to hypothesize that the increased collagen synthesis or reduced collagen degradation as possible mechanisms in the development of the disease. There are numerous biological pathways involved in the processes and, it is likely that the normal regulatory mechanisms are either down regulated or up regulated at different stages of the disease.[18]

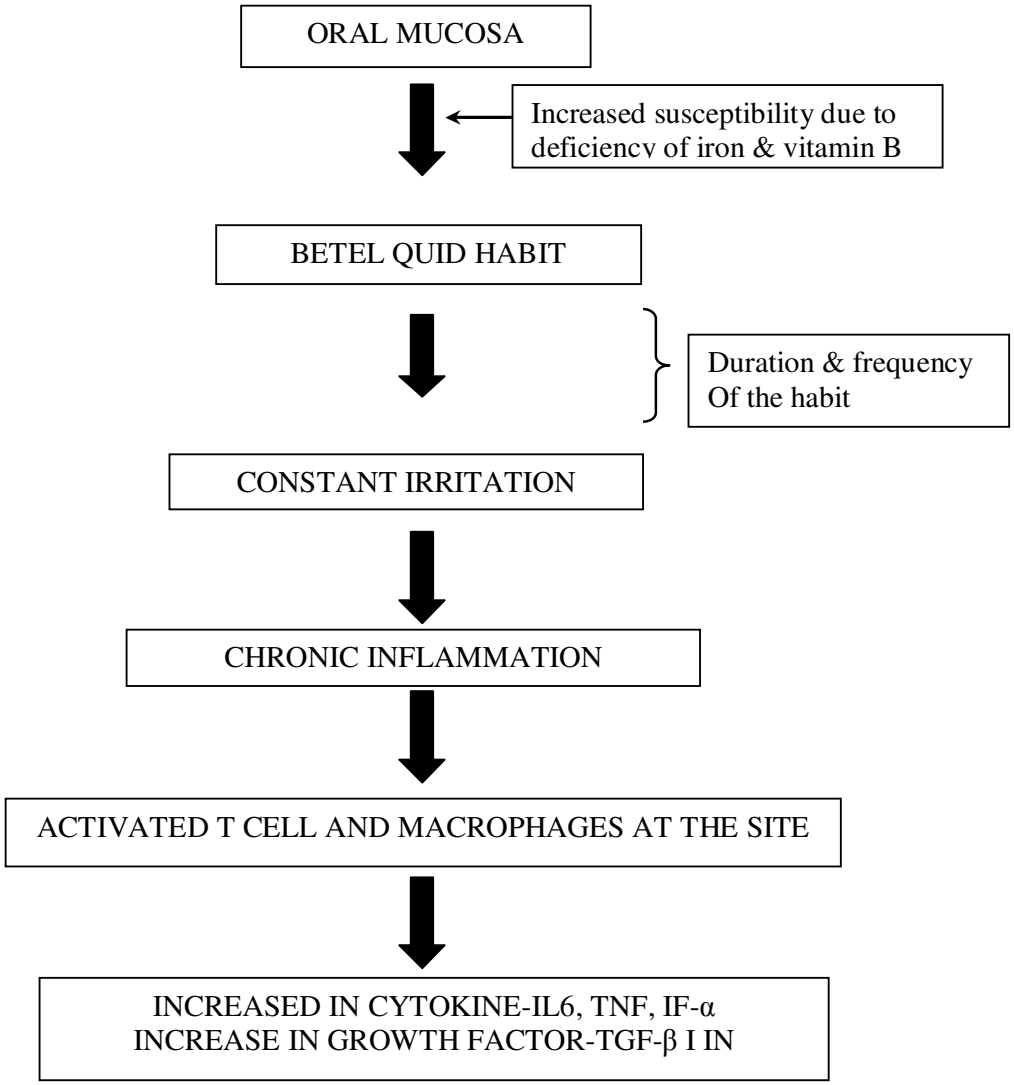

Figure 1: Initial events of the disease process of oral mucosa which is in direct contact with the betel quid due to the habit, is the site of constant irritation (Rajalalitha and Vali, 2005).[18]

Rajalalitha and Vali (2005)[18] discussed the molecular events in the pathogenesis of OSMF & have observed that the exact mechanism is not known. The flavonoids components of areca nut have been found to have some direct influence on collagen metabolism. It has been found that alkaloid exposure of buccal mucosal fibroblasts results in the accumulation of collagen. A decreased degradation of collagen due to increased cross-linking of the fibers and reduced collagenase activity are found in OSMF mucosa compared to normal oral mucosa. This evidence implies that OSMF may be considered a collagen-metabolic disorder resulting from exposure to areca nuts.

The chewing habit varies among individuals, but usually the BQ is placed in the buccal vestibule for about 15 min to an hour and repeated five to six times a day. There is constant contact between the mixture and oral mucosa. The alkaloids and flavonoids from the BQ are absorbed and undergoes metabolism. These constituents and their metabolites are a source of constant irritation oral tissues. In addition to the chemical irritation from BQ constituents and their metabolites, the coarse fibers of areca nut also cause mechanical irritation to the oral mucosa. Furthermore, the Micro trauma produced by the friction of coarse fibers of areca nut also facilitates the diffusion of BQ alkaloids and flavonoids into the sub epithelial connective tissue, resulting in juxtaepithelial inflammatory cell infiltration. Any external factor, which causes any form of injury to tissue, can elicit a protective inflammatory process. Over a period of time, due to persistent habit, chronic inflammation sets in at the site. Initial irritation leads to further atrophy and ulceration of the mucosa.[18]

Inflammation is characterized by the presence of activated T cells, macrophages, *etc*. There is an elaboration of various chemical mediators of inflammation, especially prostaglandins (PGs) plays an essential role. PGs secretion by oral keratinocytes in response to areca nut extract (ANE) has been shown. Aberrant and persistent tissue inflammation is crucial for the occurrence of cancer and tissue fibrosis. Thus, it can be considered that induction of oral mucosal inflammation by BQ ingredients to be a critical event in the pathogenesis of OSMF. Cytokines like interleukin 6, tumor necrosis factor (TNF), interferon a, *etc.* and growth factors like TGF-b are synthesized at the site of inflammation. Increased susceptibility among individuals who are anemic due to iron or vitamin B12 deficiencies has been demonstrated. This could be due to increased fragility of the mucosa by which there is more BQ absorption.

TGF-β1 is a key regulator of ECM assembly and remodeling. The action of TGF-b on the genes implicated in the formation and degradation of the ECM is mostly exerted at the transcriptional level through ill-defined intracellular pathways. The molecular events are discussed in two main sections: collagen production pathway and collagen degradation pathway, as regulated by TGF-β and the flavonoids present in areca nut.[18]

Collagen production pathway

There are three main events in this pathway; (1) activation of procollagen, (2) elevation of procollagen proteinase levels, (3) up regulation of lysyl oxidase (LOX activity).

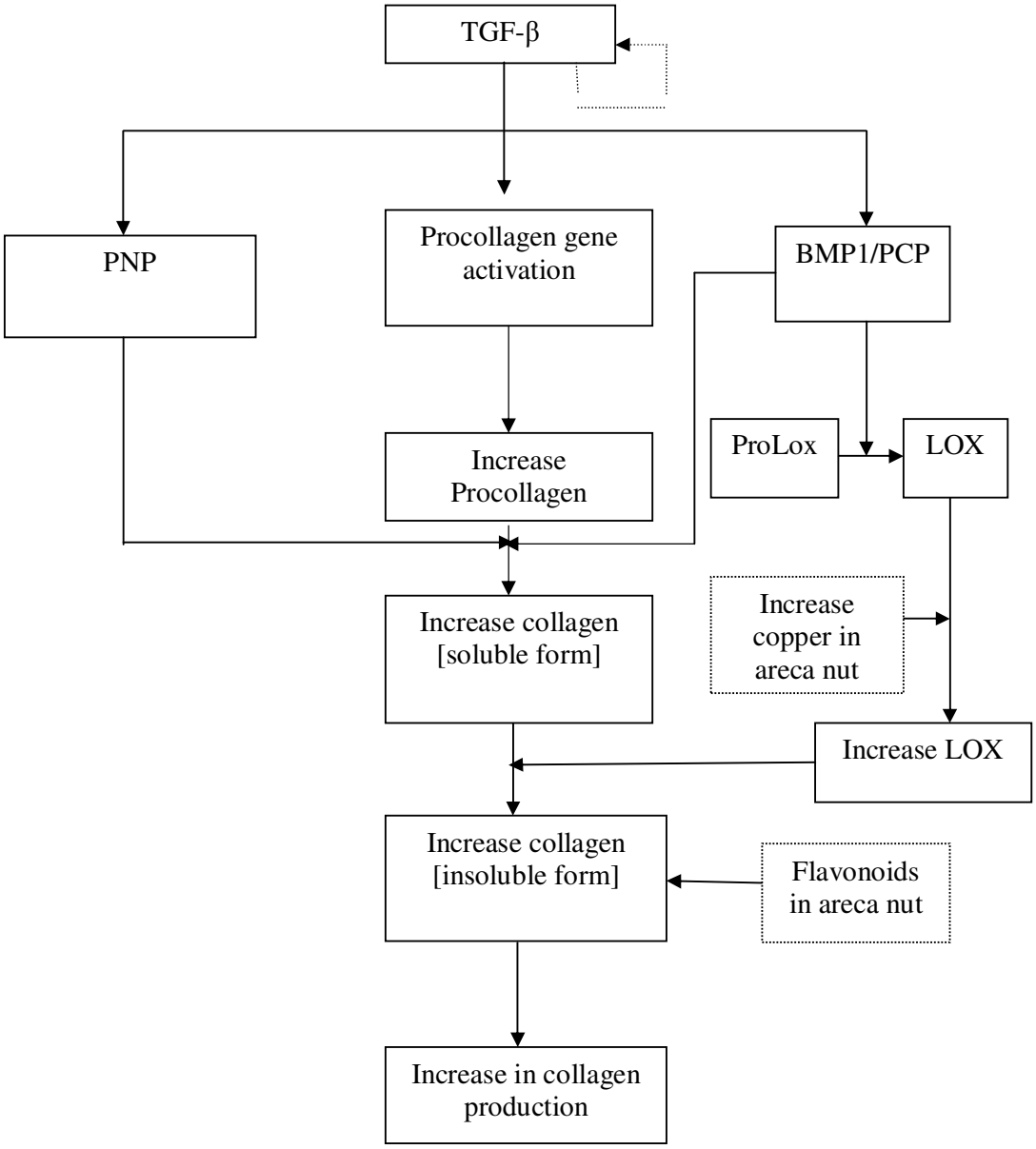

Figure 2: Collagen production pathway as regulated by TGF-β: TGF-β is a growth factor, which has autocrine activity. This activates the procollagen genes, resulting in production of more pro-collagen. It also induces the secretion of PCP and PNP, both of which are required for the conversion of pro-collagen to collagen fibrils. In OSMF, there is increased cross-linking of the collagen, resulting in increased insoluble form. This is facilitated by increased activity and production of a key enzyme – LOX. PCP/BMP1 and increased copper (Cu) in BQ stimulate LOX

activity, a key player in the pathogenesis of this disease. The flavonoids increase cross-linking in the collagen fibers. These steps results in increased collagen production. Pro-LOX: pro-lysyl oxidase; LOX: lysyl oxidase; PNP: pro-collagen N-proteinase; PCP: pro-collagen C–proteinase; BMP1: bone morphogenetic protein.[18]

(1) Activation of procollagen genes

Collagen is the most abundant protein in the human body and plays an important role in the structural element of connective tissue. They are triple helix stabilized by unusual crosslink. The processing of fibrillar collagen occurs in a stepwise manner. Procollagen genes are transcribed and translated to form procollagen monomeric chains. The genes COL1A2, COL3A1, COL6A1, COL6A3, and COL7A1 have been identified as definitive TGF-β targets. The activation of collagen I and VII collagen gene and procollagen genes by TGF-β cause an increased expression of procollagen genes and hence increase collagen level in OSMF.[18]

(2) Elevation of procollagen proteinases levels

Elevation of procollagen proteinases such as PCP that cleaves C-terminal and PNP's (PNP1 and PNP 2) cleaves N terminal play essential role in pathogenesis of OSMF. In the study conducted by **Yi-Ting *et al.*** (2009) found arecoline stimulated CTGF production in buccal mucosal fibroblasts (BMF).[37] BMFs could contribute to the pathogenesis of OSMF by producing CTGF during areca nut chewing and accompanied by an increased local TGF-β concentration due to inflammation regularly found in OSMF. Recent study has shown that curcumin (1,7-bis (4-hydroxy-3 methoxyphenyl)-1,6-heptadiene- 3,5-dione), the major yellow pigment in turmeric, curry and mustard, has high antioxidant and anti inflammatory activity and

is widely used as a flavoring and coloring agent in foods and as an herbal medicine to treat chronic inflammatory diseases suppresses the expression of extracellular matrix genes in activated hepatic stellate cells by inhibiting CTGF gene expression through suppressing ERK and NF-jB signaling.[38] Interestingly, researchers found curcumin could completely inhibit arecoline-induced CTGF synthesis and the inhibition is dose dependent.[39]

(3) Up-regulation of LOX

The association between copper and OSMF has been linked on the basis that excess copper is found in tissues of other fibrotic disorders– Wilson's disease, Indian childhood cirrhosis and primary biliary cirrhosis. The enzyme lysyl oxidase is found to be unregulated in OSMF.[39] This is a copper dependent enzyme and plays a key role in collagen synthesis and its cross linkage.[40] The possible role of copper as a mediator of fibrosis is supported by the demonstration of up regulation of this enzyme in OSMF biopsies[41] and in OSMF fibroblasts compared to normal fibroblasts grown in culture.[42]

Copper added at various concentrations *in vitro* has also been shown to increase proliferation of fibroblasts in culture. The fibroblasts in OSMF have not only increased lysyl oxidase activities but also specific growth characteristics. This was evident with the reported cell doubling time of 3.2 days for OSMF and 3.6 days for normal fibroblasts.[42] Further, OSMF fibroblasts grew more rapidly than normal as the former became confluent in 5 days compared to 6 days for the latter. As the oral mucosa is directly exposed to the copper challenge in chewers its effect may well be local. These different growth characteristics may either be due to the direct effects of ingredients of areca nut or secondary to inflammatory factors mediated by

areca nut such as IL-1, TGF-β, IGF, EG**.** The LOX activity is important for collagen synthesis and its cross linking. The process of cross linking gives tensile strength and mechanical properties to the fibers as well as makes the collagen fibers resistant to proteolysis tilting the balance towards a fibrotic condition as present in OSMF.[36]

Collagen degradation pathway

There are two main events modulated by TGF which decreases the collagen degradation;(1) activation of inhibitor of matrix metalloproteinase gene (TIMPs) (2) activation of plasminogen activator inhibitor (PAI) gene.[18]

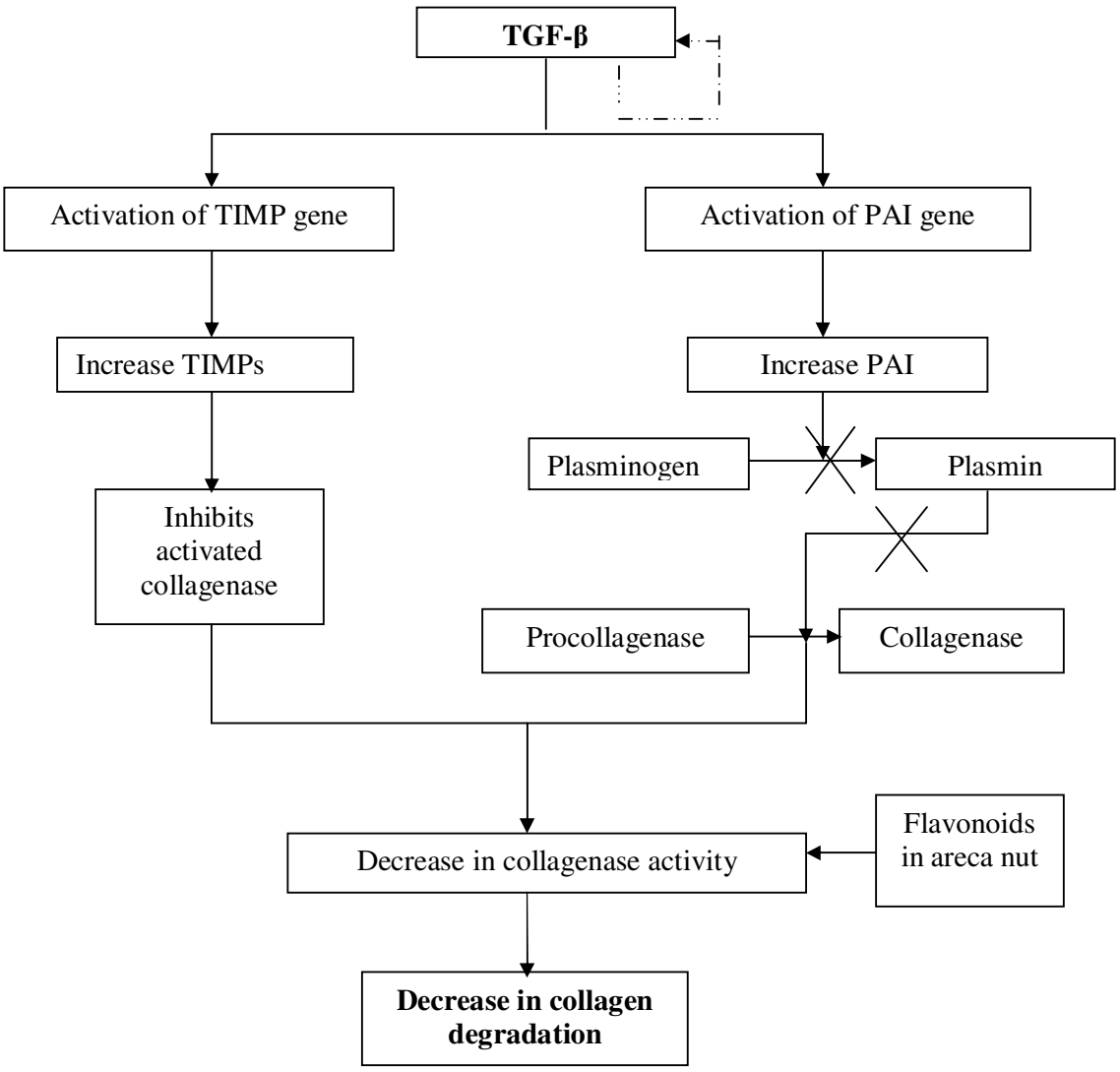

Figure 3: Collagen degradation pathway as regulated by TGF-β:TGF-β activates the genes for TIMPs; thereby more TIMP is formed. This inhibits the activated collagenase enzyme that is necessary for the degradation of collagen. It also activates the gene for PAI, which is an inhibitor of plasminogen activator, thus there is no plasmin formation. Plasmin is required for the conversion of pro collagenase to active form of collagenase and absence of plasmin results in the absence of active collagenase. The flavonoids inhibit the collagenase activity. A reduction in the activity and levels of collagenase results in a decrease in collagen degradation.[18]

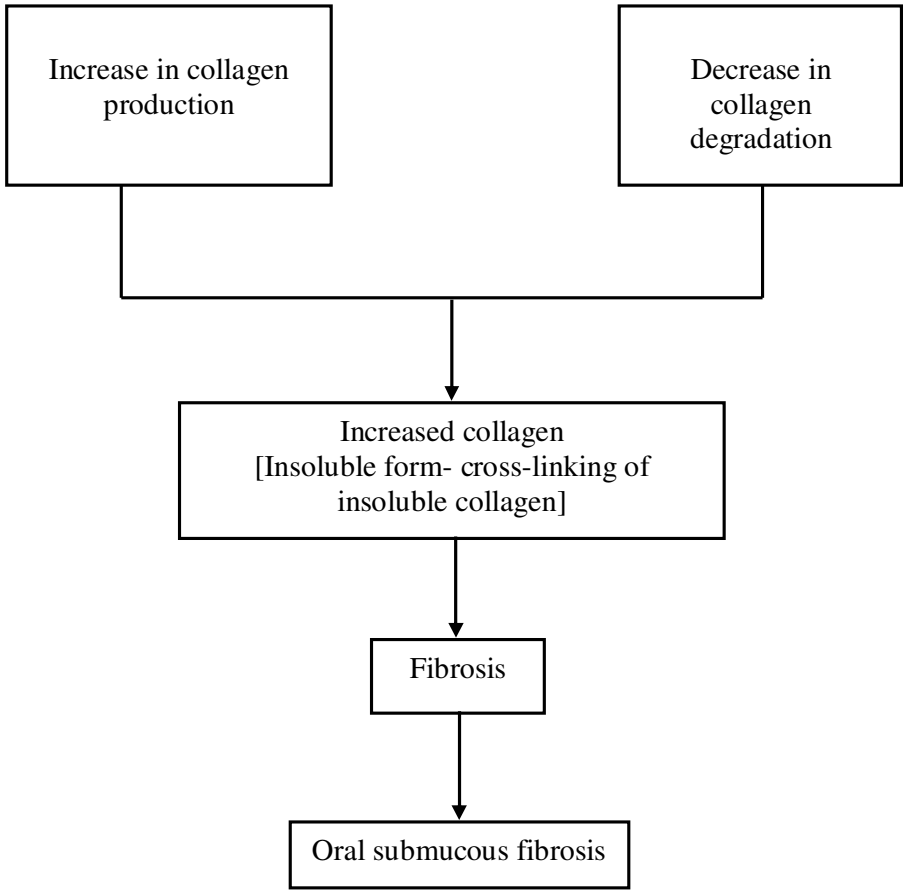

Figure 4: Effect of activated TGF-β pathway: There is an increase in collagen production and cross-linking (insoluble form) along with a decrease in collagen degradation. This produces an increased collagen deposition in the sub epithelial connective tissue layer of the oral mucosa leading to OSMF.[18]

2.3.5 Clinical Features

Onset of OSMF is insidious and is often of a long duration (2-5 years). Clinical features mainly include mucosal blanching, burning sensation, and presence of characteristic fibrous bands and is associated with gradual inability to open mouth.[12] Pindborg JJ and Sirsat SM (1966)[13] explained OSMF as a diffused condition; most often bilateral although unilateral cases have also been reported. Clinically, OSMF can be graded according to its severity. Initially the mucosa appears normal in colour. As the disease progresses the mucosa loses its resiliency, suppleness and becomes stiff. Therefore the insertion of impression trays and recording peripheral seal (border molding) becomes challenging to the prosthodontist. Stiffness of the mucosa is attributed to underlying fibrosis. In the early stages, individual fibrous bands may be palpable and, later on, fibrosis becomes so extensive that it gives a matted appearance.

Once the fibrosis gets established in the oral cavity, the oral mucosa exhibits a leathery consistency and the mobility and function of the affected part of the mucosa is reduced, viz. inability to open the mouth, limited tongue protrusion, etc. A diffused blanching occurs and, more frequently, blanching will be associated with patchy pigmentation acquiring a marble like appearance.

Prodromal symptoms

The most common initial symptom is burning sensation in the oral cavity, often experienced when the patient is eating spicy food. Other frequent early symptoms are blisters (especially on the palate), ulcerations, or recurrent stomatitis.

Excessive salivation, defective gustatory sensation, and dryness of the mouth can also occur in clinically early stages of the disease.[13]

Later symptoms

After varying periods of time, in some cases a few years after the appearance of the initial symptoms, patients complain of stiffening of certain areas of the oral mucosa, leading to (1) difficulties in opening the mouth, (2) inability to whistle or to blow out a candle, and (3) difficulties in swallowing. When the fibrosis reaches the pharynx, the patient may experience referred pain in the ears. Deafness due to occlusion of the Eustachian tubes[43] and nasal voice are the later symptoms in some patients.[44]

Clinical signs

A number of patients present vesicles, which usually are found in the soft palate, the anterior faucial pillars, the buccal mucosa, or the mucosal surfaces of the lips (especially the lower lip). These vesicles are painful, and they soon rupture, leaving small superficial ulcerations. Cultures of the vesicular fluid fail to reveal any specific organism. Although the appearance of vesicles is considered an initial symptom, vesicles may also appear as acute exacerbations in a later phase of the disease.[13]

As the disease progresses, the oral mucosa becomes blanched, slightly opaque, and white, and fibrous bands appear. The whitening often takes place in spots, so that the mucosa acquires a marble like appearance.

It was thought previously that, the palate and the faucial pillars were the areas primarily affected in cases of submucous fibrosis, but it has been observed that the

buccal mucosa and the lips also may be affected in an early stage. The oral mucosa is usually involved symmetrically. The fibrous bands in the buccal mucosa run in a vertical direction, and the fibrosis is sometimes so marked that the cheeks are almost immovable.[45]

In the soft palate the fibrous bands radiate from the pterygomandibular raphe or the anterior faucial pillar and have a, scar like appearance. The uvula is markedly involved in the later stages; it shrinks and appears as a small fibrous bud or J-shaped. The entire isthmus fancium is reduced and when the patient attempts to move the soft palate, it becomes obvious that the mobility is markedly reduced. The faucial pillars

become thick, short, and extremely hard. Sometimes the fibrosis spreads to the pharynx and down to the pyriform fossae.[46]

The lips are often affected and, upon palpation, a circular band can be felt around the entire rima oris. Clinically, the changes are quite marked in the lower lip. The circular fibrosis may cause obliquity of the rima oris and atrophy of the vermilion border.[45] Impairment of tongue movement in patients with submucous fibrosis has been observed. In order to evaluate the difficulties in opening the mouth, the degree of trismus was measured and a reduction in the patient's ability to open the mouth was found.[44]

Sinor PN *et al.* (1990) conducted a case study in Bhavnagar on 60 patients of OSMF, and observed that in all the 60 individuals the buccal mucosa and the retromolar area were involved. In 65% cases, the buccal mucosal involvement was restricted to the posterior part and in the remaining 35% posterior as well as anterior parts was affected. The soft palate was affected in 95%, and the uvula and the labial

mucosa in 50% of the cases. The tongue was affected in only one individual. In most of the patients the oral opening was severely restricted. Mucosal blanching was present in almost all cases and the most common symptom was the burning sensation on spicy food.[21]

Shah B, Lewis MAO, Bedi R (2001) presented a case report of oral submucous fibrosis occurring in an 11-year-old Bangladeshi girl who initially had recurrent oral ulcerations and discomfort with a burning sensation on eating spicy foods.[47]

Kumar A. and Bagewadi A. *et al.* (2007) in their study conducted on 58 male patients the chief findings were difficulty in swallowing and chewing, burning sensation on eating spicy foods, restricted mouth opening, palpable vertical fibrous bands, stiffness and blanching.[48]

Hazarey V.K. *et al.* (2007) in their cross-sectional study on total of 1000 OSMF cases the male to female ratio was 4.9:1 and the mean age was 28.8 years the clinical findings were reduced mouth opening, altered salivation and taste sensation were found to be significantly more prevalent in women when compared with men.[23]

2.3.6 Staging of OSMF

Since the past decades, various authors have proposed a number of staging and grading systems. Trismus, or reduction in the overall mouth opening, is one of the most reliable manifestations of oral submucous fibrosis and it has been a cardinal clinical feature for grading patients into different groups. Other classification systems were based on clinical features, histological features, and combination of above. However each system has its own drawbacks and none have been universally accepted or followed and the system varies considerably between different centers.

D Lal (1953)[49] divided the stages of involvement and progression of oral submucous fibrosis into 3 categories:

Early Stage – Mucous membrane inelastic and slightly blanched.

Advanced Stage – Marked inelasticity and opaque white blanching of the mucous membrane, almost papery white, tough on palpation, which could not be distended. Firm vertical bands felt just opposite the premolar region, and in later stage lips and palate are also involved.

Extreme Stage – Patient unable to open his mouth, the cheek and lips were found to be tightly held against teeth, in addition to the progression of the other signs and symptoms of the disease.

Haider SM *et al.* (2000) evaluated 325 OSMF patients and they staged the disease clinically and functionally as follows: [50]

Clinical staging :

Stage 1: Faucial bands only

Stage 2: Faucial and buccal bands

Stage 3: Faucial and labial bands

Functional Staging :

Stage A: Mouth opening 13- 20 mm

Stage B: Mouth opening 10-12 mm

Stage C: Mouth opening < 10 mm

They concluded that fibrotic bands are common at the posterior region of the buccal mucosa in mild cases of OSMF and as the disease increases in severity,

bands are more likely to be found anteriorly as well. They also observed that if the maximum mouth opening was 20 mm or more, there was a 4% chance that bands were present in all three regions. If, however, the maximum mouth opening was 10 mm or less, there was a 95% chance that bands were present in all three regions.[50]

2.3.7 Classification System Based On Clinical Features

Wahid PN *et al.* (1966)[51]

Grade I –

- No symptom referable to mucosal indolent.
- Focal lesions on one commonly involved site.
- Pallor or whitish discoloration
- Minimal induration of tissue present.

Grade II –

- Soreness of mucosa
- Increased sensitivity to chillies
- Diffuse whitish extensively indurate lesions on one or more sites

Grade III –

- Restriction of movements
- Fibrous bands presents
- Surface fissured or ulceration

Mathew B. *et al.* (1967)[52] classified submucous fibrosis into 3 groups.

- Mild: only blanching of oral mucosa with Palpable fibrous bands.
- Moderate: Blanching of oral mucosa with Palpable fibrous bands associated with mouth opening restricted to 2 cms.

- Severe: Extreme difficulty in opening of mouth with visible fibrous bands.

Ahuja SS and Agrawal (1971)[53]

- **Grade I (Mild):** Localized fibrous bands in cheek extending from superior to inferior vestibular fornix. These were located on lips to the mucosa in relation to first molar to second molar region.
- **Grade II (Moderate):** Generalized diffuse hardening of sub epithelial tissue extending from cheek and hard palate, uvula and pillars of fauces and sometimes pharynx.
- **Grade III (Severe) :** Combination of Grade I and Grade II with generalized diffuse form of oral submucous fibrosis.

Bhatt AP and Dholakia HM (1977)[54]

- **Grade I (Mild):** Very slight fibrous banding and little narrowing of mouth opening.
- **Grade II (Moderate):** Moderately pronounced symptoms, fibrous banding extending from cheek to Palate with moderate narrowing of the mouth opening.
- **Grade III (Severe):** Excessive amount of fibrous banding involving cheeks, palate, uvula, tongue, lips and marked narrowing of the mouth opening.

Pindborg J.J. (1989) divided OSMF based on physical findings into 3 stages as follows :

Stage 1: Stomatitis includes erythematous mucosa, vesicles, mucosal ulcers, melanotic mucosal pigmentation and mucosal petechiae.

Stage 2 : Fibrosis occurs in healing vesicles and ulcers.

- Early lesion demonstrates blanching of the oral mucosa.
- Older lesion includes vertical and circular palpable fibrous bands in the buccal mucosa and around the mouth opening or lips. This results in a mottled marble like appearance of the mucosa because of the vertical, thick, fibrous bands in association with a blanched mucosa. Specific findings includes reduction of mouth opening, stiff and small tongue, blanched and leathery floor of the mouth, 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 3 : Sequelae of OSMF are as follows :

- Leukoplakia is found in more than 25% of individuals with OSMF.
- Speech and hearing deficits may occur because of involvement of the tongue and the eustachian tubes.[55]

Lai D.R. in conducted a study and divided the OSMF population based on the interincisal width as :

- **Group A :** Mouth opening greater than 35 mm.
- **Group B :** Mouth opening between 30-35 mm.
- **Group C :** Mouth opening between 20-30 mm.
- **Group D :** Mouth opening less than 20 mm.

Classification by Lai D.R. is based on mouth opening and does not take into consideration the clinical symptom.[54]

Bailoor DN and Nagesh KS (2001)[56]suggested the following stages :

Stage I Early OSMF

- Mild blanching.
- Normal mouth opening [35 to 45 mm].
- Tongue protrusion normal [5 to 6 cm].
- Burning sensation only on taking spicy or hot food.

Stage II Moderate OSMF

- Moderate to severe blanching.
- Mouth opening and tongue protrusion reduced by 33%.
- Burning sensation even in the absence of stimuli.
- Palpable bands felt.
- Unilateral or bilateral lymphadenopathy.

Stage III Severe OSMF

- Severe burning sensation.
- Mouth opening and tongue protrusion reduced by 66%.
- Ulcerative lesions may appear on the cheek.
- Thick palpable bands.

- Lymphadenopathy bilaterally evident.

Ranganathan K. *et al*. in 2001classified[56] OSMF patients as :

- **Group 1 :** Only symptoms, with no demonstrable restriction of mouth opening.
- **Group 2 :** Limited mouth opening 20 mm and above.
- **Group 3 :** Mouth opening less than 20 mm.
- **Group 4 :** OSMF advanced with limited mouth opening, precancerous or cancerous changes seen throughout the mucosa.

Rajendran R. and **George T. (2003)**[57]

- **Early OSF:** 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 the mouth.
- **Advanced OSF:** Blanched and slightly opaque mucosa, fibrous bands in buccal mucosa running in vertical direction. Palate and the pillars are the areas first involved. Gradual impairment of tongue movement and difficulty in mouth opening.
- This classification does not have intermediate group, between early and advanced stage. An intermediate stage would be useful for effective follow up during treatment.

Khanna and **Andrade**[58] (1952) developed a classification system of OSMF based on interincisal opening :

- **Group 1:** Early OSMF without trismus (MIO >35mm)

- **Group 2:** Mild to moderate disease (MIO 26 to 35 mm)
- **Group 3:** Moderate to severe disease (MIO 15 to 25 mm)
- **Group 4a:** Severe disease (MIO <15 mm)
- **Group 4b:** Extremely severe–malignant/premalignant lesions noted intraorally.

2.3.8 Classification System Based On Histopathological Features

Pindborg J.J. and Sirsat S.M. (1966)[13] were the first to divide OSMF depending on only histopathological features:

Very early stage –

- Finely fibrillar collagen dispersed with marked edema.
- Plump young fibroblast containing abundant cytoplasm.
- Blood vessels are dilated and congested.
- Inflammatory cells, mainly polymorphonuclear leukocyte with occasional eosinophils are found.

Early stage

- Juxtaepithelial area shows early hyalinization.
- Collagen still in separate thick bundles.
- Dilated and congested blood vessels.
- Inflammatory cells are primarily lymphocytes, eosinophils, and occasional plasma cells.

Moderately advanced stage

- Collagen is moderately hyalinized.
- Thickened collagen bundles.
- Fibroblastic response is less marked.
- Blood vessels are either normal or compressed.
- Inflammatory exudates consist of lymphocyte and plasma cells.

Advanced stage

- Collagen is completely hyalinized.
- Smooth sheets with no separate bundles of collagen are seen.
- Edema is absent.
- Hyalinized area is devoid of flbroblasts.
- Blood vessels are completely obliterated or narrowed.
- Inflammatory cells found are lymphocytes and plasma cells.

2.3.9 Classification System Based On Clinical & Histopathological Features

Khanna J.N. and Andrade N.N. in 1995[13] in their study classified OSMF into different stages as follow:

Group I: Very early stage

- Mouth opening is normal.
- Burning sensation in the mouth.

- Excessive salivation.
- Acute ulceration and recurrent stomatitis.

Group II: Early stage

- Mouth opening: 26-35 mm (interincisal opening)
- Soft palate and faucial pillars are the areas primarily affected.
- Buccal mucosa appeared mottled and marble-like where dense pale, depigmented fibrosed areas alternated with pink normal mucosa.
- Widespread sheets of fibrosis palpable.

Group III: Moderately advanced stage

- Trismus evident, with mouth opening 15-25 mm (interincisal opening).
- Buccal mucosa appears pale and firmly attached to underlying tissues.
- Vertical fibrous bands palpable at the soft palate, pterygomandibular raphe, and anterior faucial pillars.
- Atrophy of vermilion border.

Group IV (a): Advanced cases

- Trismus is severe with mouth opening 2-15 mm (interincisal opening).
- Stiffness / inelasticity of oral mucosa.
- Fauces thickened, shortened and firm on palpation.
- Uvula was seen to be involved, shrunken, appears small and fibrous bud.
- Tongue movement restricted.

- Papillary atrophy (diffuse).
- Lips- circular band felt around the entire mouth.

Group lV (b): Advanced cases with premalignant and malignant changes

- Hyperkeratosis, leukoplakia or squamous cell carcinoma can be seen.[55]

2.3.10 Histopathological Features

Oral submucous fibrosis shows characteristic histological features consisting of atrophic epithelium with juxtaepithelial hyalinization and collagen of varying density. The epithelium is often keratinized. Pindborg and Sirsat (1966)[13] assessed that, in 90 per cent of the cases, the epithelium is atrophic, secondary to the connective tissue changes. The atrophic epithelium is generally without rete ridges and in advanced cases it may be ribbon-like. Epithelial dysplasia was reported to occur in as many as 26 per cent of the cases Pindborg *et al*. (1984).[24] presence of signet cells was another feature.

Structural changes in oral submucous fibrosis have been studied in detail both at the light and electron microscopic levels. Reichart PA *et al.* (1984)[59] and Van Wyk CW *et al.* (1990)[60] studied the patterns of distribution of different types of collagen in subjects with confirmed oral submucous fibrosis. The epithelial dysplasia observed in oral submucous fibrosis differs from the dysplasias seen in leukoplakia. The dysplasia seen in submucous fibrosis was characterized by marked irregular epithelial stratification, nuclear pleomorphism, and pronounced intercellular edema, whereas, in leukoplakia, the dysplasia is characterized by increased mitotic

activity, hyperchromatism, and basilar hyperplasia.[61] Interestingly, in a long-term study of various dysplastic lesions, it was noted that epithelial dysplasia in submucous fibrosis revealed a higher risk of cancer development than in dysplastic homogeneous leukoplakias.[16]

The connective tissue changes in oral submucous fibrosis are described as very early, early, moderately advanced, and advanced stages (Sirsat and Pindborg in 1966).[13] Very early changes are characterized by finely fibrillar collagen, interspersed with edema and strong fibroblastic response. The blood vessels are often dilated and congested. Inflammatory cells in this stage consisted of polymorphs and occasional eosinophils. In the early stage there is juxtaepithelial hyalinization, collagen is thickened and seen as separate bundles. In this stage the inflammatory cells consisted of lymphocytes, plasma cells, and occasional eosinophils. In moderately advanced and advanced stages there is hyalinization of the connective tissue to a variable extent. There is no edema and the predominant inflammatory cells were lymphocytes and plasma cells.[62]

Bhonsle *et al.* in 1981[63] demonstrated histological features of petechiae in oral submucous fibrosis. They consisted of juxtaepithelial, endothelial-lined lumina containing erythrocytes and the presence of juxta and intraepithelial haemorrhages. The authors pointed out that petechiae seen in this condition are due to the vascular dilatation following the loss of connective tissue support and also due to the extravasation of blood.

The histochemical alterations in oral submucous fibrosis were reported to comprise differentially stained collagen, intense metachromasia of the ground

substance, and the PAS-positive material in the connective tissue (Sirsat and Khanolkar; Sirsat and Pindborg; Hamner *et al*.).[64,65,66]

The mast cell counts in oral submucous fibrosis were reported to be high in the early stages where the reaction of the tissue to the irritant is strong and the counts were low in advanced stages when the connective tissue is hyalinized. In a detailed study by Sirsat and Pindborgh in 1967 [65] the vascular response in this condition was observed to be complex consisting of persistent dilatation of blood vessels especially in moderately advanced and advanced cases. These investigators opined that the mast cell and the vascular responses in this condition are similar to those described in certain diseases of autoimmune origin.[61]

Ultra Structural Changes

El-Labban and Canniff in 1985[67] reported the ultra structural alterations of muscle fibers in oral submucous fibrosis as consisting of degenerative changes in the muscle fibers which are more pronounced among individuals with restricted oral opening. The muscle fibers contain large pools of homogeneous material, which causes compression of their sarcomeres. The mitochondria also exhibit degenerative changes and in many instances the mitochondria are completely absent. The degenerative changes also extend to the nuclei. The plasma membrane is often disrupted, especially in those areas showing fluid-like material. The surrounding connective tissue shows degeneration. The authors concluded that these alterations might be responsible for the restricted oral opening in this condition.

Cytological Features

The cytological characteristics were described by Wahi *et al.* (1967) as anucleated squames, superficial eosinophilic cells, and large parabasal cells[19]. A rarefied chromatin pattern and a quantitative and semi quantitative increase in the alkaline phosphatase were also reported by these investigators. The authors opinioned that these features could be considered diagnostic of submucous fibrosis. In spite of these findings, however, the utility of exfoliative cytology as a diagnostic aid or to assess the precancerous nature of this disease is doubtful.

As mentioned earlier, the diagnosis of this condition should be established only on the basis of presence of palpable fibrous bands.

2.3.11 Oral Submucous Fibrosis And Malignant Potential

The precancerous nature of oral submucous fibrosis was first mentioned by Paymaster (1956)[61] who observed the development of slowly growing squamous cell carcinoma in one-third of his submucous fibrosis patients. Subsequently, Pindborg (1965)[68] reported that submucous fibrosis patients in India have a higher occurrence of leukoplakia and carcinoma than those without this disease. In South India, Pindborg and Zachariah (1965)[69] observed that 40 per cent of the oral cancer patients had oral submucous fibrosis. In another study by Pindborgh (1966) epithelial dysplasia was present in 7 per cent of OSMF patients who had no concurrent oral cancer and, among people who had associated oral cancer, epithelial dysplasia was observed in 16 per cent when biopsies were done away from the cancer site, and in 75 per cent when the biopsies were obtained nearer to the cancer location.[13]

Pindborg in **1972** summarized the criteria in support of the precancerous nature of this disease as: (1) higher prevalence of leukoplakia among submucous fibrosis patients; (2) high frequency of epithelial dysplasia; (3) concurrent finding of submucous fibrosis in oral cancer patients; (4) histological diagnosis of carcinoma without the clinical suspicion of it; and (5) incidence of oral cancer among patients with submucous fibrosis.[70]

The malignant potential of oral submucous fibrosis was assessed in a population based prospective study. In a follow-up study of 66 patients over a 15-year observation period (median 8-years), cancer developed in 4.5 per cent (Pindborg *et al.*)[71] and over a 17-year observation period (median 10 years) in 7.6 per cent (Murti *et al.*)[72]

Oral cancers originate in submucous fibrosis from diverse intraoral locations without any noticeable predilection for any specific site.[72]The malignant potential and also the origin of cancers in diverse intraoral locations in this condition is attributable to the generalized epithelial atrophy in this condition.

2.3.12 Treatment Modalities

Prevention of OSMF is more important than treatment. Since the aetiology of OSMF remains uncertain, the treatment is aimed at ameliorating the discomforting symptoms, reducing the fibrosis and improving the mouth bite. The elimination of the habit of areca nut chewing is an important preventive measure. Treatment of OSMF is a challenge, especially as the disease progresses.

Treatment is based on severity of disease. Typically, if the disease is noted before development of trismus, cessation of the betel habit will often resolve the disease. Once trismus has developed and disease is now considered mild to moderate, OSMF is irreversible, with the goal of medical and surgical therapy to maintain oral function and limit progression of disease. Treatment at this stage is focused on restoring mandibular range of motion, oral cancer surveillance, and cessation of betel nut habit. Physical therapy combined with medical treatment is often utilized. Medical therapy performed includes placental extracts, intralesional injections of collagenase or interferon gamma, and topical hyaluronidase. It is thought that the use of steroids decreases inflammation and collagen formation in the areas of injection; collagenase is hoped to break down locally areas of fibrosis; hyaluronidase, placental extracts, and interferon gamma are thought to alter collagen synthesis. Indian trials using pentoxifilline, a vasodilator, has shown some promise as an adjunct in the treatment of OSMF, as well by slowing the progression of oral mucosal fibrosis. Carbon dioxide laser, lycopene, interferon gamma, turmeric, combined therapy with, vitamins A, E and B complex, have also been tried.[73]

Vitamins, Minerals and Antioxidants

Vitamins and microelements are essential in the normal metabolism of organisms. Some studies regarded deficiencies in vitamins and minerals as promoting the initiation and development of OSMF.[74] The progressive inability to open the mouth results in difficulty in eating and consequent serious nutritional deficiencies, which will hasten the progress of the disease. Numerous studies used vitamins as a standard or adjunct therapy, and vitamins partially accelerated healing

and relieved symptoms such as burning sensations and intolerance of spicy food. Vitamin A plays an important role in maintaining the normal growth and repair of epithelial tissues. The dose of vitamin A given in OSMF is 50,000 IU daily. The vitamin B complex consists of water-soluble vitamins that work together to boost metabolism, enhance the immune system, and encourage cell growth and division.

In a study done by Maher et al. a combination of micronutrients (vitamins A, B complex, C, D, and E) and minerals (iron, calcium, copper, zinc, magnesium, and others) was evaluated for its efficacy in controlling the symptoms and signs of OSMF in 117 compliant subjects in Karachi, Pakistan. The daily oral supplementation was prescribed as follows: 3,333 IU retinol, 500 IU vitamin D, 20 mg vitamin B_1, 5 mg vitamin B_2, 10 mg vitamin B_6, 5 µg vitamin B_{12}, 50 mg nicotinamide, 10 mg vitamin E, 11.6 mg calcium D pantothenate, 150 mg ascorbic acid, 0.25 mg biotin, 1 mg folic acid, 50 mg calcium, 18.5 mg magnesium, 50 mg iron sulfate, 25.5 mg phosphorus, 1 mg copper, 0.5 mg manganese, 0.5 mg zinc, and 0.1 mg molybdenum. The subjects received supplementation for one to three years. Significant improvement in symptoms, notably intolerance to spicy food, burning sensation, and mouth opening, was observed at exit. The major outcome from this study was a beneficial clinical response in subjects with OSMF to multiple micronutrient intervention, which justifies its further evaluation in well-designed randomized controlled trials in other settings in South Asia.[75]

Vitamin C acts as a cofactor in numerous biological processes such as wound healing. It appears to be necessary for the integrity of the cellular immune response and anti-inflammatory activity. Some minerals also play essential roles in the

activities of enzymes and the synthesis of important substances. Hence minerals such as zinc and magnesium were used as an important adjunctive treatment for OSMF. Kumar et al. conducted a study on 82 patients of OSMF to evaluate the therapeutic role of zinc in OSMF. He concluded that oral zinc (220 mg) has a beneficial role in OSMF.[76]

Zinc plays essential roles in DNA synthesis and cell division, and is a constituent of many enzymes, including dehydrogenases and carbonic anhydrase. The amount of zinc greatly increases during tissue repair. Moreover, zinc is the antagonist of copper. It was suggested that substantial amounts of copper released from BQ induced lysyl oxidase activity, up-regulated collagen synthesis by fibroblasts, facilitated collagen cross-linking, and inhibited collagen degradation.[77]Magnesium ions play essential roles in many enzyme reactions, and exert stabilizing effects on excitable membranes.

Chewing Betel Quid was considered an important etiological factor of OSMF.[78] One of the most important pathogenic mechanisms is the excessive reactive oxygen species (ROS) induced by the ingredients of Betel quid. Those ROS, including oxygen ions, free radicals, and peroxides, would certainly damage crucial cellular macromolecules such as DNA, proteins, and membrane lipids. Epidemiologic studies showed that the process of carcinogenesis accompanies the generation of ROS, and a number of studies proved that the management of premalignant lesions should include antioxidants along with the cessation of unhealthy habits.[79]

Vitamin E acts as an antioxidant in a number of biological processes: it prevents the oxidation of unsaturated fatty acids, vitamin A, carotenes, and the thiol groups in certain enzymes.

Beta carotene is a powerful antioxidant, and was shown to protect against cancer and heart disease. Biologically β carotene is important as the precursor of vitamin A. Gupta S *et al.*[80] found that after 6 weeks of treatment with tablets containing mostly β-carotene and vitamin E, patients showed an effective increase in mouth opening and tongue protrusion. Moreover, the decrease in mean malondialdehyde level (a marker of free radical damage) and the increase in levels of β-carotene after treatment were found to be statistically significant ($p<0.01$ and $p<0.001$, respectively), and these factors may play an important role in treatment.

Lycopene is an open-chain unsaturated carotenoid that imparts red color to tomatoes. It possesses antioxidant and antiproliferative properties in animal and *in vitro* studies that may help combat degenerative diseases. The antioxidant activity of lycopene is at least twice as great as that of β carotene. Kumar *et al.* studied the effects of lycopene soft gels in the treatment of OSMF by giving 16 mg of lycopene daily in 2 equally divided doses. Their results indicated that lycopene was more efficacious in improving mouth opening in patients and reducing associated symptoms than was placebo treatment ($p<0.001$). They attributed this curative effect to an inhibition of abnormal fibroblasts, up-regulation of lymphocyte resistance to stress, and a suppression of the inflammatory response.[81]

Steroids

Steroids, and especially glucocorticoids, were first used in the treatment of OSMF, and now are extensively used. Cytokines and growth factors produced by inflammatory cells can promote fibrosis by inducing a proliferation of fibroblasts, up regulating collagen synthesis, and down-regulating collagenase production. Several glucocorticoids were used, such as short-acting steroids (hydrocortisone) 1.5 ml at weekly intervals, intermediate-acting steroids (triamcinolone) 10 mg /ml and long-acting steroids (betamethasone and dexamethasone) 4 mg (1 ml) biweekly sub mucosal intralesional injections or topical application of steroids may help prevent further damage.[82]

Borle and Borle found that treatment of OSMF with intralesional injections of hyaluronidase 1,500 IU on a biweekly basis and corticosteroids (triamcinolone acetonide) 10 mg/mL diluted in 1 mL immediate tissue irritation and to facilitate proper distribution of drug to all the of lidocaine 2%, to avoid sites reduced burning sensation by 86.84%.[83]

Glucocorticoids exert their anti-inflammatory activity by inhibiting the generation of inflammatory factors and increasing the apoptosis of inflammatory cells. They partially relieved patients of their symptoms at an early stage of OSMF, as confirmed in many studies. They were less useful in reversing the abnormal deposition of fibrotic tissues and recovering the suppleness of the mucosa, and thus this treatment was always associated with a high incidence of relapse. Systemic steroids were also used for the treatment of OSMF but no satisfactory results were seen. A therapy with hydrocortisone 25 mg tablet, in doses of 100 mg/day is useful in

relieving burning sensation. Triamcinolone or 90 mg of dexamethasone can be given. Moreover, prolonged use or overdose invariably produced unwanted side effects. Steroids are nonetheless useful in controlling symptoms, or as an adjunct therapy.[84]

Dipti et al in 2014 [85] conducted a study to determine the efficacy of lycopene in management of OSMF and to compare its efficacy with intralesional betamethasone injections . lycopene has been seen to have many anticariogenic properties but in combination is more efficacious as well as safe and reliable drug in management of OSMF.

Enzymes

According to several studies, a prominent characteristic of OSMF is its abundant and abnormal accumulation of collagen fibers in the lamina propria and sub mucosa of the oral mucosa, including muscle fibers and salivary glands. It is recognized that proliferative fibroblasts, inactive collagenase, and the inhibited fibrinolytic system contributed to the initiation and development of OSMF. Therefore, exogenous resolves can act as an effective drug to reverse debilitating fibrosis by degrading abnormal fibrotic tissues.[84]

Collagenase is a lysosomal enzyme, capable of degrading phosphate esters, proteins, polysaccharides, glycosides, and sulfate esters. In a controlled clinical trial, Lin and Lin found that intralesional injections of collagenase (0.5 ml of 1% solution of collagenase type I-V mixture) resulted not only in significant improvement in mouth opening, but also in a striking reduction of hypersensitivity to spices, sour, cold, and heat. These results indicated that collagenase treatment was approximately fivefold more effective than triamcinolone diacetate. After morphologic and

histological observations of biopsy specimens, Lin and Lin thought that this effect was attributable to increased vascular circulation and epithelium regeneration after lysis of the submucous fibrous tissue.[86]

Hyaluronidase 1500 IU twice weekly also showed a much quicker effect in ameliorating the burning sensation and painful ulceration than did dexamethasone, though the effect was short-term.[87] Further study found that hyaluronidase could ameliorate the symptoms and signs of OSMF by depolymerizing hyaluronic acid, which is the ground substance in connective tissue, lowering the viscosity of the intercellular cement substance, and decreasing collagen formation. Chymotrypsin, an endopeptidase, hydrolyzes ester and peptide bonds.

It was also used as a proteolytic and anti-inflammatory agent in the treatment of OSMF.[88]

Vasodilators

Pathologically, occlusive blood vessels (because of the deposition of collagen fibers and hyper coagulation status of blood) restrict nutrients and therapeutic substances from reaching the affected tissue, which may be one of the reasons for the unsatisfactory therapeutic effect of drug treatment of OSMF. Thus some drugs that were used to improve circulation and hemorrheology were tried in the treatment of OSMF.[12]

Pentoxifylline is a methylxanthine derivative, and was first used to treat intermittent claudication, sickle-cell disease, and vascular dementia because of its vasodilating properties and ability to decrease the viscosity of blood. Samlaska CP *et al.* considered pentoxifilline (400 mg tablets) an effective adjunct in the treatment

of OSMF. After 7-month trial periods and 6 to 12 months of clinical follow-up, patients in the experimental group showed more obvious symptoms and signs of amelioration than patients in the control group ($p<0.01$). The curative effect of pentoxifylline may be attributable to its properties of suppressing leukocyte function, altering fibroblast physiology, and stimulating fibrinolysis. In addition, it causes neutrophil degranulation, promotes natural killer cell activity, and inhibits T-cell and B-cell activation. But the gastrointestinal tract and central nervous system side effects of pentoxifylline were always obvious after administration.[84]

Others

Interferon-γ (INF-γ) intralesional injection of interferon gamma (0.01– 10.0 U/mL) 3 times a day for 6 months. It is an antifibrosis factor. Haque *et al.* studied its antifibrotic effect in OSMF patients in an open, uncontrolled clinical trial, and found that the net gain of mouth opening after 6 months of treatment was 8.4± 3.5 mm (mean ± SD), and that clinical symptoms also seemed to improve. Further studies attributed the antifibrotic effect to a down regulation of fibroblast proliferation and collagen synthesis, and an up-regulation of the antifibrotic cytokine and collagenase synthesis in the basal layer of the epithelium and lamina propria.[35]

Tai *et al.* (2001) treated OSMF patients with milk from cows immunized with human intestinal bacteria (immune milk) 45 g milk powder twice a day for 3 months. This may act by suppressing the inflammatory reaction and modulate cytokine production of anti-inflammatory components. After 3 months of treatment, 69.2% of patients had significantly increased their maximum mouth opening by more than 3 mm ($P <. 01$).[89]

Jirge *et al.* (2008) used levamisol to treat subjects of OSMF. The results indicated that levamisol, antioxidant and the combination of levamisol 50 mg three times daily for three alternate weeks, and antioxidant showed significant improvement in mouth opening and reduction in burning sensation. Levamisol has been found to modulate both cellular and humoral immunity in patients. The reduction in the levels of IgG, IgA and IgM were seen in the study suggests that the humoral response in OSMF was modified, probably due to slowing down the chronic inflammatory process.[90]

Treatment with intralesional placental extracts (lnj. Placentrex) 2 ml once a week (Katharia SK *et al*.)[91] resulted in significant improvement in mouth opening, color of oral mucosa, burning sensation, and reduction of fibrous bands. The action of placental extract is essentially biogenic stimulation and use is based on the tissue therapy method. This owes its inception to the corneal transplantation. According to this theory when animal and vegetable tissues are severed from the parent body and exposed to unfavorable conditions, but not mortal to their existence, undergo biogenic readjustment leading to development of substance in the state of their survival to ensure their vitality biogenic stimulators. Such tissues or their extract when implanted or injected into the body after resistance of pathogenic factors stimulates metabolic or regenerative process thereby favoring recovery.[20]

Physiotherapy

This includes measures such as forceful mouth opening and heat therapy. Heat has been commonly used. Aggressive physical therapy post surgery is essential: without physical therapy compliance, the risk of recurrent trismus is possible.

Patients should be aware that, although the trismus has resolved post surgery, their OSMF has not been cured. As such, continued physical therapy for the rest of their life is the best way to prevent recurrence of trismus. In addition, cessation of betel nut use is essential to minimise disease progression.[92]

Microwave Diathermy

Microwave diathermy has been tried and found to be valuable in the treatment of fibrosis and trismus following dental extraction and other musculoskeletal conditions. Gupta DS and Gupta MK (1980)[93] tried Physio-fibrolysis by microwave diathermy. Satisfactory results were obtained in the early and moderately advanced stages of the disease. Microtone 200 units producing microwaves of 2450MC/s was also used in treating OSMF along with injection of steroids, hyaluronidase and placental extract.

Surgical Management

As OSMF progresses to moderate to severe trismus, surgical intervention is required. It should be noted that post surgery, an aggressive regimen of physical therapy is essential to long-term resolution of trismus. Multiple surgical modalities have been attempted, from moderately invasive to significantly invasive. The most common surgical modality used initially includes release of intraoral fibrous bands, coronoidotomies, muscle of mastication myotomies, and soft tissue reconstruction with split thickness skin graft or allograft. Aggressive physical therapy post surgery is essential. Without physical therapy compliance, the risk of recurrent trismus is possible. Patients should be aware that while the trismus has resolved post surgery, their OSMF has not been cured. As such, continued physical therapy for the rest of

their life is the best way to prevent recurrence of trismus. In addition, cessation of betel nut use is essential to minimize disease progression. Finally, oral cancer surveillance is necessary for the rest of the patient's life. In the case documented in this report, this procedure was performed combined with intralesional steroid injections to minimize fibrous healing postoperatively.[74]

For cases in which initial surgical intervention is unsuccessful more aggressive surgical therapy is indicated. Again, excision of any fibrous bands intraorally, repeated masticatory muscle myotomy is required. Often in this situation, a larger soft tissue buccal defect is created, needing large soft tissue reconstruction. This can include a temporalis pedicle flap, superficial temporalis fascia pedicle flap, or a radial forearm free flap combined with split thickness skin graft coverage.[12]

2.3.13 Clinical Review of OSMF

Mehrotra R *et al*. (2009) conducted a hospital-based study on 65 clinically diagnosed and histopathologically proven patients of OSMF and 42 age and sex matched controls. In these samples serum lipids including: (i) Total cholesterol, (ii) LDL cholesterol (LDLC), (iii) HDL cholesterol (HDLC) (iv) VLDL cholesterol (VLDLC) (v) triglycerides (vi) Apo-A1 (viii) Apo-B and (viii) LPa were analyzed. A significant decrease in plasma total cholesterol, HDLC and Apo-A1 was observed in patients with OSMF as compared to the controls. Thus an inverse relationship between plasma lipid levels and patients of OSMF was found. They concluded that the lower levels of plasma cholsesterol and other lipid constituents in patients might be due to their increased utilization.[94]

Chawda JG *et al.* (2011) conducted a study on a total of 30 subjects (25 oral cancer patients and 5 controls). Fasting blood lipid profile including cholesterol (C), triglycerides (TG), high density lipoprotein (HDL), low density lipoprotein (LDL) and very low density lipoprotein (VLDL) were evaluated using spectrophotometric kits, with CHOD PAP technique. The levels of total lipids, cholesterol and HDL were significantly lower in oral cancer patients as compared to controls, but LDL and VLDL values were not significant. They concluded that there is an inverse relationship between the lipid levels and the occurrence of oral cancer.[10]

Chalkoo AH *et al.* (2011) conducted a study on 20 clinically diagnosed patients of OSMF between the age group of 20 and 50 years male patients were studied. Serum lipid including, (i) serum cholesterol, (ii) LDLC, (iii) HDLC, (iv) VLDL, (v) triglycerides, (vi) HDLC/LDLC ratio and (vii) serum cholesterol/HDLC were analyzed. Serum cholesterol and LDLC showed a significant decrease whereas serum triglycerides and HDLC were slightly increased in some patients with OSMF. Thus, the study strengthens the evidence of alterations in plasma lipid levels in OSMF patients.[95]

Kumar P *et al.* (2013) conducted a study on 30 patients were included in the study, 20 with oral submucous fibrosis and 10 healthy controls. Fasting plasma lipid profile including Total Cholesterol (TC), Very Low Density Lipoproteins (VLDL), Low Density Lipoproteins (LDL), High Density Lipoproteins (HDL) and Tri□Glycerides (TG) were measured using semiautomatic analyser. Significant decrease in plasma total cholesterol, LDL and HDL was observed in patients with OSMF as compared to the controls, but it was not statistically significant for VLDL

and TG values. Thus, The results of the present study show that there is an inverse relationship between lipid profile and the presence of oral submucous fibrosis.[96]

Radhakrishna M *et al*. (2014) conducted a study on 30 patients with oral submucous fibrosis and 19 healthy controls. Serum lipids including (i) total cholesterol, (ii) LDL cholesterol (LDLC), (iii) HDL cholesterol (HDLC), (iv) VLDL cholesterol (VLDLC) and (v) triglycerides, were analyzed using spectrophotometry kits. A significant decrease in serum total cholesterol (TC) levels, TC:HDLC ratios ($p = 0.005$, $p = 0.001$ respectively) were observed in oral submucous fibrosis patients as compared to the control group. They concluded that decrease in total cholesterol in patients with OSF could be due to the greater utilization of lipids including total cholesterol by the cells for new membrane biogenesis.[97]

Ajai K *et al*. (2014) conducted a study on 45 clinically and histopathologically diagnosed cases of OSMF and 45 age and sex matched controls. The complete lipid profile including TC, TG, HDL cholesterol, LDL cholesterol and VLDL cholesterol was analyzed. They found that serum lipid levels were significantly lower in the patients with OSMF than in the controls. When the values were compared between different disease stages, the maximum reduction of lipids was evident for stage 3 OSMF. They concluded that the level of serum lipids decreases with progression of the disease.[98]

Ranjith Kumar Kanthem, Venkateswar Rao Guttikonda (2015) conducted a study on 50 patients of OSMF, diagnosed clinically and histopathologically, were included as the study subjects. A group of 50 age and sex matched normal subjects without any oral pernicious habits were taken as controls. The serum lipid profile

consisting of total cholesterol (TC), triglycerides (TGs), high density lipoprotein (HDL), very low density lipoprotein (VLDL) and low density lipoprotein (LDL) were analyzed using Erba Chem-5 Plus Analyzer. Serum TC, HDL and LDL levels were significantly decreased in OSMF patients as compared to controls. As the clinical stage progresses, the TC and HDL levels were gradually reduced. All the lipid profile parameters such as TC, TG, HDL, VLDL and LDL progressively reduced as the histological grade advanced. They concluded that there is an inverse relationship between lipid profile and the presence of OSMF.[99]

2.4 Lipids as Biomarkers

2.4.1 Introduction

Basically lipids are defined as a very heterogenous group of biomolecules that are generally insoluble in water but which readily dissolve in non-polar solvents, such as ether and chloroform.[100] 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.[101]

2.4.2 Classification

Lipids can be classified based on their composition and the functions they perform (Figure 5). On the basis of their composition, lipids are broadly classified into simple lipids (esters of fatty acids with alcohol; these include fats, waxes), complex lipids (esters of fatty acids with alcohols containing additional groups such as phosphate, nitrogenous base, carbohydrate, protein *etc.*; these include phospholipids,

non-phosphorylated 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).[102]

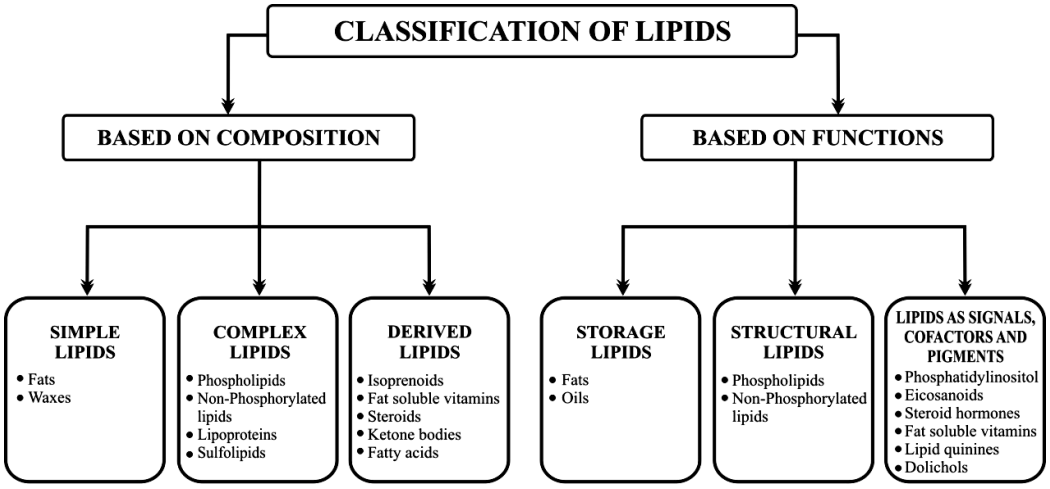

Figure 5: Classification of Lipids[102]

2.4.3 Digestion of lipids in oral cavity, stomach and small intestine

Most of the dietary lipid is in the form of triglycerides, cholesterol, and phospholipids. The digestion of lipids is initiated in the oral cavity by the action of enzyme lingual lipase, with diglycerides being the primary reaction product. The digestion

of lipids in the stomach is almost negligible, because of lack of emulsification and low pH, thus creating an unfavorable environment for the action of gastric lipase. In the small intestine emulsification takes place by three complementary mechanisms: detergent action of bile salts; surfactant action of degraded lipids; mechanical mixing due to peristalsis. This disperses lipids into smaller droplets due to reduction in the surface tension and an increase in the surface area of lipid droplets. The pancreatic enzymes are primarily responsible for the degradation of dietary triacylglycerols, cholesteryl esters and phospholipids. Pancreatic lipase cleaves triacylglycerols to produce 2-monoacylglycerol and free fatty acids. Pancreatic cholesterol esterase cleaves cholesteryl esters to produce cholesterol and free fatty acids. Phospholipids undergo hydrolysis by the action of pancreatic phospholipases.[103]

2.4.4 Absorption and transport of lipids

Bile salts act as biological detergents, converting dietary fats into mixed micelles of bile salts and triacylglycerols, thereby exerting a solubilizing effect on the lipids. The products of lipase action *i.e.* monoacylglycerols, diacyl-glycerols, free fatty acids, and glycerol diffuse into the epithelial cells lining the intestinal surface. In the cells of intestinal mucosa, long chain fatty acids are reconverted into triacylglycerols by the action of enzymes thiokinases and acyl transferases. The resynthesized lipids form lipoprotein aggregates called chylomicrons, which move from the intestinal mucosa into the lymphatic system by exocytosis from which they enter the blood and are carried to muscle and adipose tissue. In the capillaries of these tissues, the extracellular enzyme lipoprotein lipase, activated by apoC-II,

hydrolyzes triacylglycerols to fatty acids and glycerol. These fatty acids and glycerol are taken up by cells in the target tissues. In muscle, the fatty acids are oxidized for energy whereas in adipose tissue, they are reesterified for storage as triacylglycerols.

When the diet contains more fatty acids than are needed immediately for fuel or as precursors, the liver converts them to triacylglycerols, which are packaged with specific apolipoproteins into Very Low Density Lipoprotein Cholesterol (VLDL-C). The VLDL-Cs are transported in the blood to muscle and adipose tissues. In the adipose tissue, the triacylglycerols are removed and stored in lipid droplets within adipocytes, whereas in muscle fatty acids are oxidized to supply energy. The loss of triacylglycerols converts some VLDL-C to VLDL-C remnants, also called Intermediate Density Lipoprotein Cholesterol (IDL-C), and with further removal of triacylglycerol to Low-Density Lipoprotein Cholesterol (LDL-C). LDL-Cs carry cholesterol to extrahepatic tissues that have specific plasma membrane receptors for LDL-C. These receptors mediate the uptake of cholesterol and cholesteryl esters. High Density Lipoprotein Cholesterol (HDL-C) mediates the transport of excess cholesterol in extrahepatic tissues back to the liver.[103]

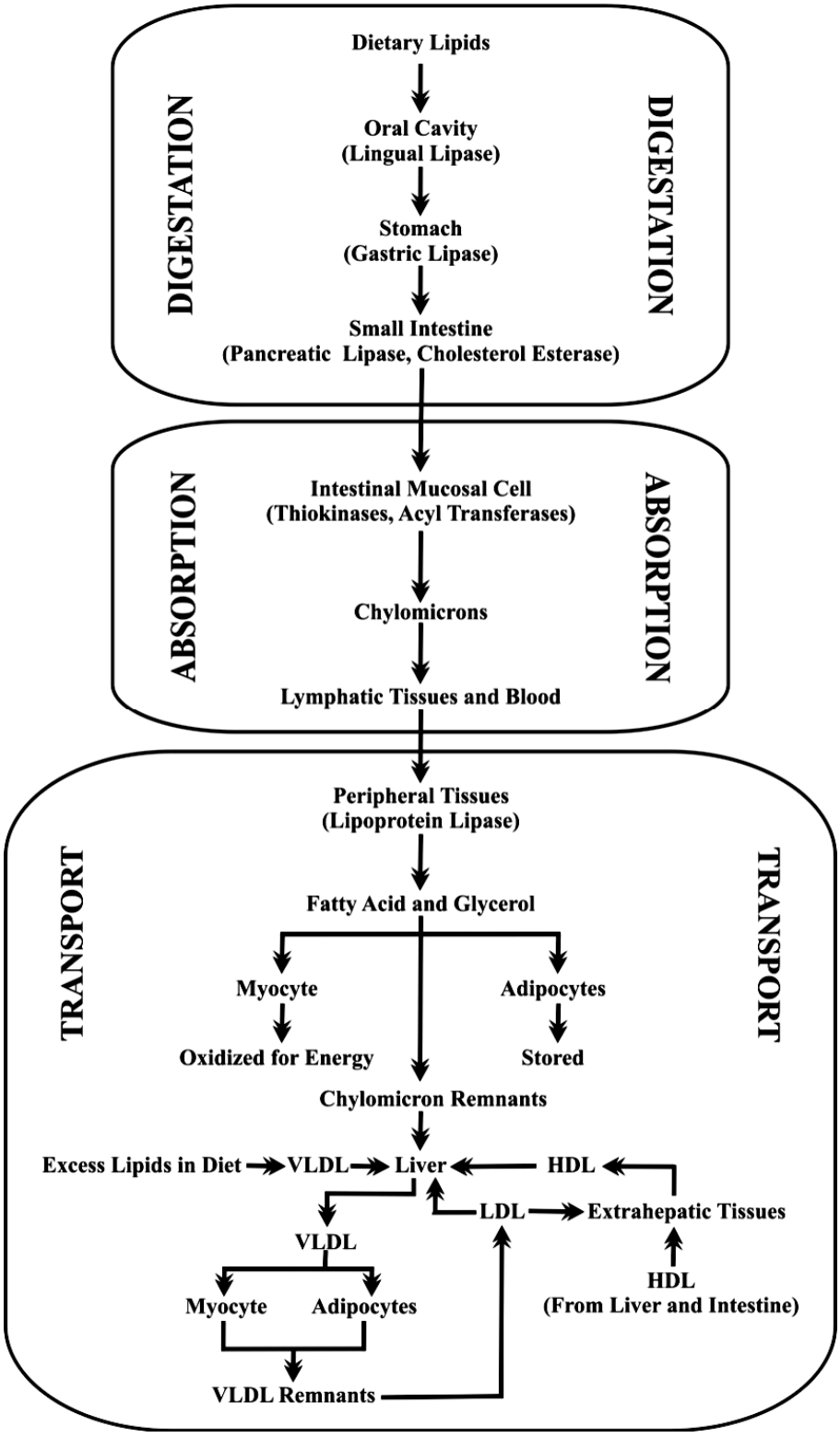

Figure 6: Lipid Metabolism[103]

2.4.5 Lipid Profile Patterns in Oral Cancer

Lipids in malignant tumors are not only necessary for providing the membrane constituents of proliferating cells but are also needed for energetic, biophysical and signaling pathways that drive tumorigenesis. Dysregulated lipid metabolism is a hallmark of cancer.[104] Cancer specific modifications of the lipid metabolism can affect the production of specific signaling lipids, such as factors derived from poly-unsaturated fatty acids and alter the availability of specific Fatty Acids (FA) pools required for protein modification.[105]

Furthermore, researchers have been intrigued in recent years with the possible role of dietary and endogenous lipids in the etiology and prognosis of cancer. Cholesterol, which is recognized to be important factor in the etiology of coronary heart disease, has recently become the focus of attention on the possible role in the etiology of cancer. There is a consistent surge of studies showing an increased mortality in cancer subjects with low plasma cholesterol levels.[106] There exists a controversy that hypocholesteremia is a predisposing factor for cancer development, or hypocholesteremia is in fact the result rather than the cause of cancer. Current theories regarding cancer causation have generated interest in variables such as levels of serum cholesterol and triglycerides as potential associations with cancer relating to dietary factors or basic constitutional factors.[107]

Cancer tissues are able to synthesize lipids de novo and it has also been demonstrated that the amount of lipid synthesis in cancer tissue is comparable to liver.[108] It has been shown that adipocytes promote homing, migration and invasion of cancer cells. They sustain cancer metastases by providing energy for rapid tumor

growth. Furthermore, co-culture of adipocytes and cancer cells demonstrate transfer of lipids from adipocytes to cancer cells, enhanced lipolysis in adipocytes and elevated β-oxidation in cancer cells.[109]

It has been consistently observed that in some malignant diseases, blood cholesterol undergoes early and significant changes. Cholesterol and Triglycerides (TGL) are important lipid constituents of the cell and are essential to carry out several vital physiological functions.

Cholesterol is essential for maintenance of the structural and functional integrity of all biological membranes. It is also involved in the activity of membrane-bound enzymes and is important for stabilization of the DNA helix. Several prospective and retrospective studies have shown an inverse association between blood lipid profile and different cancers.[1,110]

Lohe *et al.* (2010) have observed an inverse relationship between serum lipid profile and oral cancer / precancer.[5]

Patel *et al*. (2004) have also observed an inverse relationship between lower plasma lipid profile and head and neck malignancies/oral precancerous conditions. Furthermore, some investigators have also found a relation of low serum cholesterol with increased risk of cancer occurrence and mortality.[1]

It also needs to be emphasized that cellular uptake and regulation of cholesterol is mediated by lipoprotein receptors especially located on the surface of the cells. For transport in plasma, TGL and cholesterol are packaged into lipoproteins, which are then taken up and degraded by cells to fulfill the demands for cellular functions.[1]

Thus, since lipids play a substantial role in maintaining cellular integrity, it is not surprising that altered lipoprotein patterns also have been associated with malignancies. Patients suffering from Oral cancer exhibit altered levels of TC, TGL, HDL-C, LDL-C and VLDL-C[111]. Also, altered lipid profile patterns are observed in other malignancies such as hematological neoplasms, breast cancer, ovarian cancer, *etc.*[112]

2.4.6 Clinical Review of Lipids in PMDs

Gupta S, Gupta S (2011) [113] conducted a study on 25 clinically diagnosed and histopathologically proven patients of oral squamous cell carcinoma and 15 healthy controls were compared with 15 patients each of OSMF, leukoplakia, and lichen planus. In these groups, serum lipids including: (i) total cholesterol, (ii) high density lipoprotein cholesterol (HDLC), and (iii) triglycérides were analyzed. A significant decrease in plasma total cholesterol, HDLC, and triglycerides was observed in the patients with the precancerous lesions and conditions as compared to the controls. Thus, the result of present study shows an inverse relationship between plasma lipid levels and patients. However, they had higher levels of cholesterol and lower levels of HDLC and triglycérides as compared to the oral squamous cell carcinoma group.

V Reddy et al (2011) [114] conducted a study which was aimed to correlate the etiological factors to the severity of clinical grading along duration, frequency and style of chewing habit. Cross sectional study design of 390 oral submucous fibrosis (OSMF) patients who attended the dental clinic in Central India, Indore, over a period of 3 years was done. Grade I OSMF was seen in 50.51% (197), grade II OSMF in 28.20% (110) and grade III OSMF in 21.28% (83) subjects, with a male to

female ratio of 2.3:1. Gutkha and other arecanut products was practiced most commonly and showed significant risk in the severity of OSMF it was concluded in this study that the relative risk of OSMF increased with duration, frequency and style of chewing habits for longer duration and swallowing it without spitting.

A study by Kumar P et al (2012)[115] was aimed to evaluate the alteration in plasma lipid profile in oral submucous fibrosis (OSF) patients. A total of 30 patients were included in the study, 20 with oral submucous fibrosis and 10 healthy controls. Fasting plasma lipid profile including Total Cholesterol (TC), Very Low Density Lipoproteins (VLDL), Low Density Lipoproteins (LDL), High Density Lipoproteins (HDL) and Tri-Glycerides (TG) were measured using semiautomatic analyser. The data obtained were analysed using independent sample 't' test. A statistically significant decrease in plasma total cholesterol, LDL and HDL was observed in patients with OSMF as compared to the controls, but it was not statistically significant for VLDL and TG values. It was concluded that there is an inverse relationship between lipid profile and the presence of oral submucous fibrosis. Hence, alteration in plasma lipid profile may have a diagnostic role in the future and can be used as a biochemical indicator to detect the initial changes seen in the neoplastic process.

Sharma G et al (2013)[116] conducted a study in 10 clinically diagnosed patients of OSMF. Fasting blood samples were collected in plain vials and their lipid profiles were analysed and then compared with the normal standardized values showed a significant decrease in serum cholesterol LDLC and LDL/HDLC ratio, whereas

HDLC was found to be raised in some patients in the study group as opposed to controls.

Maximum patients were in 3rd to 4th decade and male predominance was seen. Their lipid profile when compared with the normal standardized values showed a significant decrease in serum cholesterol, LDL cholesterol and LDL/HDL ratio. The LDL cholesterol was significantly lower in OSMF patients alongwith VLDL and LDL/HDL ratio. They concluded that the lower lipid status may be considered as a useful indicator for initial changes occurring in the neoplastic cells.

Singh S *et al*. (2013) [117] conducted a study on 75 subjects, 50 individuals were oral carcinoma patients and 25 individuals were healthy controls. The parameters assessed included total cholesterol (TC), high□density lipoprotein-cholesterol (HDLC), low-density lipoprotein-cholesterol, very low-density lipoprotein- cholesterol and triglycerides (TGL). These groups were subdivided into subjects with no habit of tobacco (NHT) and subjects with habit of tobacco (WHT) conducted a study on 30 patients which were included in the study, 20 with oral submucous fibrosis and 10 healthy controls. Fasting plasma lipid profile including Total Cholesterol (TC), Very Low Density Lipoproteins (VLDL), Low Density Lipoproteins (LDL), High Density Lipoproteins (HDL) and Tri□Glycerides (TG) were measured using semiautomatic analyser. Significant decrease in plasma total cholesterol, LDL and HDL was observed in patients with OSMF as compared to the controls, but it was not statistically significant for VLDL and TG values. Thus, the results of the present study show that there is an inverse relationship between lipid profile and the presence of oral submucous fibrosis.

Gupta N *et al.* (2014)[118] conducted a study on 95 subjects who were clinically diagnosed as patients of head and neck cancer and oral submucous fibrosis & age and sex matched healthy controls. The lipid profile values including (i) serum cholesterol (ii) low density lipoprotein cholesterol (iii) high density lipoprotein cholesterol (iv) very low density lipoprotein cholesterol (v) serum triglyceride were estimated. Also the lipid profile values in TNM (primary tumor, regional lymph node, distant metastasis) staging in head & neck cancer patients and functional staging of oral submucous fibrosis patients were also estimated. They found that mean lipid levels were found to be maximum in oral cancer patients for all the parameters except serum high density lipoprotein cholesterol. For all the variables except serum low density lipoprotein cholesterol, minimum values were observed in oral submucous fibrosis patients. For serum low density lipoprotein cholesterol minimum values were observed in control group. They concluded a direct relationship between lipid profile, cancer patients and an inverse relationship between lipid profile and oral submucous fibrosis patients.

Goel P *et al.* (2015) [119] conducted a study on 20 clinically diagnosed patients of OSF, 20 biopsy-proven cases of leukoplakia, 20 biopsy-proven cases of lichen planus and 20 subjects in the control groups were studied and serum lipids including the following were analyzed: (i) serum cholesterol, (ii) serum triglyceride, (iii) low-density lipoprotein (LDL), (iv) high-density lipoprotein (HDL), and (v) very low-density lipoprotein (VLDL). They found that Serum lipid profile had inverse relationship with oral precancerous conditions/lesions. Serum triglycerides and VLDL levels showed significant reduction in patients with leukoplakia and lichen

planus as compared with controls. No significant correlation of the lipid has been found in the OSF patients.

Poorey V.K, Thakur P (2015)[120] conducted a study on newly diagnosed and histologically confirmed, 100 head and neck malignancy cases. Fasting blood samples were collected and the lipid profile studied. They found that there is a preponderance of head and neck malignancy in the age group of 41–60 years, males having the higher incidence. Malignancy involving oral cavity were the commonest and majority were well differentiated. Statistically, there was a highly significant reduction of mean serum total cholesterol (TC), triglycerides and high density lipoproteins (HDL) in the subjects of head and neck malignancy as compared to the control group.

Neeta Misra et al (2017)[121] in conducted a study including 50 subjects ,25 subjects with OSMF and 25 subjects having habit of tobacco but no OSMF .She concluded that lower serum lipid status may be considered as a useful indicator for initial changes occurring in the neoplastic cells

CHAPTER-2

MATERIALS AND METHODOLOGY

3.1 INTRODUCTION

This study was conducted in Department of Oral Medicine and Radiology of Babu Banarasi Das College of Dental Sciences, Lucknow. Ethical clearance for the study was obtained from the institutional ethical committee.

The study population was drawn from the patients attending the outpatient Department of Oral Medicine & Radiology. In the present study 100 subjects out of with clinically diagnosed Oral Submucous Fibrosis were enrolled and 100 controls with no apparent lesions of the oral mucosa having tobacco habit.

The criteria for diagnosis of OSMF were restricted mouth opening, presence of palpable vertical fibrous band, stiffness and blanching, W.H.O criteria [122]

3.2 ARMAMENTARIUM [Figure-7(a)]

3.2.1 Examination of patient

1. Electrically operated dental chair with illumination.
2. A pair of sterile disposable gloves
3. Disposable mouth mask
4. Stainless steel kidney tray
5. Mouth mirror (No. 5)
6. Straight probe

7. Tweezers and
8. Explorer.
9. Sterile Gauze piece and cotton swab.
10. Vernier caliper

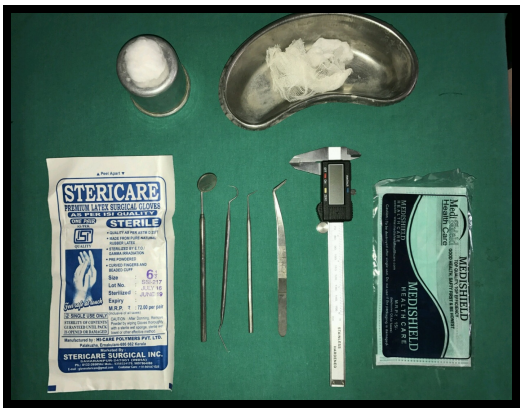

Figure 7(a) : Armamentarium

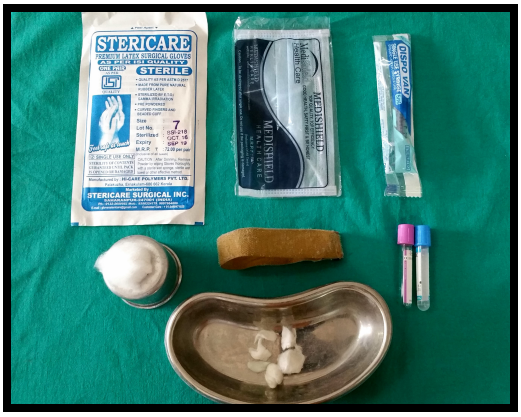

Figure 7(b) : Armamentarium

Figure 8: Measurement of mouth opening

3.2.2 Collection of serum samples [Figure-7(b)]

1. A pair of sterile disposable gloves
2. Disposable mouth mask
3. Sterile cotton
4. Tourniquet
5. Sterile disposable syringe -5ml
6. Sterile disposable needle 23gauge×25mm (Dispovan)
7. Test tubes 12×75mm
8. Centrifugation machine (REMI R8C Laboratory Cenrifuge) 3000 rpm.

Figure 9: Centrifugal Machine

3.2.3 Estimation of lipid profile

1. ERBA CHEM-5 chemical analyzer
2. Des kits from Erba Diagnostics, Daman
3. Micro pipette with tip (250121, 1000 pl)

3.3 Patient Selection

The study subjects were made to sit comfortably on a dental chair. Patients were examined under artificial illumination using sterile gloves. The clinical examination was carried out following the method described by Kerr, Ash and Millard[122] and relevant data were entered into the proforma. Detailed interview with emphasis on recording any oral habits of chewing areca nut, paan (betel quid), and gutkha (commercially available processed tobacco containing preparations), the duration of habit and the frequency of each habit per day. Each patient was informed about the protocol and was given appropriate instructions after obtaining a written consent.

3.3.1 Inclusion Criteria

1. Subjects who are healthy and well oriented to time, place and person.
2. Subjects of either sex aged between 25 -55 yrs.
3. Subjects with habit of tobacco consumption in any form.
4. Subjects with clinically diagnosed oral submucous fibrosis.

3.3.2 Exclusion Criteria

1. Subjects who have undergone any treatment for OSMF previously.
2. Subjects suffering from any known systemic disease that can alter lipid profile.
3. Pregnant, menstruating and subjects on oral contraceptives.
4. Subjects failing to give their consent.

Following the establishment of the diagnosis, each patient was educated about the nature of the condition, its precancerous potential and motivated to discontinue the use of areca nut, tobacco or any abusive habit in any form.

All patients underwent an oral prophylaxis procedure to remove extrinsic stains. This was done to motivate the patient toward recovery and to know if the patient resumed the habit.

Khanna and Andrade[58] classification system of OSMF based on interincisal opening was used for the study. (Figure 8)

- Group 1 : Early OSMF without trismus (MIO >35mm)
- Group 2 : Mild to moderate disease (MIO 26 to 35 mm)
- Group 3 : Moderate to severe disease (MIO 15 to 25 mm)
- Group 4a: Severe disease (MIO <15 mm)
- Group 4b: Extremely severe–malignant/premalignant lesions noted intraorally.

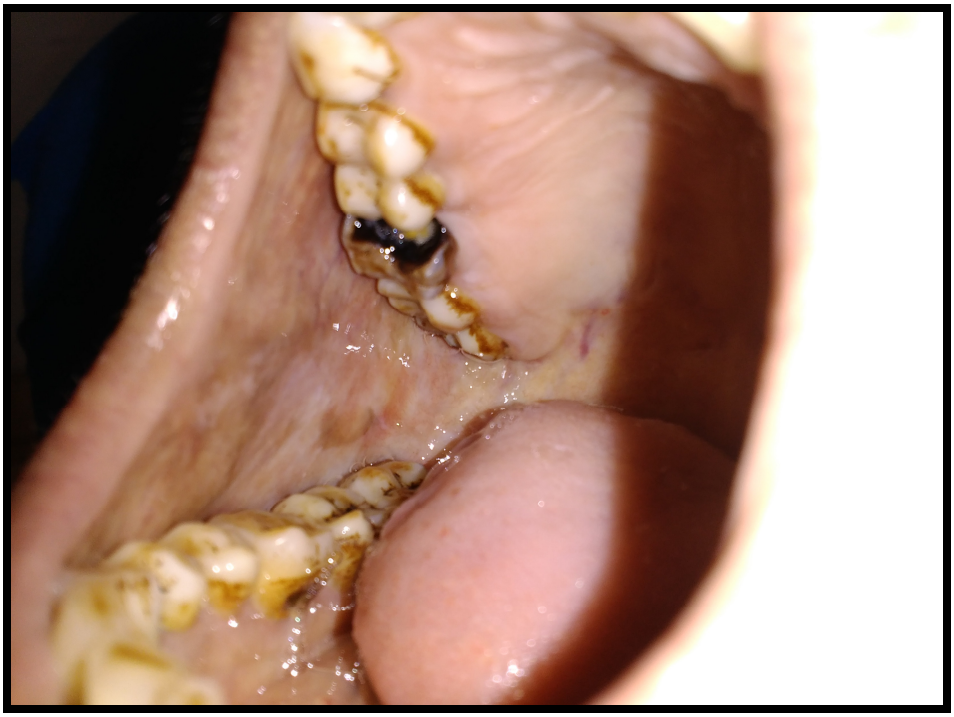

Figure 10: Blanching of right buccal mucosa

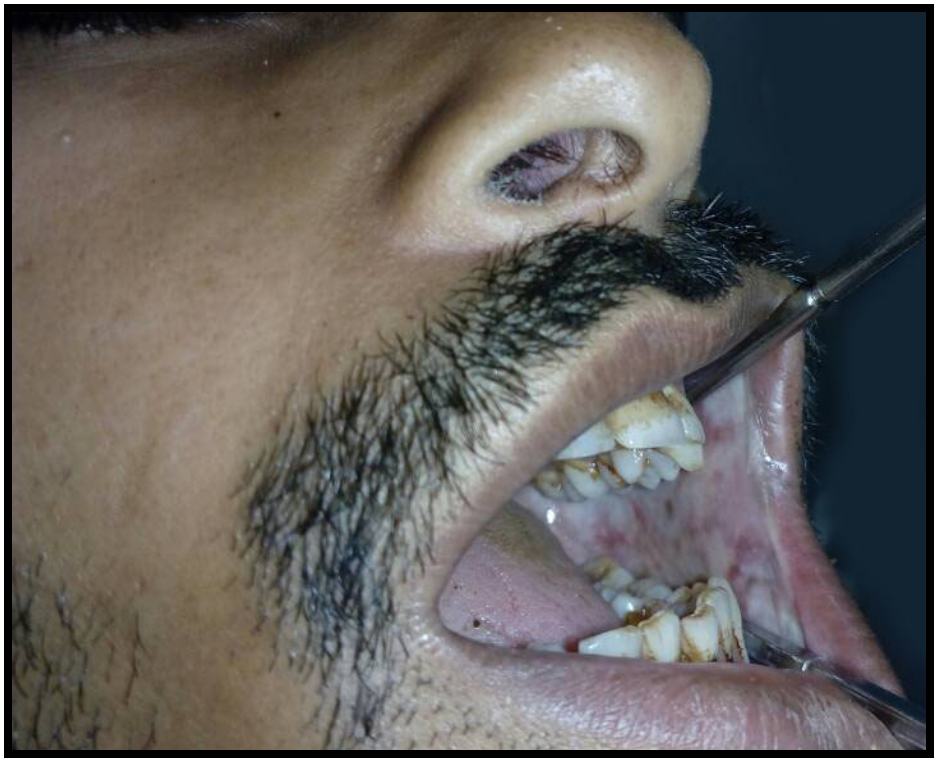

Figure 11: Blanching of left buccal mucosa

Mouth opening (Figure 8)

This was assessed as the interincisal distance as measured from the mesioincisal edge of the upper left central incisor tooth to the mesioincisal edge of the lower left central incisor tooth. The measurement was made using a vernier caliper and was recorded in millimeters.

Burning sensation

The intensity of burning sensation was determined using a Visual Analogue Scale (VAS) of 0-100, where 0 is no burning sensation and 100 is the worst possible burning sensation. All the relevant data were entered in the proforma.

3.4 Method of serum sample collection

Patient was explained regarding the procedure. The patients cubital fossa was disinfected with cotton swab dipped in spirit. Then 5m1 of blood was withdrawn from each subject from anti-cubital vein by using disposable syringe of 23 gauge needle. (Figure 12) Drawn blood was kept in the test tubes (Figure 13) and allowed to clot for 30 minutes before centrifugation for 15 minutes at 3000 rpm and preserved in a frozen state at 2 to 8°C for 5 days until analysis. Serum was then separated carefully after clot formation and was used for the study. Fresh, clean and non haemolyzed serum from patients was used for the assay.

3.5 Method of Estimation of Plasma Lipid Profile

3.5.1 Total cholesterol

Plasma levels were estimated using cholesterol des kits obtained from Erha Diagnostics, Mannheim. Methodology used was dynamic extended stability, CHODPAP method, end point with lipid clearing agent. Methodology: Modified Roeschlau S method (Roeschlaus P. *et al.* 1974).[123]

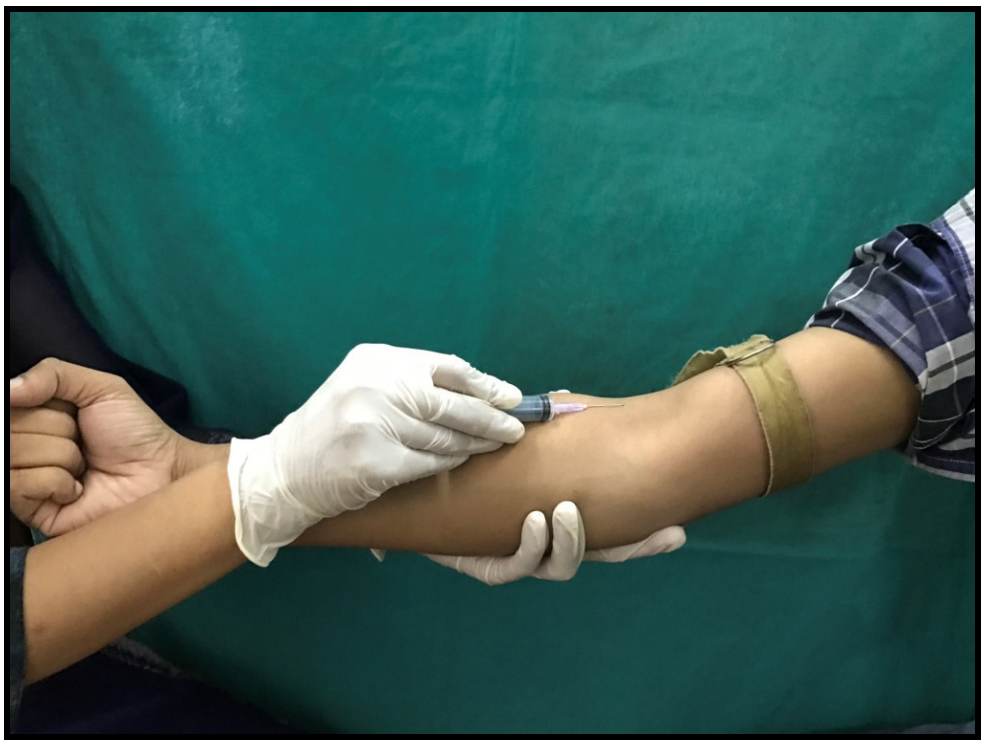

Figure 12: Collection of venous blood

Figure 13: Serum Samples

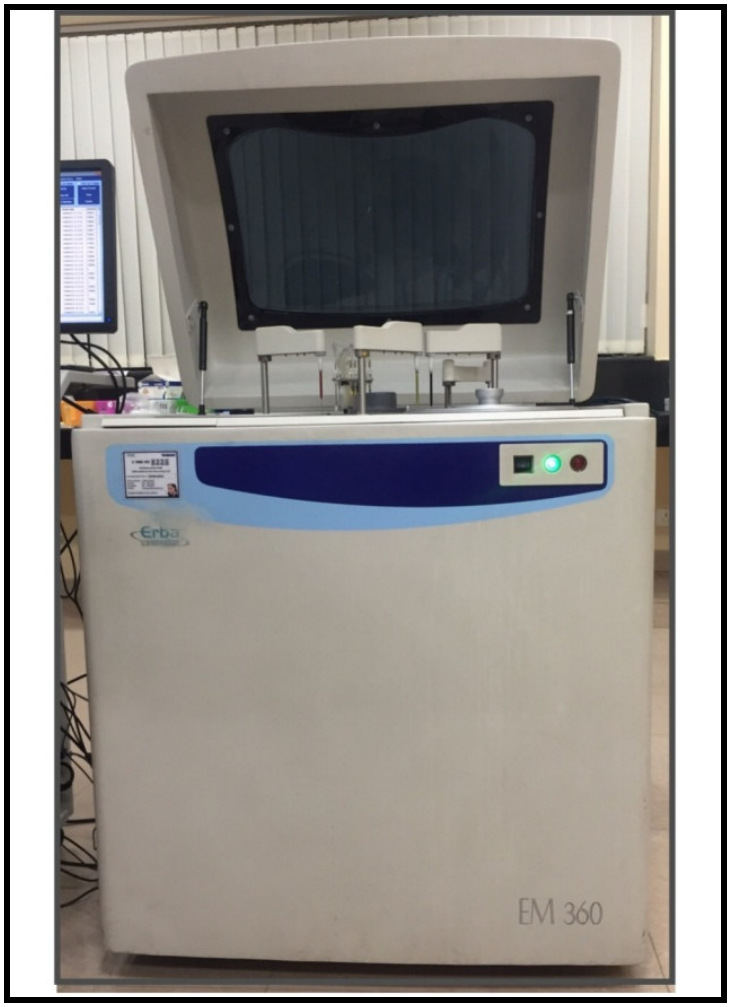

Figure 14: Anlalysis Armamentarium

Principle

The estimation of cholesterol involves the following enzyme catalyzed reactions.

1. Cholesterol ester is converted into cholesterol and fatty acid in presence of cholesterol esterase.
2. Cholesterol then combines with oxygen in the presence of cholesterol oxidase and forms cholest-4-en-3-one and H_2O_2.

3. 2 molecules of H_2O_2 combines with 4aminoantipyrine and phenol and forms 4 molecules of water and quinoeimine. Absorbance of quinoeimine so formed is directly proportional to cholesterol concentration.

Reagent composition:

Reagent 1: cholesterol reagent

Cholesterol esterase > 200 IU/L

Cholesterol oxidase >150 IU/L

Peroxidase (horseradish) > 2000 1U/L

Sodium phenolate 20 mmol/L

4 aminoantipyrine 0.5 mmol/L

Phosphate buffer (pH: 6.5±0.1) 68 mmol/L

Lipid clearing agent

Reagent 2: Cholesterol 200 mg /dl

5.2 m molt L

Reagent 3: aqua 4

Double deionized, 0.2 micron, membrane filtered, particle free water for reconstitution of reagent 1

Reagent Constitution

Allow reagent 1 and reagent 3 to attain room temperature. Add the amount of aqua 4 indicated on the label to contents of each vial of reagent 1, swirl to dissolve, do not shake vigorously.

A blank solution is prepared by adding 1000µl of reagent to 20µl of distilled water. A standard solution is prepared by adding 1000µl of reagent to 20µl of cholesterol standard. A test solution is prepared by adding 1000µl of reagent to 20µl of sample. Blank is aspirated followed by standard and tests. The mixture is shook well and incubated at 370c. The three solutions are read by using the analyzer. The blank solution is read first followed by standard and test solution.

3.5.2 High Density Lipoprotein Cholesterol

Plasma levels were measured using HDL cholesterol kits obtained from Erba Diagnostics, Mannheim.

Methodology: Phosphotungstic acid method, end point.

Principle

Chylomicrons, LDL and VLDL are precipitated from serum by phosphotungstate in the presence of divalent cations such as magnesium. The HDL cholesterol remains unaffected in the supernatant and is estimated using Erba Cholesterol reagent. Serum / plasma in the presence of phosphotunstate and magnesium ions is dissociated to form HDL (supernatant) and LDL + VLDL + chylomicrons (precipitate).

Reagent composition

Reagent 1: Precipitating agent

Phosphotungstic acid 2.4mmol/L

Magnesium chloride 40 mmol/L

Reagent 2 : HDL cholesterol standard 25 mg/dl (Figure 15)

Reagent preparation

The reagent is ready for use –

Supernatant of sample is obtained by adding 500µl of precipitant reagent to 250µl of sample. Mixture is shook well and allowed to stand for 10 minutes at room temperature. Then the mixture is centrifuged at 4000 rpm for 10 minutes and clear supernatant fluid is obtained which is used to determine the concentration of HDL cholesterol in the sample. A blank solution is prepared by adding 1000µl of reagent to 50µl of distilled water. A standard solution is prepared by adding 1000µl of sample to 50µl of HDL cholesterol standard. A test solution is prepared by adding 1000µl of sample to 50µl of supernatant. The solutions are mixed well and incubated at 370c for 10 minutes. The solutions are read using the analyzer. The blank solution is read first followed by standard and test solution.

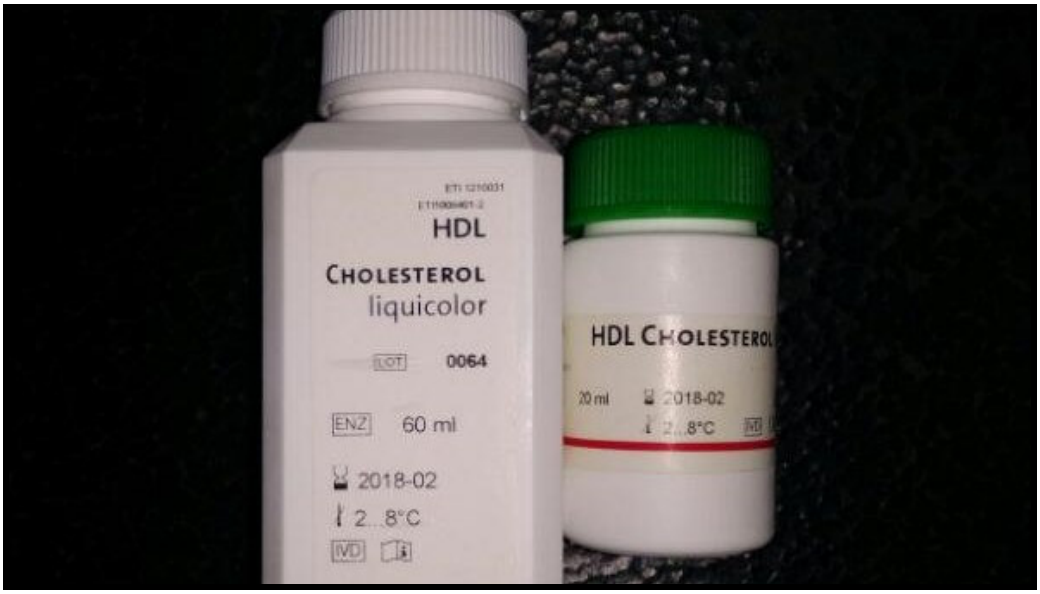

Figure 15: Reagent preparation –HDL Cholesterol

3.5.3 Triglyceride

- Plasma levels were estimated from triglyceride des kit obtained from Erba.
- Diagnostics Mannheim.

Methodology: Dynamic extended stability with lipid clearing agent, GPO- Winder method, end point.

Principle :

1. Triglyceride combines with water in presence of lipase and forms glycerol and free fatty acids.
2. Glycerol along with ATP in presence of glycerol kinase forms glycerol 3 phosphate and ADP.
3. Glycerol 3 phosphate along with oxygen in presence of glycerol phosphate oxidase firms dihydroxyacetone phosphate and H202.
4. H_2O_2 along with 4 aminoantipyrine and 3, 5 dichloro-2-hydroxybenzene sulfonate in presence of peroxidase forms quinoneimine dye and 2 molecules of water. The intensity of chromogen (quinoneimine) formed is proportional to the triglyceride concentration in the sample.

Reagent composition: (Figure 16)

Reagent 1: triglyceride des reagent

Active ingredient Concentration:

- ATP 2.5 mmol/L
- Mg2+ 2.5 mmol/L

- 4 Aminoantipyrinc 0.8 mmol/L
- 3,5 dichloro-2-hydroxybenzene sulfonate 1 mmol/L
- Peroxidase > 2000 U/L
- Glycerol kinase > 550 U/L
- Glycerol phosphate oxidase > 8000 U/L
- Lipoprotein lipase > 3500 U/L
- Buffer (p1-1: 7.0 + 0.1 at 200C 53 mmol/L

Also contains non reactive fillers, stabilizers and surfactants

Reagent 2: Triglyceridc standard

Triglyceride standard 200 mg/dL

Figure 16: Reagent preparation – Triglyceridc standard

Reagent reconstitution

Allow the reagent bottle and aqua 4 (supplied in the kit) to attain room temperature. Add the amount of aqua 4 indicated on the label to the contents of each vial. Swirl to dissolve, allow to stand for 10 minutes at room temperature and do not shake vigorously. A blank solution is prepared by adding 100µl of reagent to 10µl of distilled water. A standard solution is prepared by adding 1000µl of reagent to 10µl of triglyceride standard. A test solution is prepared by adding 1000µl of reagent to 1000µl of sample. The solutions are mixed well and incubated at 370c for 10 minutes.

The three solutions are read by using the analyzer. Blank solution is read first followed by standard and test solution. Very low density lipoprotein cholesterol: calculated by the following formula:

VLDL = Triglyceride / 5

Low density lipoprotein cholestero1: was calculated by using the following formula: LDL = Total cholesterol-(VLDL- HDL).

The normal values established in our laboratory are :

Parameter Normal serum concentration (mg%)– According to Friedawald WT, 1972. 124

Total cholesterol 189 ± 33

- TGL 73± 9
- HDL 53 ± 13
- LDL 122 ± 28
- VLDL 14±9

The study group was compared with above established normal values. The results were tabulated and statistically analyzed.

3.6 Statistical Analysis

The results are presented in frequencies, percentages and mean±SD. The Chi-square test was used to compare the categorical/dichotomous variables between cases and controls. The Unpaired t-test was used to compare the continuous variables between cases and controls. The univariate and multivariate binary logistic regression was used to find the risk factors associated with disease. The unadjusted and adjusted odds ratio (OR) with its 95% confidence interval (CI) was calculated. Pearson correlation coefficient was calculated. The p-value<0.05 was considered significant. All the analysis was carried out on SPSS 16.0 version (Chicago, Inc., USA).

Formula

3.6.1 Mean and standard deviation (SD)

The *sample mean* is the average and is computed as the sum of all the observed outcomes from the sample divided by the total number of events. We use x as the symbol for the sample mean. In math terms,

$$\bar{x} = \frac{1}{n}\sum_{i=1}^{n} x \qquad (3.1)$$

where n is the sample size and the x correspond to the observed valued.

We define the *variance* to be

$$s^2 = \frac{1}{n-1}\sum_{i=1}^{n}(x-\bar{x})^2 \qquad (3.2)$$

and the *standard deviation* to be

$$s = \sqrt{\frac{1}{n-1}\sum_{i=1}^{n}(x-\bar{x})^2} \qquad (3.3)$$

3.6.2 Chi-square test

The test statistic is a chi-square random variable (X^2) defined by the following equation.

$$X^2 = \Sigma\ [\ (O_{r,c} - E_{r,c})^2 / E_{r,c}\ ] \qquad (3.4)$$

where $O_{r,c}$ is the observed frequency count at level *r* of Variable A and level *c* of Variable B, and $E_{r,c}$ is the expected frequency count at level *r* of Variable A and level *c* of Variable B.

$$intercept = b = \bar{y} - m\bar{x} \qquad (3.5)$$

3.6.3 Binary logistic regression

Logistic regression analysis is a popular and widely used analysis that is similar to linear regression analysis except that the outcome is dichotomous (e.g., success/failure or yes/no or died/lived). The epidemiology module on Regression

Analysis provides a brief explanation of the rationale for logistic regression and how it is an extension of multiple linear regression. In essence (see page 5 of that module). In essence, we examine the odds of an outcome occurring (or not), and by using the natural log of the odds of the outcome as the dependent variable the relationships can be linearized and treated much like multiple linear regression.

Simple logistic regression analysis refers to the regression application with one dichotomous outcome and one independent variable; multiple logistic regression analysis applies when there is a single dichotomous outcome and more than one independent variable. Here again we will present the general concept. Hosmer and Lemeshow provide a very detailed description of logistic regression analysis and its applications.[3]

The outcome in logistic regression analysis is often coded as 0 or 1, where 1 indicates that the outcome of interest is present, and 0 indicates that the outcome of interest is absent. If we define p as the probability that the outcome is 1, the multiple logistic regression model can be written as follows:

$$\hat{p} = \frac{\exp(b_0 + b_1X_1 + b_2X_2 + ... + b_pX_p)}{1 + \exp(b_0 + b_1X_1 + b_2X_2 + ... + b_pX_p)} \qquad (3.6)$$

$\hat{p}$ is the expected probability that the outcome is present; X_1 through X_p are distinct independent variables; and b_0 through b_p are the regression coefficients. The multiple logistic regression model is sometimes written differently. In the following form, the outcome is the expected log of the odds that the outcome is present,

$$\ln\left(\frac{\hat{p}}{(1-\hat{p})}\right) \tag{3.7}$$

$$\ln\left(\frac{\hat{p}}{(1-\hat{p})}\right) = b_0 + b_1X_1 + b_2X_2 + \ldots + b_pX_p \tag{3.8}$$

Notice that the right hand side of the equation above looks like the multiple linear regression equation. However, the technique for estimating the regression coefficients in a logistic regression model is different from that used to estimate the regression coefficients in a multiple linear regression model. In logistic regression the coefficients derived from the model (e.g., b_1) indicate the change in the expected log odds relative to a one unit change in X_1, holding all other predictors constant.

3.6.4 Unpaired t-test

The unpaired t method tests the null hypothesis that the population means related to two independent, random samples from an approximately normal distribution are equal.

$t=x_1-x_2/(sqrt(1/n_1+1/n_2)$

$s=[sum(x_j-x_1)^2+sum(x_i-x_2)^2]/(n_1+n_2-2)$

where x_1 and x_2 are the sample means, s^2 is the pooled sample variance, n_1 and n_2 are the sample sizes and t is a Student t quantile with $n_1 + n_2 - 2$ degrees of freedom.

3.6.5 Pearson correlation coefficient

Correlation between sets of data is a measure of how well they are related. The most common measure of correlation in stats is the Pearson Correlation (r). The full name is the Pearson Product Moment Correlation or PPMC. It shows the linear relationship between two sets of data.

$$r = \frac{n(\Sigma xy) - (\Sigma x)(\Sigma y)}{\sqrt{[\, n\Sigma x^2 - (\Sigma x)^2 \,][\, n\Sigma y^2 - (\Sigma y)^2 \,]}} \qquad (3.9)$$

CHAPTER-4

OBERVATIONS AND RESULTS

4.1 Age distribution

Table-1: Age distribution of cases and controls

Age in years	Cases (n=100)		Controls (n=100)		p-value[1]
Age in years	**No.**	**%**	**No.**	**%**	
<30	38	38.0	35	35.0	0.81
30-40	30	30.0	36	36.0	
41-50	29	29.0	27	27.0	
>50	3	3.0	2	2.0	
Mean±SD (Range)	36.14±9.36		34.59±8.48		

[1]Chi-square test

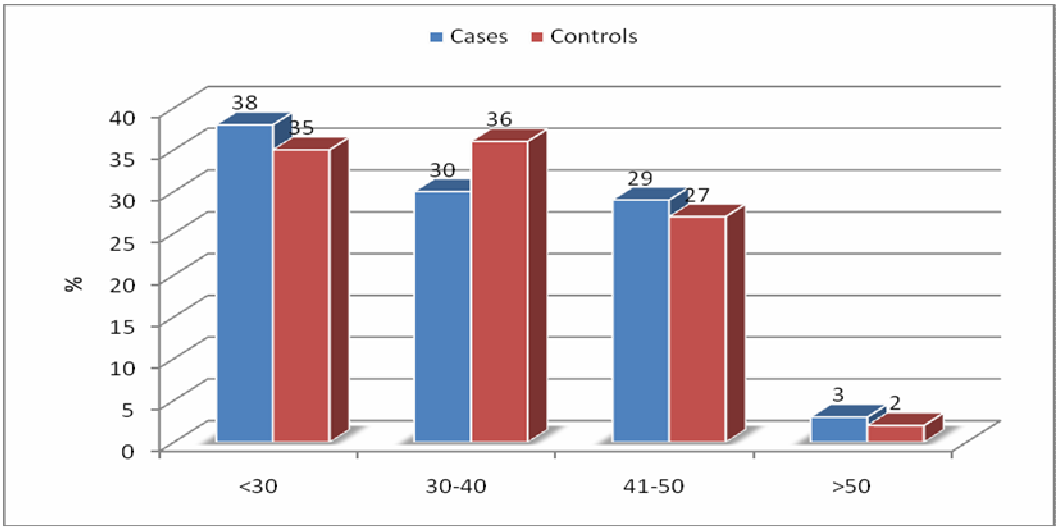

Figure 17: Age distribution of cases and controls

Table 1 & Figure 17 shows age distribution of cases and controls. More than one third of cases (38%) and 35% of controls were below 30 years of age. However, 30% of cases and 36% of controls were between 30-40 years. The mean age of cases and controls was 36.14±9.36 and 34.59±8.48.

4.2 Gender distribution

Table-2: Gender distribution of cases and controls

Gender	Cases (n=100)		Controls (n=100)		p-value[1]
	No.	%	No.	%	
Male	97	97.0	98	98.0	0.65
Female	3	3.0	2	2.0	

[1]Chi-square test

Table 2 shows gender distribution of cases and controls. Majority of both cases (97%) and controls (98%) were males. There was significant (p>0.05) association of age and gender between cases and controls.

4.3 Smokeless tobacco habit between cases and controls

Table-3: Comparison of smokeless tobacco habit between cases and controls

Smokeless tobacco habit	Cases (n=100)		Controls (n=100)		OR (95% CI)	p-value[1]
	No.	%	No.	%		
User	92	92.0	77	77.0	3.43 (1.45-8.11)	0.003*
Non-user	8	8.0	23	23.0	1.00 (Ref.)	

OR-Odds ratio, CI-Confidence interval, [1]Chi-square test, *Significant, Ref.-Reference category

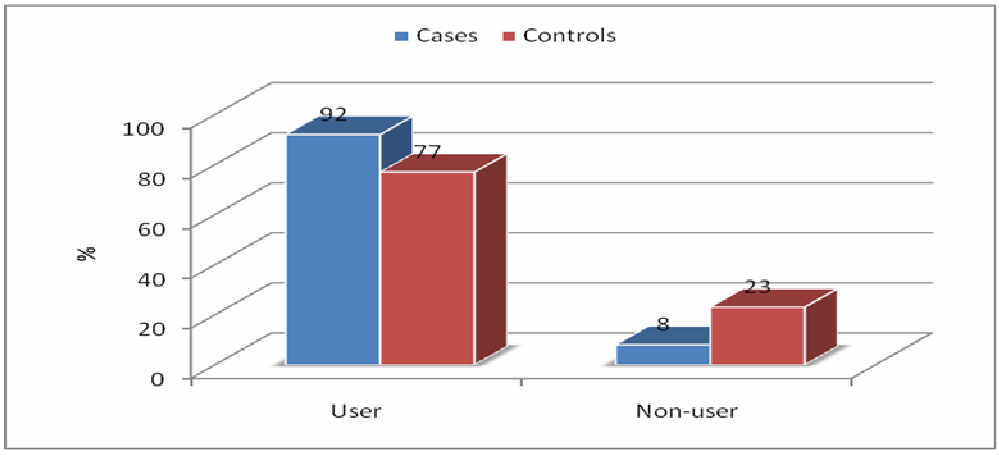

Figure 18: Comparison of smokeless tobacco habit between cases and controls

Table 3 & Figure 18 shows the comparison of smokeless tobacco habit between cases and controls. The percentage of smokeless tobacco users in the cases (92%) was higher than controls (77%). The smokeless tobacco users were 3.43 times significantly higher in cases than controls (OR=3.43, 95%CI=1.45-8.11, p=0.003).

4.4 Smoking habit between cases and controls

Table-4: Comparison of smoking habit between cases and controls

Smoking	Cases (n=100)		Controls (n=100)		OR (95% CI)	p-value[1]
	No.	%	No.	%		
Smoker	28	28.0	39	39.0	0.60 (0.33-1.10)	0.09
Non-smoker	72	72.0	61	61.0	1.00 (Ref.)	

OR-Odds ratio, CI-Confidence interval, [1]Chi-square test, *Significant, Ref.-Reference category

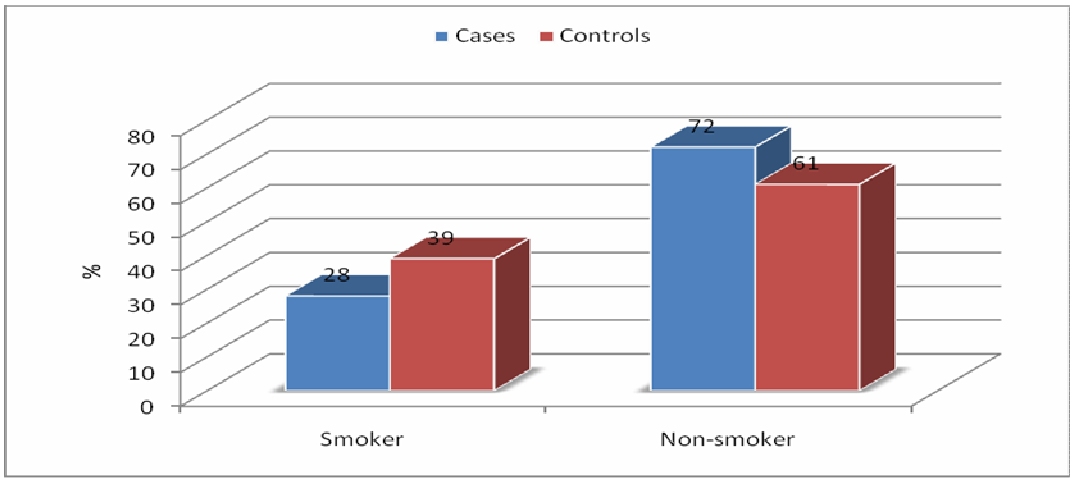

Figure 19: Comparison of smoking habit between cases and controls

Table 4 & Figure 19 shows the comparison of smoking habit between cases and controls. More than half of cases (28%) and 39% of controls were smoker. There was no significant (p>0.05) association of smoking habit between cases and controls.

4.5 Multiple tobacco use between cases and controls

Table-5: Comparison of multiple tobacco use between cases and controls

Multiple tobacco use	Cases (n=100)		Controls (n=100)		OR (95% CI)	p-value[1]
	No.	%				
Both chewer and smoker	20	20.0	16	16.0	1.31 (0.63-2.71)	0.46
Single habit	80	80.0	84	84.0	1.00 (Ref.)	

OR-Odds ratio, CI-Confidence interval, [1]Chi-square test, *Significant, Ref.-Reference category

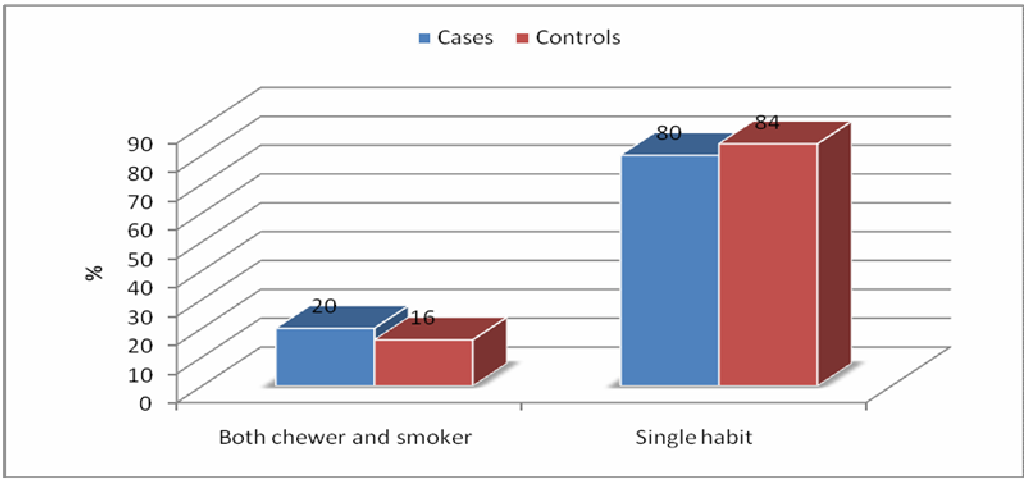

Figure 20: Comparison of multiple tobacco use between cases and controls

Table 5 & Figure 20 shows the comparison of multiple tobacco habit between cases and controls. One fifth of cases (20%) and 16% of controls were both chewer & smoker. The association was statistically insignificant (p>0.05).

4.6 Duration of tobacco use between cases and controls

Table-6: Comparison of duration of tobacco use between cases and controls

Groups	Duration in years (Mean±SD)
Cases	4.55±1.42
Controls	4.27±2.26
p-value[1]	0.29

[1]Unpaired t-test

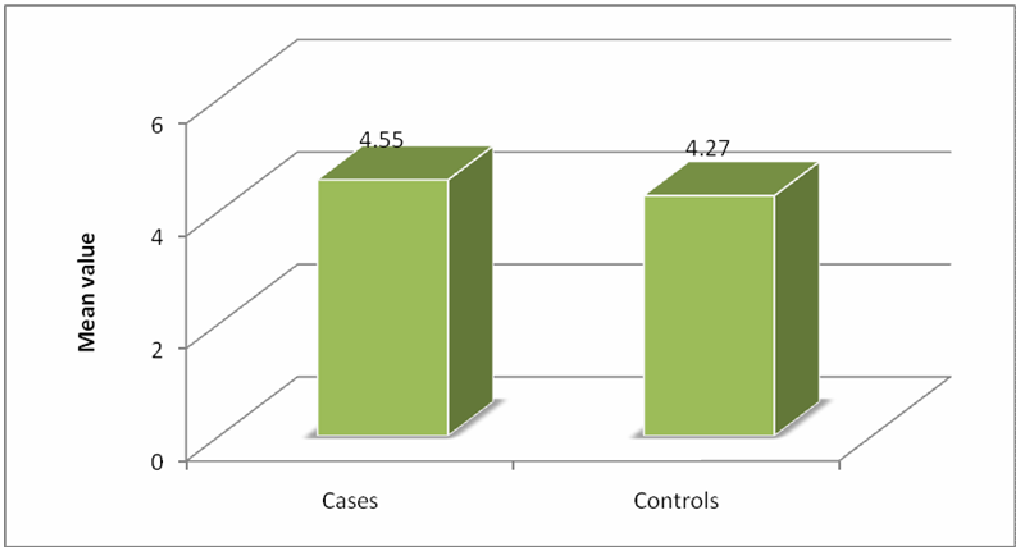

Figure 21: Comparison of duration of tobacco use between cases and controls

Table 6 & Figure 21 shows the comparison of duration of tobacco use between cases and controls. The duration of tobacco use was insignificantly ($p>0.05$) higher among the cases (4.55±1.42) compared to controls (4.27±2.26).

4.7 Duration of tobacco use among male and females between cases and controls

Table-7: Comparison of duration of tobacco use among male and females between cases and controls

Gender	Duration of tobacco use in years		OR (95% CI)	p-value[1]
	Cases	Controls		
Male	4.56±1.43	4.21±2.24	-	-
Female	4.33±1.15	7.00±0.00	Ref.	

[1]Binary logistic regression, OR-Odds ratio, CI-Confidence interval, *Significant

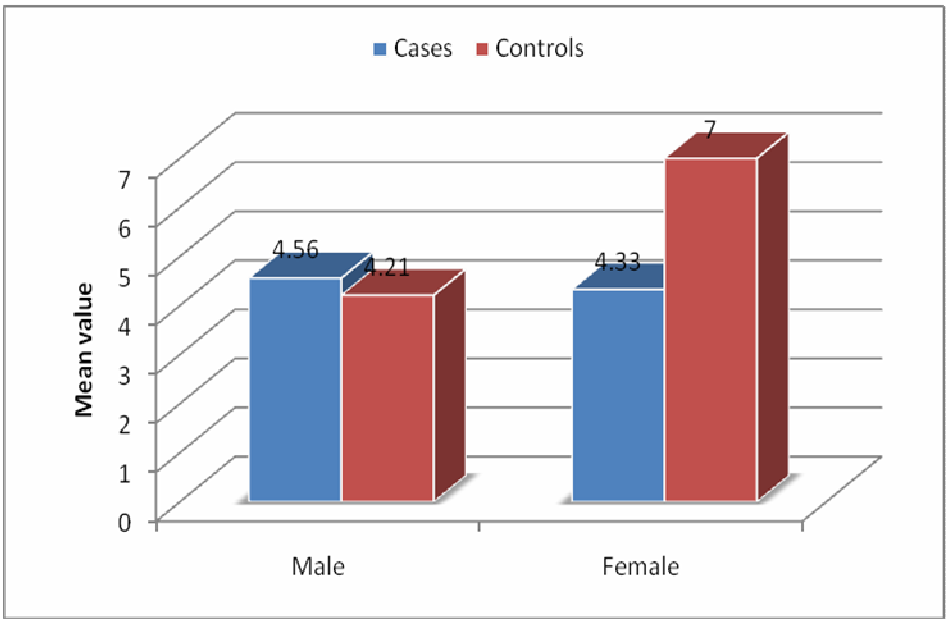

Figure 22: Comparison of duration of tobacco use among male and females between cases and controls

Table 7 & Figure 22 shows the comparison of duration of tobacco use between cases and controls among male and females. The duration of tobacco use was in male cases and control was 4.56±1.43 and 4.21±2.24 years respectively. However, the

duration of tobacco use was in female cases and control was 4.33±1.15 and 7.00±0.00 years respectively.

4.8 Lipid levels between cases and controls

Table-8: Comparison of lipid levels between cases and controls

Lipid profile	Cases	Controls	OR (95% CI)	p-value[1]
Total Cholesterol (Mg%)	138.34±20.86	153.40±23.35	0.97 (0.95-0.98)	0.0001*
HDL Cholesterol (Mg%)	33.66±10.02	42.82±7.22	0.88 (0.84-0.92)	0.0001*
Triglycerides (Mg%)	105.85±57.39	132.85±40.25	0.98 (0.97-0.99)	0.0001*
LDL Cholesterol (Mg%)	81.09±13.44	95.46±13.08	0.90 (0.87-0.93)	0.0001*
VLDL Cholesterol (Mg%)	28.88±10.51	27.89±8.65	1.01 (0.98-1.04)	0.46

[1]Binary logistic regression, OR-Odds ratio, CI-Confidence interval, *Significant

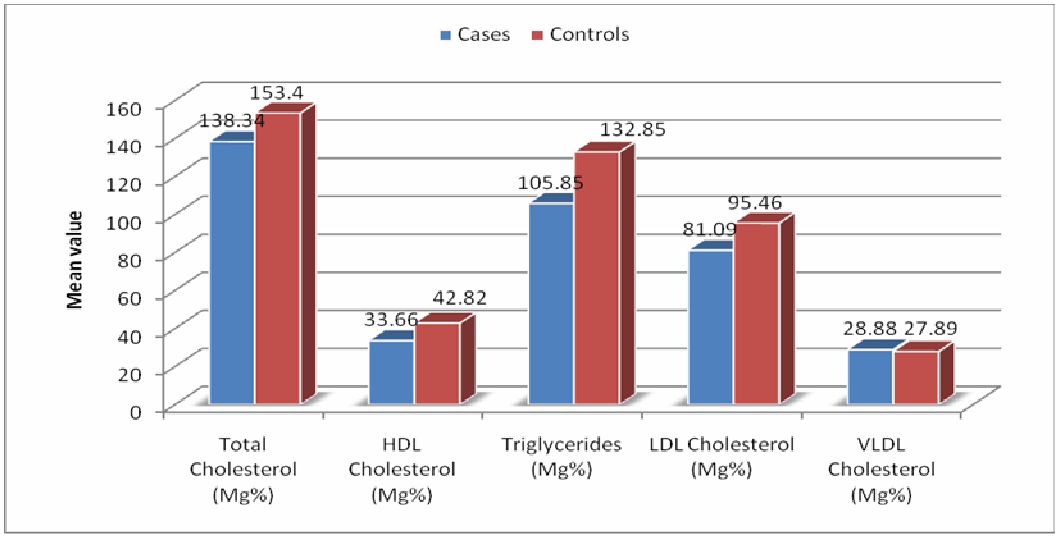

Figure 23: Comparison of lipid levels between cases and controls

Table 8 & Figure 23 shows the comparison of profile between cases and controls. TC was found to be significantly (p=0.0001) lower among cases (138.34±20.86) compared to controls (153.40±23.35). TC was 3% lower among cases than controls (OR=0.97, 95%CI=0.95-0.98). HDL was also found to be lower among cases compared to controls and this was 12% significantly lower among cases than controls (OR=0.88, 95%CI=0.84-0.92, p=0.0001). TG was only 2% lower among cases than controls (OR=0.98, 95%CI=0.97-0.99, p=0.0001). LDL was found to be 10% lower among cases compared to controls (OR=0.90, 95%CI=0.87-0.93, p=0.0001). However, VLDL was found to be insignificantly (p>0.05) higher among cases than controls.

4.9 Lipid ratio between cases and controls

Table-9: Comparison of lipid ratio between cases and controls

Lipid ratio	Cases	Controls	OR (95% CI)	p-value[1]
TC/HDL cholesterol ratio	4.37±1.10	3.67±0.76	2.19 (1.57-3.05)	0.0001*
LDL/HDL ratio	2.58±0.75	2.27±0.38	2.41 (1.46-3.98)	0.001*

[1]Binary logistic regression, OR-Odds ratio, CI-Confidence interval, *Significant

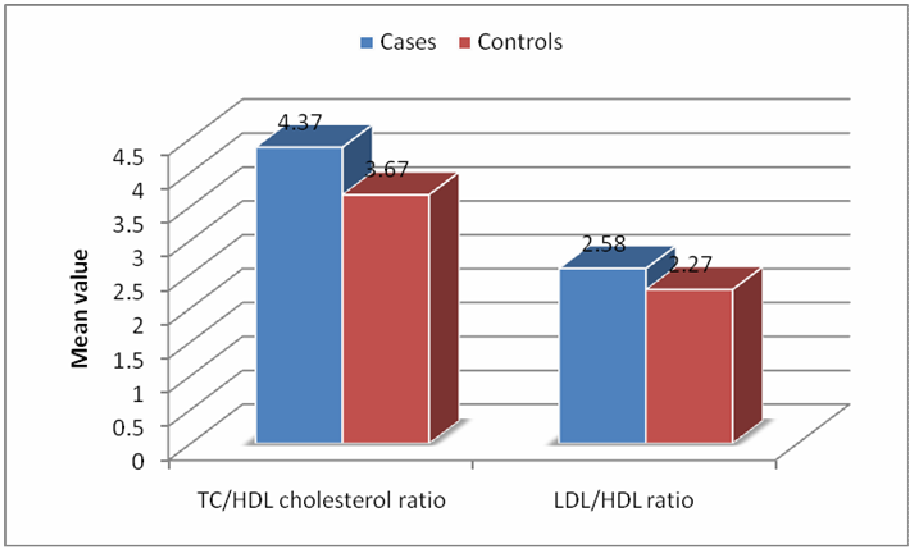

Figure 24: Comparison of lipid ratio between cases and controls

Table 9 & Figure 24 shows the comparison of profile between cases and controls. TC/HDL ratio was 2.19 folds was higher in cases than controls (OR=2.19, 95%CI=1.57-3.05, p=0.0001). LDL/HDL ratio was 2.41 folds was higher in cases than controls (OR=2.41, 95%CI=1.46-3.98, p=0.0001).

4.10 Lipid profile among males between cases and controls

Table-10: Comparison of lipid profile among males between cases and controls

Lipid profile	Males		OR (95% CI)	p-value[1]
	Cases	Controls		
Total Cholesterol (Mg%)	137.77±20.42	152.69±23.05	0.96 (0.95-0.98)	0.0001*
HDL Cholesterol (Mg%)	33.79±10.14	42.76±7.28	0.88 (0.85-0.92)	0.0001*
Triglycerides (Mg%)	104.24±67.67	132.58±40.62	0.99 (0.98-0.99)	0.001*
LDL Cholesterol (Mg%)	80.65±13.23	95.08±12.94	0.89 (0.86-0.93)	0.0001*
VLDL Cholesterol (Mg%)	28.74±10.64	27.86±8.74	1.01 (0.98-1.03)	0.52

[1]Binary logistic regression, OR-Odds ratio, CI-Confidence interval, *Significant

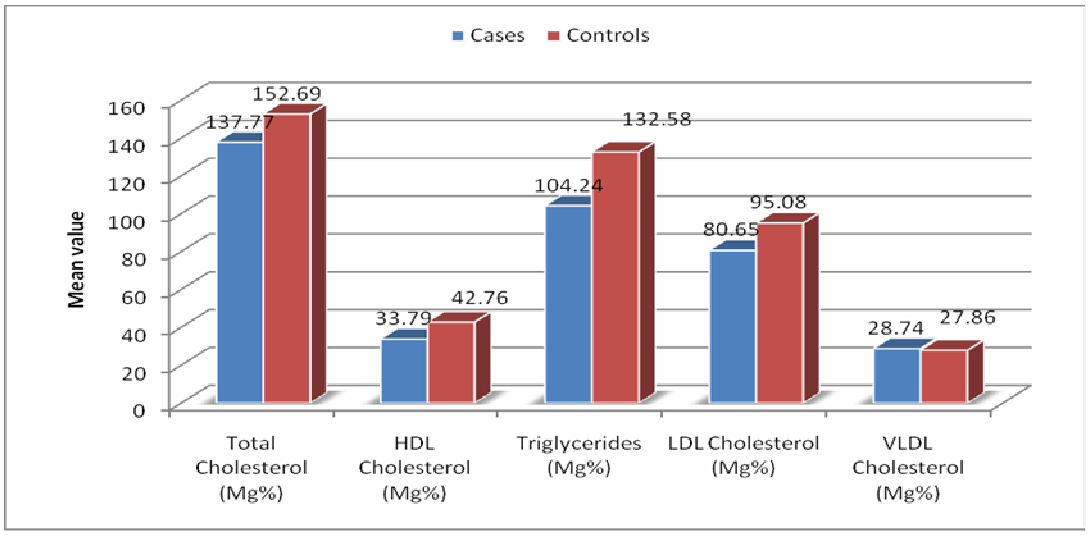

Figure 25: Comparison of lipid profile among males between cases and controls

Table 10 & Figure 25 shows the comparison of lipid profile between cases and controls among males. TC was found to be significantly (p=0.0001) lower among cases (137.77±20.42) compared to controls (152.69±23.05) in males. TC was 4% lower among cases than controls (OR=0.96, 95%CI=0.95-0.98) in males. HDL was also found to be lower among cases compared to controls and this was 12% significantly lower among cases than controls (OR=0.88, 95%CI=0.85-0.92, p=0.0001) in males. TG was only 1% lower among cases than controls (OR=0.99, 95%CI=0.98-0.99, p=0.0001) in males. LDL was found to be 11% lower among cases compared to controls (OR=0.89, 95%CI=0.86-0.93, p=0.0001) in males. However, VLDL was found to be insignificantly (p>0.05) higher among cases than controls in males

4.11 Lipid profile among females between cases and controls

Table-11: Comparison of lipid profile among females between cases and controls

Lipid profile	Females		OR (95% CI)	p-value[1]
	Cases	Controls		
Total Cholesterol (Mg%)	156.57±31.75	188.50±0.70	0.95 (0.93-0.97)	0.0001*
HDL Cholesterol (Mg%)	29.33±2.88	45.50±0.71	0.82 (0.80-0.91)	0.0001*
Triglycerides (Mg%)	132.58±6.92	146.00±0.70	0.83 (0.81-0.90)	0.0001*
LDL Cholesterol (Mg%)	95.33±15.01	114.50±0.70	0.81 (0.80-0.96)	0.0001*
VLDL Cholesterol (Mg%)	33.37±1.55	29.85±0.91	1.02 (0.95-1.04)	0.53

[1]Binary logistic regression, OR-Odds ratio, CI-Confidence interval, *Significant

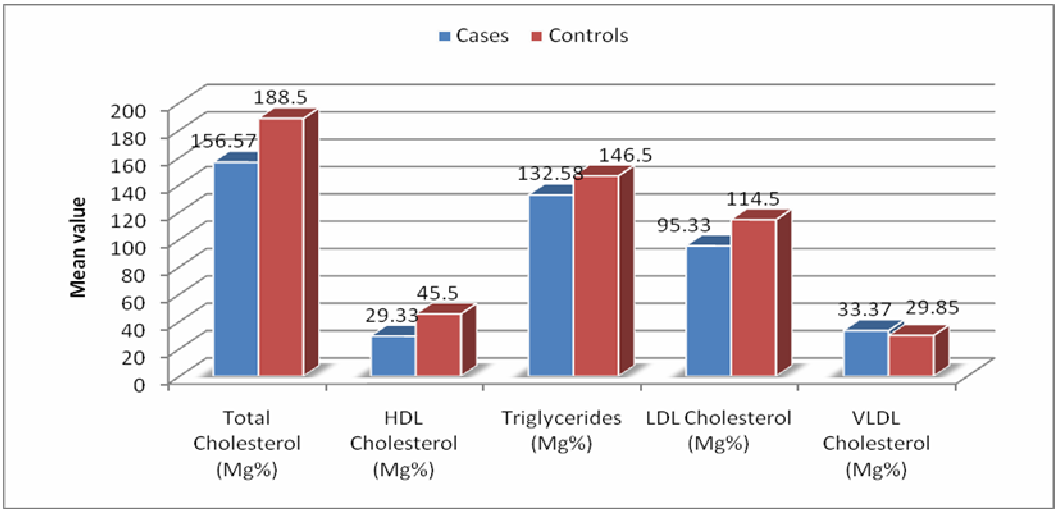

Figure 26: Comparison of lipid profile among females between cases and controls

Table 11 & Figure 26 shows the comparison of lipid profile between cases and controls among females. TC was found to be significantly (p=0.0001) lower among

cases (156.57±31.75) compared to controls (188.50±0.70) in females. TC was 5% lower among cases than controls (OR=0.95, 95%CI=0.93-0.97) in females. HDL was also found to be lower among cases compared to controls and this was 12% significantly lower among cases than controls (OR=0.88, 95%CI=0.80-0.91, p=0.0001) in females. TG was only 17% lower among cases than controls (OR=0.83, 95%CI=0.81-0.90, p=0.0001) in females. LDL was found to be 19% lower among cases compared to controls (OR=0.81, 95%CI=0.80-0.96, p=0.0001) in females. However, VLDL was found to be insignificantly (p>0.05) higher among cases than controls in females.

4.12 Lipid ratio among males between cases and controls

Table-12: Comparison of lipid ratio among males between cases and controls

Lipid ratio	Males		OR (95% CI)	p-value[1]
	Cases	Controls		
TC/HDL cholesterol ratio	4.26±1.12	3.62±0.75	2.13 (1.53-2.98)	0.0001*
LDL/HDL ratio	2.58±0.79	2.21±0.39	2.30 (1.39-3.80)	0.001*

[1]Binary logistic regression, OR-Odds ratio, CI-Confidence interval, *Significant

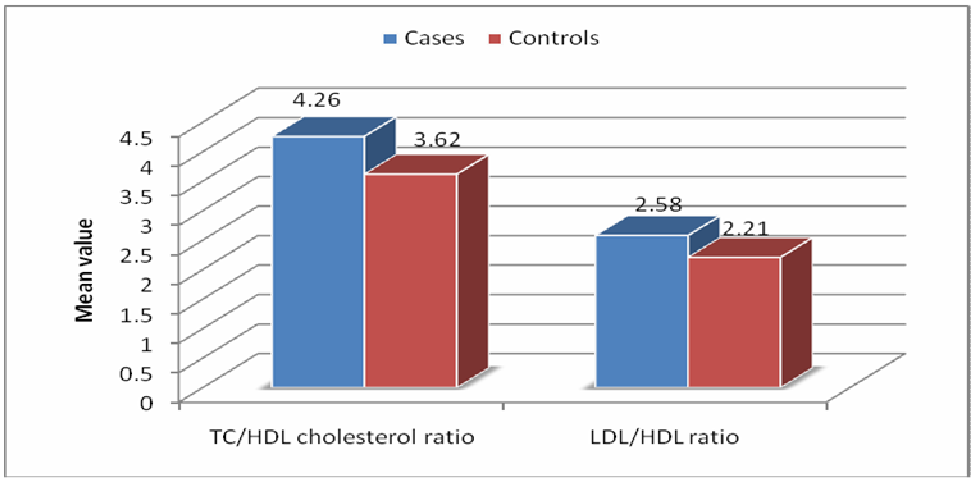

Figure 27: Comparison of lipid ratio among males between cases and controls

Table 12 & Figure 27 shows the comparison of lipid ratio between cases and controls among males. TC/HDL cholesterol ratio was significantly (p=0.0001) higher among cases (4.26±1.12) than controls (3.62±0.75) in males. TC/HDL cholesterol ratio was 2.13 times higher among cases than controls in males (OR=2.13, 95%CI=1.53-2.98) in males. LDL/HDL ratio was also higher among cases (2.58±0.79) compared to controls (2.21±0.39) in males and this 2.30 (OR=2.30, 95%CI=1.39-3.80, p=0.001) times higher among cases than controls in males.

4.13 Lipid ratio among females between cases and controls

Table-13: Comparison of lipid ratio among females between cases and controls

Lipid ratio	Females		OR (95% CI)	p-value[1]
	Cases	Controls		
TC/HDL cholesterol ratio	5.28±0.59	5.00±1.41	1.13 (1.07-2.98)	0.0001*
LDL/HDL ratio	3.22±0.19	2.40±0.69	1.40 (1.20-3.56)	0.0001*

[1]Binary logistic regression, OR-Odds ratio, CI-Confidence interval, *Significant

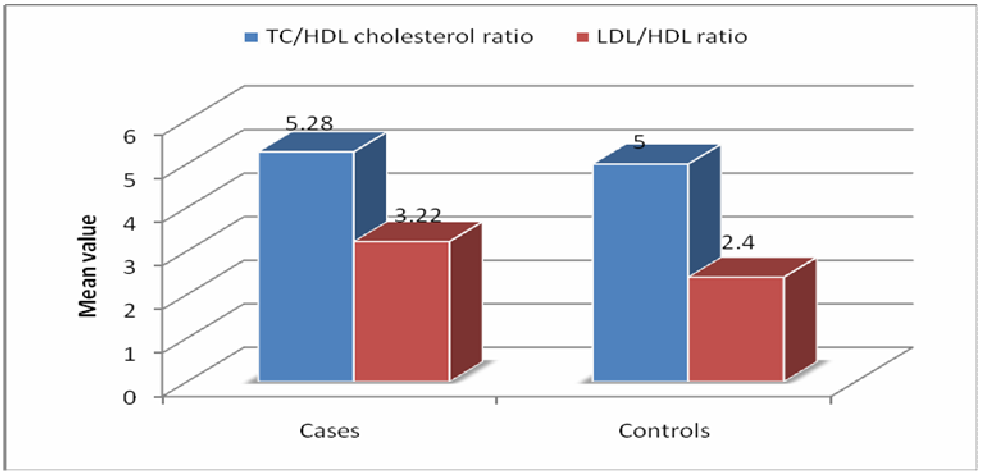

Figure 28: Comparison of lipid ratio among females between cases and controls

Table 13 & Figure 28 shows the comparison of lipid ratio between cases and controls among females. TC/HDL cholesterol ratio was significantly (p=0.0001) higher among cases (5.28±0.59) than controls 5.00±1.41) in females. TC/HDL cholesterol ratio was 1.13 times higher among cases than controls in females (OR=1.13, 95%CI=1.07-2.98) in females. LDL/HDL ratio was also higher among cases (3.22±0.19) compared to controls (2.40±0.69) in females and this 1.40 times higher among cases (OR=1.40, 95%CI=1.20-3.56, p=0.0001) than controls in females.

4.14 Correlations

Table-14: Correlation of duration of habit with lipid profile among cases and controls

Lipid profile	Cases		Controls	
	Correlation coefficient	p-value	Correlation coefficient	p-value
Total Cholesterol (Mg%)	0.15	0.13	0.04	0.63
HDL Cholesterol (Mg%)	0.10	0.28	0.09	0.34
Triglycerides (Mg%)	-0.03	0.84	0.15	0.13
LDL Cholesterol (Mg%)	0.10	0.32	-0.09	0.35
VLDL Cholesterol (Mg%)	0.10	0.30	-0.04	0.62

*Significant

Table 14 shows the correlation of duration of habit with lipid profile among cases and controls. There was poor correlation of duration of habit with lipid profile among cases and controls.

Table-15: Correlation of duration of habitwith lipid ratio among cases and controls

Lipid ratio	Cases		Controls	
	Correlation coefficient	p-value	Correlation coefficient	p-value
TC/HDL cholesterol ratio	0.04	0.68	0.01	0.90
LDL/HDL ratio	-0.02	0.80	-0.11	0.27

Table 15 shows the correlation of duration of habit with lipid ratio among cases and controls. There was poor correlation of duration of habit with lipid ratios among cases and controls.

4.15 Independent risk factors for OSMF

Table-16: Independent risk factors for OSMF

Factors	Adjusted OR	95.0% C.I.for OR		p-value[1]
		Lower	Upper	
HDL Cholesterol	0.94	0.91	0.99	0.04*
Triglycerides	0.93	0.90	0.99	0.006*
LDLCholesterol	0.79	0.71	0.88	0.0001*
LDL/HDL	79.20	56.70	178.12	0.001*

[1]Multivariate logistic regression, OR-Odds ratio, CI-Confidence interval, *Significant

Table 18 show independent risk factors for OSMF. The multivariate logistic regression analysis revealed that HDL, TG, LDL and LDL/HDL ratio were found to be independent risk factors of the cases. HDL Cholesterol was 6% lower among cases compared to controls (Adjusted OR=0.94, 95%CI=0.91-0.99, p=0.04). TG was found to be 7% lower among cases than controls (Adjusted OR=0.93, 95%CI=0.90-0.99, p=0.006). LDL was 21% lower among cases compared to controls (Adjusted OR=0.79, 95%CI=0.71-0.88, p=0.001). LDL/HDL was significantly higher among cases than controls, however, confidence interval was too high.

CHAPTER-5

DISCUSSION

5.1 Introduction

Oral submucous fibrosis (OSMF) is a chronic, premalignant condition of the oral mucosa Pindborg (1966) defined OSMF 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 fibroelastic change of the lamina propria, with epithelial atrophy leading to stiffness of the oral mucosa and causing trismus and inability to eat". [12,13]

Its incidence is particularly high in India, some other countries in Asia. In India, the chewing of tobacco products, in addition to smoking in various forms, is primarily responsible for the high incidence. The WHO have estimated that 90% of oral cancers in India among men are attributable to the chewing and smoking of tobacco. [75]

All clinical Presentations that carry a risk of cancer defined under the term "potentially malignant disorders." OSMF has been identified as a potential malignant disorder with the highest rate of malignant transformation. Chewing betel quid has been recognized as one of the main risk factors. Carcinogens in these substances generate reactive oxygen species (ROS) and lipid peroxides thereby leading to tissue

injury as a result of elevated lipid peroxidation, further damaging the cellular structural block, namely lipids. Carcinogenesis leads to low levels of cholesterol in the proliferating tissues and blood. There are reports of early and significant changes in phospholipids and cholesterol.

Cholesterol and triglycerides are the important lipid constituents of cells and are essential for various physiological functions. Cholesterol is essential for maintenance of structure and functional integrity of all biological membranes and also important for stabilization of DNA helix.

The lipoproteins help in the transport of cholesterol and triglycerides in plasma as they are hydrophobic. The major functions of the plasma lipoproteins are transport of triglycerides and cholesterol from sites of synthesis to sites of storage, energy use or metabolism. The VLDL transports the endogenous synthesized triglycerides, LDL are the biologic vehicle to provide cholesterol to the peripheral tissues, HDL transports cholesterol from peripheral tissues to liver for degradation.[139]

In the search for possible causes of malignancies on the one hand and the need for the modality affording early diagnosis, attention was paid to the role played by lipids in malignancies.

Increasing incidence of oral cancer and precancer in India necessitates probing for its early diagnosis and prognosis. Thus, there is need to develop specific and faster tests as an aid in their early diagnosis. Early detection is also called as secondary prevention. It is therefore important to identify few diagnostics and predictive approaches.

Hence, the present study was aimed to evaluate alteration of serum lipid profile in potentially malignant disorders patients. The plasma lipid profile was estimated by the enzymatic method and the parameters that were considered were total cholesterol, High density lipoprotein cholesterol, Low density lipoprotein cholesterol, Very low density lipoprotein, Triglyceride levels, TC/HDL ratio and LDL/HDL ratio

5.2 Age distribution

More than one third of cases (38%) and 35% of controls were below 30 years of age. However, 30% of cases and 36% of controls were between 30-40 years. The mean age of cases and controls was 36.14±9.36 and 34.59±8.48. (Table 1; Figure 19)

Our findings were consistent with the study done by **Sharma G. *et al.***[116] where they had their maximum patients in the 3rd and 4th decade of life.

The common age range which is seen in our study was in 3rd to4th decade and which can be due to changing lifestyle of youngsters and increased in the usage of Ghutkha/ pan masala/ smoking as per the regional variation. (Pandya et al)

5.3 Gender distribution

In our study majority of both the cases (96%) and controls (98%) were males and it shows that the male/female ratio was similar ($p>0.05$) in cases and controls, thus both the groups were comparable in terms of gender. (Table-2).

A similar male predominance was reported by Radhakrishna M *et al.*[97] (out of 30 cases all 30 were males), Goel P *et al.*[142] (out of 60 cases 51 were males and 9 were females), Neerupakam M *et al.*[121] (male to female ratio was 4:1), Kumar P *et al.*[96] (out of 30 subjects 22 were males and 8 were females) Reddy V *et al.*[147] (male to female ratio 2.36:1) Misra et al [121]

We found that male predominance can be due to easy accessibility for males to use areca nut and its products more frequently than females in our society. Moreover males are the working gender and money earner among Indian subcontinent. Areca nut/betel quid, gutkha is chewed for variety of reasons such as stress reliever, mouth freshener, improving concentration and digestion after food. Whereas, females are more conscious about their esthetic values and it is considered socially unacceptable for a female to get gutkha from gutkha vendors.

5.4 Habit History

5.4.1 Smokeless tobacco habit between cases and controls

The present study shows that most of the cases (92%) and controls (77%) were using smokeless tobacco and majority of them were using Gutkha. However, there was no significant ($p>0.05$) difference in the habit of smokeless tobacco between cases and controls. (Table-3; Figure 20)

Similar results were observed by Chalkoo A.H[95] where 45% of the subjects were consuming smokeless tobacco in the form of Betel leaves, slaked lime, areca nut along with tobacco. In our study the percentage of smokeless tobacco users in the

cases (92%) was higher than controls (77%). The smokeless tobacco users were 3.43 times significantly higher in cases than controls. This was in conjuction with Chalkoo AH's study where 95% patients included in the study were using smokeless tobacco.[95]

Similar findings were reported by Sinor P.N[21], Shah & Sharma P.P[20], who found that most of their subjects had the habit of chewing gutkha.

Pindborg J *et al.*[70], Gupta M.K *et al.*[126], reported that areca nut is the main causative agent in the etiopathogenesis of OSMF.

It was observed in our study that occurrence of OSMF was seen significantly in those having one or the other forms of areca nut. This occurrence has shown that the products which leach out from areca nut would be responsible for the development of OSMF. In the areca nut chewers the lysyl oxidase activity is upregulated to alter fibroblast metabolism producing more collagen. This may add to the conclusion of the occurrence of OSMF due to the products leaching out from areca nut causes OSMF and not from those leaching out from tobacco.

5.4.2 Smoking habit between cases and controls

Cigarette was used by more than half of the cases and controls. More than half of cases (28%) and 39% of controls were smoker. There was no significant ($p>0.05$) association of smoking habit between cases and controls. This was in conjuction with Chalkoo AH's study where 5% patients included in the study were smokers. (93) (Table 4; Figure 21)

A similar study was done by Gupta MK[126] where strong evidence has been found for the role of smoking in the development of both oral cancer and oral leukoplakia. Epidemiologic patterns of cigarette smoking show a steep increase in central European countries.

5.4.3 Multiple tobacco use between cases and controls

Table 5; Figure 22 shows the comparison of multiple tobacco habit between cases and controls. One fifth of cases (20%) and 16% of controls were both chewer & smoker. The association was statistically insignificant ($p>0.05$). Multiple habit chewing and smoking did not have any correlation in our study but guthka was the most significant factor for OSMF but no correlation was found with lipid profile.

5.4.4 Duration of tobacco use between cases and controls

The present study shows that most of the cases (58%) and controls (53%) were using tobacco since 3-5 years and 42% of cases had habit more than 5 years. Comparison of duration of tobacco use between cases and controls. 23 shows the comparison of duration of tobacco use between cases and controls. The duration of tobacco use was insignificantly ($p>0.05$) higher among the cases (4.55 ± 1.42) compared to controls (4.27 ± 2.26).

This finding was consistent with study conducted by Mehrotra R *et al.*[94] where most of the patients with OSMF consumed tobacco for 6-10 yrs and Reddy V *et al.*[147] and Aher V *et al.*[157] who concluded that subjects consuming gutkha only or

areca nut only showed high occurrence of OSMF in short duration (mean duration 8.2 and 10.9 years, respectively)

We concluded that duration of chewing gutkha has a definite role in increased severity of developing OSMF.

5.4.5 Duration of tobacco use among male and females between cases and controls

Table 7; Figure 24 shows the comparison of duration of tobacco use between cases and controls among male and females.

The duration of tobacco use was in male cases and control was 4.56±1.43 and 4.21±2.24 years respectively.

However, the duration of tobacco use was in female cases and control was 4.33±1.15 and 7.00±0.00 years respectively.

5.5 Lipid levels

5.5.1 Lipid levels between cases and controls

The present study evaluated the lipid levels between cases and controls shows the comparison of profile between cases and controls (Table 8, Figure 25). TC was found to be significantly (p=0.0001) lower among cases (138.34±20.86) compared to controls (153.40±23.35). TC was 3% lower among cases than controls (OR=0.97, 95%CI=0.95-0.98). HDL was also found to be lower among cases compared to

controls and this was 12% significantly lower among cases than controls (OR=0.88, 95%CI=0.84-0.92, p=0.0001). TG was only 2% lower among cases than controls (OR=0.98, 95%CI=0.97-0.99, p=0.0001). LDL was found to be 10% lower among cases compared to controls (OR=0.90, 95%CI=0.87-0.93, p=0.0001). However,

There was no significant (p>0.05) difference in VLDL between cases and controls. Kratika Ajai et al. [96] observed similar results in their study. They found that the mean serum TC, serum HDL, serum LDL, serum VLDL and serum TG levels in oral submucous fibrosis group were 151.07 mg/dL, 37.80 mg/dL, 95.15 mg/dL, 18.11 mg/dL and 94.18 mg/dL respectively. However, in the control group the corresponding values were 191.42 mg/dL, 51.36 mg/dL, 113.64 mg/dL, 26.43 mg/dL and 132.16 mg/dL respectively. A statistically significant reduction [P<0.001] was noted between the control group and OSMF cases.

Similar results were observed in the study conducted by Chalkoo AH et al. [95] where it was found that the lipid profile of OSMF patients when compared with standardized values showed a significant decrease in serum cholesterol, LDL and LDL/HDL ratio. Whereas serum triglyceride and HDL was found to be raised in some patients which was in contrast to this study.

Gupta N et al. [2] conducted a study on Alterations in serum lipid profile patterns in head & neck cancer and oral submucous fibrosis patients and observed similar results as the current study. Mean lipid levels were found to be maximum in oral cancer patients and OSMF patients for all the parameters like serum cholesterol, LDL, VLDL and serum triglyceride except HDL which was slightly raised. The results were statistically significant.

Another study conducted by Poorey VK and Thakur P [8] on the alteration of lipid profile in patients with head and neck malignancy revealed similar results as the present study. Highly significant reduction was observed in the levels of mean serum total cholesterol, triglycerides and high density lipoproteins (HDL) in the subjects of head and neck malignancy as compared to the control group.

Gupta S and Gupta S [113] also performed a similar study on the alterations in serum lipid profile patterns in oral cancer and oral precancerous lesions and conditions and found results which were similar to our study. They found a significant decrease in plasma total cholesterol, HDLC, and triglycerides were observed in the patients with the precancerous lesions and conditions as compared to the controls.

Our results are comparable with study done by Mehrotra R *et al.*[94] where they found significant decrease in TC and HDL Cholesterol in OSMF patients.

Similar study conducted by Ajai K *et al.*[97]in which they found decrease serum lipid level in OSMF patients including TC, HDL, LDL, VLDL which is similar with our study .

Similar study done by Radhakrishna M *et al.*[97] where they observed decrease level of serum lipid profile in OSMF patients which is comparable to our study and decreased TC / HDLC which is not comparable we found high TC/HDLC ratio (4.29±1.13) and LDL/HDL ratio (2.60±0.79).

5.5.2 Lipid ratio between cases and controls

Table 9; Figure 26 shows the comparison of profile between cases and controls. TC/HDL ratio was 2.19 folds was higher in cases than controls (OR=2.19, 95%CI=1.57-3.05, p=0.0001). LDL/HDL ratio was 2.41 folds was higher in cases than controls (OR=2.41, 95%CI=1.46-3.98, p=0.0001).

this study was in accordance to the study done by Gupta S. Gupta S in 2011 [138] , Singh S et al [139] , Gupta N et al (2014)[140] , Goel P et al (2015)[119] , Poorey V.K Thakurin 2015[143]

LDL was also 13% lower among the cases compared to controls. the duration of tobacco use was two times higher among the cases than controls. Similar results were seen in study done by Chalkoo AH[95]

5.5.3 Lipid profile among males between cases and controls

Further analysing the data we compared the lipid profile among the gender which was not done by previous researchers

Table 10; Figure 27 shows the comparison of lipid profile between cases and controls among males. TC was found to be significantly (p=0.0001) lower among cases (137.77±20.42) compared to controls (152.69±23.05) in males. TC was 4% lower among cases than controls (OR=0.96, 95%CI=0.95-0.98) in males. HDL was also found to be lower among cases compared to controls and this was 12% significantly lower among cases than controls (OR=0.88, 95%CI=0.85-0.92, p=0.0001) in males. TG was only 1% lower among cases than controls (OR=0.99,

95%CI=0.98-0.99, p=0.0001) in males. LDL was found to be 11% lower among cases compared to controls (OR=0.89, 95%CI=0.86-0.93, p=0.0001) in males. However, VLDL was found to be insignificantly (p>0.05) higher among cases than controls in males

5.5.4 Lipid profile among females between cases and controls

Table 11; Figure 28 shows the comparison of lipid profile between cases and controls among females. TC was found to be significantly (p=0.0001) lower among cases (156.57±31.75) compared to controls (188.50±0.70) in females. TC was 5% lower among cases than controls (OR=0.95, 95%CI=0.93-0.97) in females. HDL was also found to be lower among cases compared to controls and this was 12% significantly lower among cases than controls (OR=0.88, 95%CI=0.80-0.91, p=0.0001) in females. TG was only 17% lower among cases than controls (OR=0.83, 95%CI=0.81-0.90, p=0.0001) in females. LDL was found to be 19% lower among cases compared to controls (OR=0.81, 95%CI=0.80-0.96, p=0.0001) in females. However, VLDL was found to be insignificantly (p>0.05) higher among cases than controls in females.

5.5.5 Lipid ratio among males between cases and controls

Table 12; Figure 29 shows the comparison of lipid ratio between cases and controls among males. TC/HDL cholesterol ratio was significantly (p=0.0001) higher among cases (4.26±1.12) than controls (3.62±0.75) in males. TC/HDL cholesterol

ratio was 2.13 times higher among cases than controls in males (OR=2.13, 95%CI=1.53-2.98) in males. LDL/HDL ratio was also higher among cases (2.58±0.79) compared to controls (2.21±0.39) in males and this 2.30 (OR=2.30, 95%CI=1.39-3.80, p=0.001) times higher among cases than controls in males. Higher lipid ratio indicates risk factor for ischemic heart disease

5.5.6 Lipid ratio among females between cases and controls

In the present study while comparing the female among the cases suffering from OSMF had higher lipid ratio than control group

Table 13; Figure 30 shows the comparison of lipid ratio between cases and controls among females. TC/HDL cholesterol ratio was significantly (p=0.0001) higher among cases (5.28±0.59) than controls 5.00±1.41) in females. TC/HDL cholesterol ratio was 1.13 times higher among cases than controls in females (OR=1.13, 95%CI=1.07-2.98) in females. LDL/HDL ratio was also higher among cases (3.22±0.19) compared to controls (2.40±0.69) in females and this 1.40 times higher among cases (OR=1.40, 95%CI=1.20-3.56, p=0.0001) than controls in females.

5.6 Correlation of duration of habit with lipid profile & lipid ratio among cases and controls

Table 14 & 15 shows the correlation of duration of habit with lipid profile among cases and controls. There was poor correlation of duration of habit with lipid

profile among cases and controls.these variables were not correlated by previous researchers.

5.7 Independent risk factors for OSMF

Table 16 show independent risk factors for OSMF. The multivariate logistic regression analysis revealed that HDL, TG, LDL and LDL/HDL ratio were found to be independent risk factors of the cases. HDL Cholesterol was 6% lower among cases compared to controls (Adjusted OR=0.94, 95%CI=0.91-0.99, p=0.04). TG was found to be 7% lower among cases than controls (Adjusted OR=0.93, 95%CI=0.90-0.99, p=0.006). LDL was 21% lower among cases compared to controls (Adjusted OR=0.79, 95%CI=0.71-0.88, p=0.001). LDL/HDL was significantly higher among cases than controls, however, confidence interval was too high

Guthka chewing is a major risk factor for health, with The combination of risk factors like guthka and tobacco chewing, lower lipid profile is an independent risk factor for osmf.

CHAPTER-6

CONCLUSION

Oral potentially malignant disorders have received considerable attention in the recent past because of its chronic debilitating and resistant nature. The potential for malignant transformation is considered high, and the disease affects person of all ages and both genders.

One of the important components responsible for the maintenance of cell integrity is lipids, which are required for various biological functions like cell division and growth of normal and malignant tissues. Tobacco carcinogens causes peroxidation of polyunsaturated fatty acid and this affects the essential constituents of the cell membrane and might be involved in carcinogenesis/tumorigenesis. Recently much attention has been given towards detection of lipid profile in oral cancer and precancerous states because of the encouraging results of the studies on head and neck carcinoma.

Blood or serum-based diagnostic and predictive approaches which are safe, economical and amenable to repeated sampling are the need of the hour in early detection of these premalignant lesions and conditions.

In our study there was a significant reduction in the serum lipid levels (TC, HDL, LDL, triglycerides in all the patients of OSMF The TC/HDL and HDL/LDL ratio was higher patients compared to controls but VLDL no changes.

The reduced levels of serum lipid profile may be a consequence of disease, probably mediated by the greater utilization of lipids for new membrane biogenesis.

From these findings it appears that the decrease in the lipid levels may be considered as a useful marker in the early diagnosis of oral premalignant conditions and lesions like OSMF.

Lipid profile can be added on to other tests as an additional indicator and can serve as another evaluating parameter to observe early changes occurring during carcinogenesis.

To conclude, in the present study we have attempted to summarize the basics of lipids and have thrown a light on the various possible associations between the serum lipid profile and OSMF. This study supports the evidence of an inverse relationship between serum lipid profile and OSMF lipid status may be used as a useful indicator for early changes occurring in neoplastic cells and serve as one of the early diagnostic aids in oral potentially malignant disorders.

REFERENCES

1. Patel PS, Shah MH, Jha FP, Raval GN, Rawal RM, Patel MM, Patel JB, Patel DD. Alterations in plasma lipid profile pattern in head and neck cancer and oral pre cancer conditions. *Indian Journal of Cancer* 2004; 41(1): 25-31.

2. Gupta S. Alterations in serum lipid profile patterns in oral cancer and oral precancerous lesions and condition a clinical study. *Indian Journal of Dentistry* 2011; 2(2):1-7.

3. Kumar P, Augustine J, Arora S, Gupta S, Mohanty V.R. Serum lipid profile in oral cancer and leukoplakia: Correlation with tobacco abuse and histological grading. *Journal of cancer and research therepeutics* 2012; 8(3):348-388.

4. Bansal S.K, Sharma Saloni, Singh Harkanwalpreet. Evaluation of serum lipid profile in untreated forms of head and neck cancer. *Journal of Biosciences and Informatics* 2013; 4(2):179-189.

5. Lohe V.K, Dedwekar S.S, Bhowateb R.R, Kadu R.P, Dangore S.B. Evaluation of correlation of serum lipid profile in patients with oral cancer & precancer & its association with tobacco abuse. *J Oral Patho Med* 2010;39: 141-148.

6. Goyal S, C Vani, K Srikant, C.H Lalitha . Serum lipid profile in patients with oral tobacco habits and oral precancer lesions and conditions. *Webmed Central Oral Medicine* 2013; 4(2):WMC004034.

7. Warnakulasuriya S, Johnson W.N, Waal Van Der I. Nomenclature and classification of potentially malignant disorders of the oral mucosa. *J of Oral Patho Med* 2007;36:575-80.

8. Murti P.R, Bhonsle R.B, Pindborg J.J, Daftary D.K, Gupta P.C & Mehta F.S. Malignant transformation rate in oral submucous fibrosis over a 17 year period. *Community Dent Oral Epidemiol* 1985; 13(6):340-341.

9. Nayak P,Nayak S, Darafsh MD. Alterations in plasma lipid profile in precancerous conditions. *Journal of Nepal Dental Sciences* 2010;11(1):40-45.

10. Chawda G.J, Jain S.S *et al*. The relationship between serum lipid levels and the risk of oral cancer. *Indian journal of medical and paediatric oncology* 2011; 32(1):34-37.

11. Riana C, Raizada RM, Chaturvedi VN, Harinath BC, Puttewar MP, Kennedy AK. Clinical Profile and Serum Beta- Carotene Levels in Oral Submucous Fibrosis. *IJOHNS* 2005;57(3):191-5.

12. Rajendran R. Oral submucous fibrosis. *J Oral Maxillofac Pathol* 2003;7:1-4.

13. Pindborgh JJ, Sirsat SM. Oral Submucous Fibrosis. *J. of Oral Surg Oral Med Oral Pathol* 1966;22:764-9.

14. Abrol B.M. Clinicopathological, biochemical and immunological studies in syndrome of idiopathic oral fibrosis. *The Bombay Hospital Journal* 1977; 19(1): 50-60.

15. Jayanthi V, Probert CSJ, Sher KS, Mayberry JF. Oral submucous fibrosis a preventable disease. *Gut* 1992;33:4-6.

16. Gupta PC, Mehta FS, Daftary DK. Incidence rates of oral cancer and natural history of oral precancerous lesions in a 10-year follow-up study of Indian villagers. *Community Dent Oral Epidemiol* 1980;8:287–333.

17. Shafer WG, Hine MK, Levy BM. A Textbook of Oral Pathology. 4th ed. Pennsylvania: *Saunders*; 2003.

18. Rajalalitha P, Vali S. Molecular pathogenesis of oral submucous fibrosis- a collagen metabolic disorder. *J Oral Pathol Med* 2005;34:321–8.

19. Prabhu SR, Wilson DF, Daftary DK, Johnson NW. Oral Diseases in the tropics. 1st ed. New Delhi: Oxford; 1993.

20. Shah N, Sharma PP: Role of chewing and smoking habits in the etiology of oral submucous fibrosis: a case control study. *J Oral Pathol Med* 1998;27:475-9.

21. Sinor PN, Gupta PC, Murti PR, Bhonsle RB, Daftary DK, Mehta ES, PindborgJJ: A case-control study of oral submucous fibrosis with special reference to the etiology role of areca nut. *J Oral Pathol Med* 1990;19:94-8.

22. Kumar A, Sharma SC, Sharma P, Chandra OM, Singhal KC, Nagar A. Beneficial effect of oral zinc in the treatment of oral submucous fibrosis. *Indian J Pharmac* 1991;23:236-41.

23. Hazarey VK, Erlewad DM, Mundhe KA, Ughade SN. Oral submucous fibrosis: study of 1000 cases from central India. *J Oral Pathol Med* 2007; 36:12–7.

24. Pindborg J. Oral Submucous Fibrosis as a Precancerous Condition. *Scand J Dent Res* 1984 ;92(3):224-9.

25. Van Wyk CW. Grobler-Rabie AE. Martell RW. Hammond MG; HLA-antigens in oral submucous fibrosis. *J Oral Pathol Med* 1994;23:23-7.

26. Ahmad MS, Ali SA., Ali AS, Chaubey KK. Epidemiological and etiological study of oral submucous fibrosis among Gutkha chewers of Patna, Bihar, India. *J Indian Soc Pedod Prev Dent* 2006:84-9.

27. Saraswathi TR, Ranganathan K, Shanmugam S, Sowmya R, Narasimhan PD, Gunaseelan R. Prevalence of oral lesions in relation to habits: Cross-sectional study in South India. *Indian J Dent Res* 2006;17:121-5.

28. Chiang CP, Hsieh RP, Chen TH, Chang YF, Liu BY. High incidence of auto-antibodies in Taiwanese patients with oral submucous fibrosis. *J Oral Pathol Med* 2002;31:402-9.

29. Tilakaratne WM, Klinikowski MF, Takashi S, Peters TJ, Warnakulsuriya S. Oral submucous fibrosis: Review on aetiology and pathogenesis *Oral Oncol* 2006;42:561–8.

30. Maher R, Lee AI, Warnakulasuriya KAAS, Lewis JA, Johnson NW: Role of areca nut in the causation of oral submucous fibrosis: a case-control study in Pakistan. *J Oral Pathol Med* 1994;23:65-9.

31. Kackar P.K, Puri R.K. & Venkatachalam V.P Oral submucous fibrosis- Treatment with hylase. *The Journal of Laryngol and Otol* 1985;99:57-9.

32. Murti PR, Bhonsle RB, Gupta PC, Daftary DK, Pindborg JJ, Mehta FS: Etiology of oral submucous fibrosis with special reference to the role of areca nut chewing. *J Oral Pathol Med* 1995;24:145-52.

33. Yadav A, Kumar L, Misra N, Ummapathi D, and GC Shiva Kumar. Estimation of serum zinc, copper and iron in patients of OSMF. *Natl J Maxillofac Surg* 2015;6(2) : 190-193.

34. Haque MF, Harris M, Meghji S, Speight PM: An immunohistochemical study of oral submucous fibrosis. *J Oral Pathol Med* 1997;26:75-82.

35. Haque MF, Meghji S, Nazir R, Harris M. Interferon gamma may reverse oral submucous fibrosis. *J Oral Pathol Med* 2001;30:12-21.

36. Chen HM[1], Hsieh RP, Yang H, Kuo YS, Kuo MY, Chiang CP. HLA typing in Taiwanese patients with oral submucous fibrosis. J Oral Pathol Med. 2004;33:191-9.

37. Yi-Ting D, Chen HM, Cheng SJ, Chiang CP, Kuo MY. Arecoline stimulated connective tissue growth factor production in human buccal mucosal fibroblasts: Modulation by curcumin. *Oral Oncol* 2009;45:99-105.

38. Goel A, Kunnumakkara AB, Aggarwal BB. Curcumin as Curecumin": from kitchen to clinic. *Biochem Pharmacol* 2008;75:787-809.

39. Chen A, Zheng S. Curumin inhibits connective tissue growth factor gene expression in activated hepatic stellate cells *in vitro* by blocking NFkappa B and ERK signaling. *Br. J Pharmacol* 2008;153:557-67.

40. Trivedy C, Warnakulasuriya KA, Hazare VK, Tavassoli M, Sommer P, *et al.* The up regulation of lysyl oxidase in oral submucous fibrosis and squamous cell carcinoma. *J Oral Pathol. Med* 1999;28:246-51.

41. Kagen HM, Trackenman PC. Properties and function of lysyl oxidase. *Am J Respir Cell Mol Biol* 1991;5:206-10.

42. Ma RH, Tsai CC, Shieh TY. Increased lysyl oxidase activity in fibroblasts cultured from oral submucous fibrosis associated with betel nut chewing in Taiwan. *J Oral Pathol Med* 1995;24:407-12.

43. Rao, A.B.N. Idiopathic palatal fibrosis. *Br J Surg* 1962;50:23–5.

44. Millard PR. Submucous fibrosis. *Br J Dermatol*. 1966;78:305-7.

45. Z Clinical Aspects of Oral Submucous Fibrosis, *Acta odont. Scandinav* 1964;22: 679-91.

46. Joshi SG. Submucous Fibrosis of the Palate and Pillars. *Indian J. Otolaryng* 1953;4:1-4.

47. Shah B, Lewis M A O, Bedi R, Oral submucous fibrosis in a 11-year-old Bangladeshi girl living in the United Kingdom. *BDJ.* 2001;191:130-2.

48. Kumar A, Bagewadi A, Keluskar V, Singh M. Efficacy of lycopene in the management of oral submucous fibrosis. *J. Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2007; 103: 207-13.

49. Lal D. Diffuse Sub Mucus Fibrosis. *JIDA* 1953; 26: 1-5.

50. Haider SM, Merchant AT, Fikree FF, Rahbar M H. Clinical and functional staging of oral submucous fibrosis. *Br J Oral Maxillofac Surg* 2000;38:12–5.

51. Wahid PN, Kapur Vl, Luthra UK, Srivastava MC Submucous fibrosis of the oral cavity. 2. *Studies on epidemiology Bull WHO* 1966: 35: 793-799.

52. Mathew B, Warier PRK, Zachariah j. Oesophageal changes in oral submucous fibrosis. *Ind J Path & Bact* 1967; 10(4): 349 – 353.

53. Ahuja SS, Agrawal. SMF of oral mucosa. *J Oral Med* 1971; 26: 35.

54. Bhat AP, Dholakia HM. Mast cell density in oral submucous fibrosis. *JIDA* 1977; 49: 187 – 191.

55. Ranganathan K, Mishra G. An overview of classification schemes for oral submucous fibrosis. *J Oral Maxillofac Pathol* 2006;10(2):55-8.

56. Bailoor DN, Nagesh KS. Oral precancer. Fundamentals of Oral Medicine and Rad (2^{nd} edn) 2001: 282 – 289.

57. Rajendran R. George T. Morphohistometric analysis of advancing tumour fronts in malignancies associated with oral submucous fibrosis. *Indian J Dent Res* 2003; 14(4): 194-205.

58. Khanna JN, Andrade NN: Oral submucous fibrosis: A new concept in surgical management Report of 100 cases. *Int J Oral Maxillofac Surg* 1995.

59. Reichart PA, Mohr U, Srisuwan S, Geerlings H, Theetranont C, Kangwanpong T. Precancerous and other oral mucosal lesions related to chewing, smoking and drinking habits in Thailand. *Community Dent Oral Epidemiol* 1987;15(3) :152-60.

60. Van Wyk CW, Seedat HA, Phillips VM. Collagen in submucous fibrosis: an electron microscopic study. *J Oral Pathol Med* 1990; 19(4): 182-187.

61. Paymaster, J.C. Cancer of the buccal mucosa. A clinical study of 650 cases in Indian patients. *Cancer* 1965;9:431–5.

62. Zittermann SI, Issekutz AC. Basic fibroblast growth factor (bFGF, FGF-2) potentiates leukocyte recruitment to inflammation by enhancing endothelial adhesion molecule expression. *J Pathol* 2006;168:835-46.

63. Bhonsle RB, Murti PR, Daftary DK,. Focal vascular dilatations and petechiae in oral submucous fibrosis. Scand. *J Dent Res* 1981;89:270-4.

64. Sirsat SM, Khanolkar VR. A histochemical and electron microscopic study of submucous fibrosis of the palate. *Indian J Pathol Bacteriol* 1957;73:439-42.

65. Sirsat SM, Pindborg JJ: Subepithelial changes in oral submucous fibrosis. Acta. *Pathol Microbiol Scand* 1967;70(2):161-73.

66. Hamner JE, Mehta FS, Pindborg JJ, Daftary K.. Altered staining reaction of connective tissues in 53 submucous fibrosis patients. *J. Dent Res* 1971;50:388-92.

67. El-Labban NG, Canniff JP. Ultra structural findings of muscle degeneration in oral submucous fibrosis. *J Oral Pathol* 1985;14(9):709–17.

68. Pindborgh JJ, Kalapesi HK, Kale SA, Singh B, Taleyarkhan BN. Frequency of oral leukoplakia and related condition among 10000 Bombayites, Preliminary report. *J Indian Dent Assoc* 1965;37:228-9.

69. Pindborgh J, Zachariah J. Frequency of oral submucous fibrosis among 100 Indian with oral cancer. *Bulletin of the World Health Organization* 1965;32: 750-3.

70. Pindborg JJ. Is submucous fibrosis a pre-cancerous condition in the oral cavity? *Int Dent J* 1972;22:474-80.

71. Pindborg JJ, Murti PR, Bhonsle RB, Gupta PC, Daftary DK, Mehta FS.. Oral submucous fibrosis as a pre-cancerous condition. Scand. *J Dent Res* 1984;92:224-9.

72. P.R. Murti,R.B. Bhonsle, J. Pindborg, D.K. Daftary, P.C. Gupta, Fali S. Mehta. Malignant transformation rate in oral submucous fibrosis over a 17-year period. *Community Dent Oral Epidemiol* 1985;13:340-1.

73. Aziz SR. Lack of reliable evidence for oral submucous fibrosis treatments. *EBD* 2009;10(1):8-9.

74. Gupta PC, Hebert JR, Bhonsle RB, Sinor PN, Mehta H, Mehta FS. Dietary factors in oral leukoplakia and submucous fibrosis in a population based case control study in Gujarat, India. *J of Oral Dis* 1998;4(3):200-6.

75. Maher R, Aga P, Johnson NW, Sankaranarayanan R, Warnakulasuriya S Evaluation of multiple micronutrient supplementation in the management of oral submucous fibrosis in Karachi, Pakistan. *Nutr Cancer* 1997;27:41-7.

76. Kumar A, Sharma SC, Sharma P, *et al.*: Beneficial effect of oral zinc in the treatment of oral submucous fibrosis. *Indian J Pharmacol* 1991;23:236-41.

77. Trivedy C, Baldwin D, Warnakulasuriya S, *et al.*: Copper content in Areca catechu (betel nut) products and oral submucous fibrosis. *Lancet* 1997;349: 1447.

78. Chiu CJ, Chang ML, Chiang CP, *et al.*: Interaction of collagen related genes and susceptibility to betel quid-induced oral submucous fibrosis. *Cancer Epidemiol Biomarkers Prev* 2002;11:646-53.

79. Singh M[1], Krishanappa R, Bagewadi A, Keluskar V.: Efficacy of oral lycopene in the treatment of oral leukoplakia. *Oral Oncol* 2004;40:591-6.

80. Gupta S, Reddy MVR, Harinath BC. Role of oxidative stress and antioxidants in aetiopathogenesis and management of oral submucous fibrosis. *IJCB* 2004; 19(1):138-41.

81. Jiang X, Hu J. Drug treatment of Oral Submucous Fibrosis: A Review of the Literature. *J Oral Maxillofac Surg* 2009;67:1510-15.

82. Pindborg JJ. Oral epithelial changes in thirty Indians with oral cancer and submucous fibrosis. *Cancer* 1967;20:1141-46.

83. Borle RM, Borle SR. Management of oral submucous fibrosis: a conservative approach. *J Oral Maxillofac Surg* 1991;49(8):788–91.

84. Samlaska CP, Winfield EA: Pentoxifylline—Clinical review. *J Am Acad Dermatol* 1994;30:603-21.

85. Dipti Singh, Mathod C Shashikant, Neeta Misra, Sudhanshu Aggrawal. Lycopene And Intralesional Betamethasone Injections In The Management of OSMF. *JIAOMR* 2014; 26(3) :264-268.

86. Lin HJ, Lin JC: Treatment of oral submucous fibrosis by collagenase: Effects on oral opening and eating function. *Oral Dis* 2007;13:407-13.

87. Kakar PK, Puri RK, & Venkatachalam VP: Oral submucous fibrosis Treatment with hyalase. *J Laryngol Otol* 1985;99:57-9.

88. Gupta D, Sharma SC: Oral submucous fibrosis—A new treatment regimen. *J Oral Maxillofac Surg* 1988;46:830-3.

89. Tai YS *et al.*: Oral administration of milk from cows immunized with human intestinal bacteria leads to significant improvements of symptoms and signs in patients with oral submucous fibrosis. *J Oral Pathol Med* 2001;30:618-25.

90. Jirge V, Shashikanth MC, Ali IM, Anshumalee N. Levamisol and antioxidants in the management of oral submucous fibrosis: A comparative study. *J Indian Acad Oral Med Radiol* 2008;20:135-40.

91. Katharia SK, Singh SP, Kulshreshtha VK. The effects of placenta extract in the management of oral submucous fibrosis. *Indian J Pharmacol* 1992;24:81-3.

92. Anshumalee N, Shashikanth MC, Shambulingappa P, Deepak U. Lycopene: A Promising Antioxidant. *JIAOMR* 2007;19(04):458-63.

93. Gupta DS, Gupta MK. Oral submucous fibrosis–clinical study and management by phsiofibrolysis *JIDA* 1980;52:375.

94. Ravi Mehrotra,Shruti Pandya,Ajay Kumar Chaudhary,Himanshu Pratap Singh, Ritesh Kumar Jaiswal,Mangal Singh, SC Gupta and Mamta Singh. Lipid profile in OSMF. *Lipids in health and disease* 2009; 8(29):1-7.

95. Chalkoo H.A . A study on alterations in plasma lipid profile patterns in OSMF patients. *Journal of Indian Academy of Oral Medicine and Radiology* 2011;23(1):36-38.

96. Pramod Kumar, Amit Singh,Bharat Sankhla, and Anjali Naraniya. Alteration in plasma lipid profile in oral submucous fibrosis patients: A case control study. *South Asian Journal of Cancer* 2013; (3):147-149.

97. Mayeesh Radhakrishna, Jose Joy Idiculla and Aiswarya CJ. Alterations in serum lipid profile patterns in patients with oral submucous fibrosis. *J. of Health Sciences.* 2014;1(3):JS001G.

98. Ajai K, Panat SR, Aggarwal A, Agarwal N, Upadhyay N, Joshi A. Estimation of serum lipids in patients with Oral Submucous Fibrosis in India. *J Clin Exp Dent.* 2014;6(3):237-42.

99. Ranjith Kumar Kanthem, Venkateswar Rao Guttikonda. Serum lipid profile in oral submucous fibrosis: A clinico pathological study. *Journal of Oral and Maxillofacial Pathology*. 2015;19(2)S:139-14.

100. Apps DK, Cohen BB, Steel CM (Editors) Biochemistry a concise text for medical students (5th edn.) Great Britain: Mackays of Catham; 1992, pp. 94-144.

101. Satyanarayana U, Chakarapani U .Biochemistry (3rd edn.) Kolkata: Arunabha Sen Books and Allied Pvt. Ltd. 2006;28-42.

102. West ES, Todd WR, Mason HS, Van Bruggen JT. Textbook of Biochemistry (4th edn.) New Delhi: Oxford & IBH Publishing Co. Pvt. Ltd.; 1974, pp. 123-172.

103. Nelson DL, Cox MM. Lehninger principles of biochemistry (3rd edn.) New York: Worth Publishers; 2004; 598-622.

104. Nomura DK, Cravatt BF. Lipid metabolism in cancer. *Biochimica Biophysica Acta* 2013; 1831: 1497-1498.

105. Baenke F, Peck B, Miess H, Schulze A. Hooked on fat: the role of lipid synthesis in cancer metabolism and tumour development. *Disease Models & Mechanisms* 2013; 6: 1353-1363.

106. Feinleib M. Review of the epidemiological evidence for a possible relationship between hypocholesterolemia and cancer. *Cancer Research* 1983; 43: 2503-2507.

107. Raste AS, Naik PP. Clinical significance of lipid profile in cancer patients. *Indian Journal of Medical Sciences*. 2000; 54: 435-441.

108. Medes G, Thomas A, Weinhouse S. Metabolism of neoplastic tissue. IV. A study of lipid synthesis in neoplastic tissue slices in vitro. *Cancer Research* 1953; 13: 27-29.

109. Nieman KM1, Kenny HA, Penicka CV, Ladanyi A, Buell- Gutbrod R, Zillhardt MR, *et al*. adipocytes promote ovarian cancer metastasis and provide energy for rapid tumor growth. *Nature Medicine* 2011; 17: 1498-1503.

110. Allampallam K, Dutt D, Nair C, Shetty V, Mundle S, Lisak L, *et al*. The clinical and biologic significance of abnormal lipid profiles in patients with myelodysplastic syndromes. *Journal of Hematotherapy and Stem Cell Research* 2000; 9: 247-255.

111. Singh S, Ramesh V, Premalatha B, Prashad KV, Ramadoss K. Alterations in serum lipid profile patterns in oral cancer. *Journal of Natural Science, Biology and Medicine* 2013; 4: 374-378.

112. Kuliszkiewicz-Janus M, Malecki R, Mohamed AS. Lipid changes occurring in the course of hematological cancers. *Cellular & Molecular Biology Letters* 2008; 13: 465-474.

113. Gupta S . Alterations in serum lipid profile patterns in oral cancer and oral precancerous lesions and conditions—a clinical study. *Indian Journal of Dentistry* 2011; 2(2):1-7.

114. Reddy V. Oral Submucous Fibrosis: Correlation of Clinical Grading to various habit factors. *International Journal of dental clinics* 2011:3(1): 21-24.

115. Priya Kumar**,** J Augustine**,** Aadithya B Urs**,** Shelly Arora**,** Shalini Gupta**,** Vikrant R Mohanty. Serum lipid profile in oral cancer and leukoplakia: Correlation with tobacco abuse and histological grading. *Journal of Cancer Research and Therapeutics* 2012; 8 (3):344-348.

116. Gopal Sharma, 2Dr. Deepa Das, 3Dr. Jaya Mukherjee, 4Dr. Bhagyashri Purandare. Lipid Profile in Oral Submucous Fibrosis Patients in India -A Pilot Study. *Indian Journal of Basic & Applied Medical Research* 2013; 7(2): 790-796.

117. Singh S(1), Ramesh V, Premalatha B, Prashad KV, Ramadoss K.. Alterations in serum lipid profile patterns in oral cancer. *J Nat Sci Biol Med* 2013;4(2): 374–378.

118. Gupta N. Alterations in serum lipid profile patterns in head & neck cancer and oral submucous fibrosis patients. *International Dental Journal of student's Research.* 2014;2(3):17-24.

119. Prachi Goel, Ranjana Garg, Vijay Raghavan. Lipid profile in oral potentially malignant disorders. *Journal of Indian Academy of Oral Medicine & Radiology*. 2014;26(4):374-378.

120. Vijay kumar Poorey, Pooja Thakur. Alteration of lipid profile in patients with head and neck malignancies. *Indian J of Otolaryngol Head Neck Surg*. 2015. doi10.1007/s12070-015-0829.4.

121. Neeta Misra, Anuj Maheshwari, Shivani Pandey, pradyumna Misra, Amanpreet Kaur. Alterations in serum lipid profile patients in patients with OSMF. *OMJ*. 2017;7(1):page no 7-14.

122. D.A. Kerr, M. Ash, H.D. Millard. Oral Diagnosis. 4th ed. Saint Louis: Mosby; 1974.

123. Roeschlau P, Bernt E, Gruber W. Enzymatic determination of total cholesterol in serum. *Z Klin Chem Klin Biochem*. 1974 May;12(5):226.

124. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 1972;18(6):499-502.

ANNEXURES

Annexure-I

CASE HISTORY PROFORMA

Title : A comparative study of serum lipid profile in patients with oral submucous fibrosis .

DEPARTMENT OF ORAL MEDICINE & RADIOLOGY
Babu Banarasi Das College of Dental Sciences, Lucknow (U.P.)

OPD No : Case No :

Name : Age /Sex :

Occupation: Address :

Contact No :

Chief Complaint :

HISTORY OF PRESENT ILLNESS

Burning Sensation	Duration	VAS

PAST MEDICAL, DENTAL, DRUG & FAMILY HISTORY

PERSONAL HISTORY

Abusive habits : No Abusive habits :

Abusive Habit	Duration	Frequency Side	Duration in the oral cavity
Smokeless tobacco			
Smoking tobacco			
Alcohol			

INTRA ORAL EXAMINATION

- **Soft Tissue Examination**

Lips			
Site	Blanching	Stiffness and Fibrous Bands	Any White or Red lesion
Upper Labial Mucosa			
Lower labial Mucosa			
Right Buccal Mucosa			
Left Buccal Mucosa			
Vestibule			
Palate			
Floor of Mouth			
Retromolar Pad Area			

Uvula	Inflammation	Blanched	Shrunken

Tongue	Protrusion

Investigations :

Reports:

Lipid Profile	Level
Total Cholesterol	
HDL	
LDL	
VLDL Trigycerides	

Treatment Planning

Signature of Student ***Signature of Guide***

Annexure-II

CONSENT FORM

Title of the Study……..……..……..……..……..……..……..…...

Study Number……..……..……..……..……..……..……..……

Subject's Full Name…….……..……..……..……..……..……..…….

Date of Birth/Age ……..……..……..……..……..……..……...

Address of the Subject……..……..……..……..……..

Phone no. and e-mail address……..……..……..…….....

Qualification ……..……..……..……..……..……...

Occupation: Student / Self Employed / Service / Housewife/ Other……..…….....

(Please tick as appropriate)

Annual income of the Subject……..

Name and of the nominees(s) and his relation to the subject…… (For the purpose of compensation in case of trial related death).

- I confirm that I have read and understood the Participant Information Document dated ……..for the above study and have had the opportunity to ask questions.

  OR I have been explained the nature of the study by the Investigator and had the opportunity to ask questions.

2. I understand that my participation in the study is voluntary and given with free will without any duress and that I am free to withdraw at any time, without giving any reason and without my medical care or legal rights being affected.
3. I understand that the sponsor of the project, others working on the Sponsor's behalf, the Ethics Committee and the regulatory authorities will not need my permission to look at my health records both in respect of the current study and

any further research that may be conducted in relation to it, even if I withdraw from the trial. However, I understand that my Identity will not be revealed in any information released to third parties or published.

4. I agree not to restrict the use of any data or results that arise from this study provided such a use is only for scientific purpose(s).
5. I permit the use of stored sample (tooth/tissue/blood) for future research.

Yes [ ] No [ ] Not Applicable [ ]

6. I agree to participate in the above study. I have been explained about the complications and side effects, if any, and have fully understood them. I have also read and understood the participant/volunteer's Information document given to me.

Signature (or Thumb impression) of the Subject/Legally Acceptable Representative:……...

Signatory's Name……. Date …….

Signature of the Investigator…… Date……..

Study Investigator's Name..... Date……..

Signature of the witness…… Date……..

Name of the witness……

Received a signed copy of the PID and duly filled consent form

Signature/thumb impression of the subject or legally Acceptable representative Date……..

सहमति पत्र

अध्ययन शीर्षक.....

अध्ययन संख्या .....

प्रतिभागी के पूर्ण नाम .....

जन्मतिथि/आयु .....

फोन नंम्बर और ई–मेल पता.....

योग्यता .....

व्यवसाय : छात्र/स्व कार्यरत/सेवा/गृहिणी.....

अन्य (उचित रूप में टिक करे).....

प्रतिभागी की वार्षिक आय .....

प्रत्याशीयों के नाम और प्रतिभागी से सम्बन्ध..... (परीक्षण से सम्बन्धित मौत के मामले में मुआवजे के प्रयोजन के लिये)

1. मेरी पुष्टि है कि मैने अध्ययन हेतु सूचना पत्र दिनांक ..... को पढ़ व समझ लिया था मुझे प्रश्न पूछने या मुझे अध्ययन अन्वेषक ने सभी तथ्यों को समझा दिया है तथा मुझे प्रश्न पूछने के समान अवसर प्रदान किये गये।
2. मैने यह समझ लिया है कि अध्ययन में मेरी भागीदारी पूर्णातः स्वैच्छिक है और किसी भी दबाव के बिना स्वतन्त्र इच्छा के साथ दिया है किसी भी समय किसी भी कारण के बिना मेरे ईलाज या कानूनी अधिकारों को प्रभावित किये बिना अध्ययन में भाग न लेने के लिये स्वतन्त्र हूँ।
3. मैने यह समझ लिया है कि अध्ययन के प्रयोग तक, प्रयोजक की तरफ से काम करने वाले लोग, अचार समिति और नियामक अधिकारियों को मेरे स्वास्थ्य रिकार्ड को वर्तमान अध्ययन या आगे के अध्ययन के सन्दर्भ देखने के लिये मेरी अनुमति की जरूरत नहीं है, चाहें मैने इस अध्ययन से नाम वापस ले लिया है। हालाकि मैं समझता/समझती हूँ कि मेरी पहचान को किसी भी तीसरे पक्ष या प्रकाशित माध्यम में नहीं दिया जायेगा।
4. मैं इससे सहमत हूँ कि कोई भी डेटा या परिणाम जो इस इस अध्ययन से प्राप्त होता है उसका वैज्ञानिक उद्देश्य (ओं) के उपयोग के लिये मेरी तरफ से कोई प्रतिबन्ध नहीं है।
5. मैं परीक्षण की अनुमति देता/देती हूँ। मुझे इसके द्वारा यदि कोई परेशानी होती है, तो इसके बारे में जानकारी दे दी गयी है। मैने रोगी की जानकारी सूचना पत्र को पढ़ तथा समझ लिया है।

प्रतिभागी/कानूनी तौर पर स्वीकार प्रतिनिधि का हस्ताक्षर या (अंगूठे का निशान).....

हस्ताक्षरकर्ता का नाम..... दिनांक .....

अन्वेषक के हस्ताक्षर ..... दिनांक .....

अन्वेषक का नाम .....

गवाह के हस्ताक्षर ..... दिनांक .....

गवाह का नाम .....

मैने पी0आई0डी0 और विधिवत भरे सहमति फार्म का एक हस्ताक्षर की नकल प्राप्त की।

प्रतिभागी कानूनी तौर पर प्रतिनिधि के हस्ताक्षर या अंगूठे का निशान ..... दिनांक .....

Annexure-III

Babu Banarasi Das College of Dental Sciences
(A constituent institution of Babu Banarasi Das University)
BBD City, Faizabad Road, Lucknow - 227105 (INDIA)

Participant Information Document (PID)

1. Study title

Study of Alteration of Lipid Profile in Patients Suffering from Oral Submucous Fibrosis.

2. Invitation paragraph

You are being invited to take part in a research study. lt is therefore important for you to understand why the study is being done and what it will involve. Please take time to read the following information carefully. Ask us for any clarifications or further information. Whether or not you wish to take part is your decision.

3. What is the purpose of the study?

The purpose of this study is to evaluate alteration of lipid in oral submucous fibrosis patients.

4. Why have I been chosen?

You have been chosen for this study as you are fulfilling the required criteria for this study.

5. Do I have to take part?

Your participation in the research is entirely voluntary. If you do, you will be given this information sheet to keep and will be asked to sign a consent form. During the study you still are free to withdraw at any time and without giving a reason.

6. What will happen to me if I take part?

You will have to undergo blood examination where 2.5ml of your blood will be taken by syringe.

7. What do I have to do?

You do have to change your regular lifestyles for the investigation of the study.

8. What is the procedure that is being tested?

A 2.5 ml of your blood will be taken and value obtained then the study will be done on alteration of lipid in oral sub mucous fibrosis patients.

9. What are the interventions for the study?

We will observe the alteration of lipid in oral sub mucous fibrosis patients.

10. What are the side effects of taking part?

There are no side effects on patients of this study.

11. What are the possible disadvantages and risks of taking part?

There are no risks or disadvantages of taking part in this study.

12. What are the possible benefits of taking part?

Alteration of lipid may serve as a biomarker to analyze the progression of the disease and its malignant transformation and outcome for the treatment may be improved.

13. What if new information becomes available?

If additional information becomes available during the course of the research you will be told about these and you are free to discuss it with your researcher, your researcher will tell you whether you want to continue in the study. If you decide to withdraw, your researcher will make arrangements for your withdrawal. If you decide to continue in the study, you may be asked to sign an updated consent form

14. What happens when the research study stops?

If the study stops/finishes before the stipulated time, this will be explained to the patient/volunteer.

15. What if something goes wrong?

If any severe adverse event occurs, or something goes wrong during the study, the complaints will be handled by reporting to the institution (s), and Institutional ethical committee.

16. Will my taking part in this study be kept confidential?

Yes it will be kept confidential.

17. What will happen to the results of the research study?

Result is the soul properties of the department of the Oral medicine and Radiology, BBDCODS Lucknow

Your identity will be kept confidential in case of any report/publications.

18. Who is organizing the research?

This research study is organized by Department of Oral medicine and Radiology, BBDCODS Lucknow

19. Will the results of the study be made available after study is over?

Yes.

20. Who has reviewed the study?

The study has been reviewed and approved by the Head of the Department of Oral medicine and Radiology,and the (IEC) (IRC) of the institution.

21. Contact for further information

Dr. Neeta Misra (PhD Scholar) Head of Department

Department of Oral medicine and Radiology Babu Banarasi College of Dental Sciences. BBDU Lucknow-227105 Mob- 8004006622

Dr Laxmi Bala, Head of Department Department of Biochemistry Member Secretary IEC, Babu Banarasi College of Dental Sciences. BBDU Lucknow- 227105 bbdcods.iec@qmail.com

दंत चिकित्सा विज्ञान के बाबू बनारसी दास कॉलेज
(बाबू बनारसीदास विश्वविद्यालय के एक घटक संस्था)
बीबीडी सिटी, फैजाबाद रोड, लखनऊ–227105

प्रतिभागी जानकारी दस्तावेज (पीआईडी)

1. अध्ययन खिताब

स्टडी ऑफ ऑटरेशन ऑफ लिपिड़ प्रोफाइल इन पेसेन्ट सफरिंग फ्राम ओरल सब म्यूकस फाइब्रोसिस।

2. निमंत्रण पैरा

आप एक शोध अध्ययन में भाग लेने के लिए आमंत्रित हैं। इसलिए यह महत्वपूर्ण है कि आप समझे कि आप ये अध्ययन क्यों करने जा रहे है और इसमें क्या शामिल होगा। ध्यान से पढ़ने के लिए समय ले। किसी भी जानकारी या स्पष्टीकरण के लिए हमसे पूछे।

3. अध्ययन का उद्देश्य क्या है?

ओरल सबम्यूकस फाइब्रोसिस के मरीजों का लिपिड़ प्रोफाइल का आकलन करना।

4. मुझे क्यों चुना गया है?

आपको इस अध्ययन के लिए चुना गया क्योंकि आप इस अध्ययन के लिए आवश्यक मानदण्डों को पूरा कर रहे हैं। आपको गुटखा खाने के वजह से ओरल सबम्यूकस फाइब्रोसिस हो गया है।

5. क्या मैं भाग लूँ?

अनुसंधान के क्षेत्र में भागीदारी पूरी तरह स्वैच्छिक है। यदि आप कहते है तो आपको इस जानकारी को रखने के लिए पत्र दिया जाएगा और एक सहमति पत्र पर हस्ताक्षर करने के लिए कहा जाएगा। अध्ययन के दौरान आप कभी भी किसी भी समय और बिना कारण दिये वापस होने के लिए स्वतंत्र है।

6. यदि मैं भाग लू तो मुझे क्या होगा?

आपकी जांच के लिये 2.5 मिली0 रक्त लिया जायेगा।

7. मुझे क्यो–क्या करना होगा?

आप अध्यन की जांच के लिए अपने नियमित जीवन शैली बदलने की जरूरत नहीं है।

8. परीक्षण की क्या प्रक्रिया है क्या किया जा रहा है?

आपके दिये हुये 2.5 मिली0 रक्त को लिपिड़ प्रोफाइल के आकलन के लिये भेजा जायेगा।

9. अध्ययन के हस्तक्षेप क्या है?

प्राप्त लिपिड प्रोफाइल का ओरल सबम्यूकस फाइब्रोसिस मरीज में आंकलन किया जायेगा।

10. भाग लेने के दुष्प्रभाव क्या है?

इस अध्ययन के मरीजों पर कोई दुष्प्रभाव नहीं है।

11. संभावित नुकसान और भाग लेने का जोखिम क्या है?

इस अध्ययन में भाग लेने में कोई जोखिम या संभावित नुकसान नहीं है।

12. भाग लेने के संभावित लाभ क्या है?

इस अध्ययन से हमें लिपिड़ प्रोफाइल के आल्टरेशन से बीमारी का जल्दी पता चलेगा, जिससे इलाज जल्दी सम्भव हो सकेगा।

13. यदि क्या नई जानकारी उपलब्ध हो जाती है?

अनुसंधान अतिरिक्त जानकारी आपको इनके बारे में बताया जाएगा और आप अपने शोधकर्ता के साथ इस पर चर्चा करने के लिए स्वतन्त्र हैं, शोधकर्ता आपको बता देगा कि आप अध्ययन में जारी रखना चाहते हैं। आप वापस लेने का फैसला करते हैं, तो आपके शोधकर्ता अपनी वापसी के लिए व्यवस्था कर देगा। आप अध्ययन में जारी रखने का फैसला करते हैं, तो आप एक अद्यतन सहमति पत्र पर हस्ताक्षर करने के लिए कहा जा सकता है।

14. जब शोध अध्ययन बंद हो जाता है तो क्या हो सकता है?

यदि अध्ययन बंद हो जाता है निर्धारित समय से पहले खत्म हो जाता है तो यह मरीज/स्वयंसेवक को समझाया जाएगा।

15. क्या कुछ गलत हो जाता है?

किसी भी गंभीर प्रतिकूल घटना होती है, या कुछ और अध्ययन के दौरान गलत हो जाता है, शिकायतों संस्था(ओ) के लिए रिपोर्टिंग और संस्थागत नैतिक समिति द्वारा नियंत्रित किया जाएगा।

16. इस अध्ययन में मेरी भागीदारी लेना गोपनीय रखा जाएगा ?

हाँ, यह गोपनीय रखा जाएगा।

17. शोध अध्ययन के परिणामों का क्या होगा?

परिणाम ओरल मेडिसिन एण्ड रेडियोलॉजी विभाग, दंत चिकित्सा विज्ञान के बाबू बनारसी दास कॉलेज लखनऊ के विभाग के आत्मा के गुण है। आपकी पहचान किसी भी रिपोर्ट/प्रकाशनों के मामले में गोपनीय रखी जायेगी।

18. किसने अनुसंधान का आयोजन किया है?

यह शोध अध्ययन ओरल मेडिसिन एण्ड रेडियोलॉजी विभाग, दंत चिकित्सा विज्ञान के बाबू बनारसी दास कॉलेज लखनऊ द्वारा आयोजित किया गया है।

19. क्या अध्ययन खत्म होने के बाद अध्ययन के परिणामों को उपलब्ध कराया जाएगा?

हाँ।

20. किसने अध्ययन की समीक्षा की है?

अध्ययन की समीक्षा और मंजूरी ओरल मेडिसिन एण्ड रेडियोलॉजी विभाग के प्रमुख (संस्था आईईसी और आईआरसी) ने दी है।

21. अधिक जानकारी के लिए सम्पर्क

डॉ0 नीता मिश्रा (विभागाध्यक्ष)
ओरल मेडिसिन एण्ड रेडियोलॉजी विभाग
डेंटल साइंसेज बाबू बनारसी दास कॉलेज।
लखनऊ–227105
मो0 नं0– 8004006622

डॉ0 लक्ष्मी बाला
सदस्य सचिव आईसी
डेंटल साइंसेज बाबू बनारसी दास कॉलेज।
लखनऊ
Bbdcods.iec@gmail.com

पीआई के हस्ताक्षर ..... ।
नाम ..... ।
तारीख ..... ।

Annexure-IV

Babu Banarasi Das College of Dental Sciences
(A Constituent Institution of Babu Banarasi Das University)
BBD City, Faizabad Road, Lucknow – 227105 (INDIA)

Dr. Lakshmi Bala
Professor and Head Biochemistry and
Member-Secretary, Institutional Ethics Committee

Communication of the Decision of the I[st] Institutional Ethics Committee

IEC Code: 26 **BBDCODS/ 02 /2013**

Agenda Item No. 1

Title of the Project: Study of alternation of lipid profile in patients suffering from Oral Submucus Fibrosis.

Principal Investigator: Dr. Neeta Misra **Department:** Oral Medicine & Radiology

Name and Address of the Institution: BBD College of Dental Sciences Lucknow

Type of Submission: New

Date of IEC Meeting: 03/09/2013

Subject: IEC Approval

Dear Dr. Neeta Misra

Institutional Ethics Committee reviewed and discussed your submitted documents of the research study during the IEC meeting held on 3[rd] Sept 2013.

The following are the members of the Institutional Ethics Committee.

1	Dr. B.N. Dhawan, Former Director, CDRI, Lucknow	Chairman
2	Dr. Nuzhat Husain, Director and Dean., RMLIMS, Lucknow	Member
3	Dr. O.P. Asthana, Former Deputy Director, CDRI, Lucknow	Member
4	Dr. J.S. Srivastava, Deputy Director, Chief Scientist, CDRI, Lucknow	Member
5	Shri. S.N. Tandon, Former Judge, Lucknow	Member
6	Shri. Alok Saran, Senior Advocate, Lucknow	Member
7	Dr. Manisha Chowdhry, Professor, Endodontics, BBDCODS	Member
8	Dr.Vivek Govila, Professor Head, Periodontics, BBDCODS	Member
9	Dr. Lakshmi Bala, Professor and Head, Biochemistry, BBDCODS	Member-Secretary

However, Dr. O. P. Asthana, Dr. Nuzhat Hussain, Mr. Alok Saran could not attend due to other prior commitments.

Following Points must be noted:

1. The IEC is organized and functions in accordance with GLP-CDSCO/ICMR/Schedule Y guidelines/ICH-GCP.
2. The decision was taken with thorough consensus. PI or any of the Co-PI were not present during the decision making of the IEC.

3. The Principal Investigator should mention in their proposals the duration of the study. The IEC Clearance is normally provided for one year/duration of the study which so ever is tlonger. The IEC would also evaluate the correctness of the duration. The PI should inform the date of initiation of the study to the Member-Secretary on prescribed format with-in 6 months of the initiation of the study failing that the approval would relapse.
4. The PI will submit half yearly progress report of the project to the Member Secretary for its review by IEC., Consent should be taken from the volunteer/patient to use their stored samples for future research. However, a new study (protocol) for using stored samples will require fresh IEC approval. The IEC approval is also desired for any future study which uses such data retrospectively.
5. The PI, Co-PI and other investigators should co-operate with IEC functioning.
6. In the event of any amendments/changes/deviations of the protocol, IEC must be informed and the changes should be highlighted clearly.
7. In the event of any SAE, or any new information which could affect SAE must be communicated to IEC and sponsors. For IEC approved studies, the PI should inform and report SAEs within 7 days of the occurrence of the SAE to the Institution and to IEC. If the SAE is 'Death', the IEC should receive the SAE reporting within 24 hrs of the occurrence.

Decisions of the IEC: IEC has taken following decisions for the current protocol study/trial;

The committee approved the above project from ethics point of view, with the following conditions

Comments:

I. IEC clearance and a Co-guide from KGMU required
II. A person/ biochemist should be involved as Co- Guide to ensure proper evaluation of lipid profile.
III. Separate Information documents required for patients and control subjects
IV. New CF required under DCGI regulation should be used

The approval will be granted subject to the compliance with all the above suggestions of the IEC.

Kindly resubmit the 4 copies of revised proposal of the documents.

Please reply by: 26-Oct-13

Lakshmi Bala

(Dr. Lakshmi Bala)
Member Secretary
IEC

Member Secretary
Institutional Ethic Committee
BBD College of Dental Sciences
BBD University
Faizabad Road, Lucknow-226028

Annexure-V

Published Articles

ORISSA MEDICAL JOURNAL

Alteration in Serum Lipid Profile Patterns in Patients of Oral Submucous Fibrosis

Guest Article

Neeta Misra[1], Anuj Maheshwari[2], Shivani Pandey[3], Pradyumna Misra[4], Amanpreet Kaur[5]

Abstract :

Background : Altered lipid profile patterns have been associated with pre-malignancies and malignancies because lipids play a vital role in the maintenance of cell integrity. Tobacco carcinogens induce generation of free radicals and reactive oxygen species, which cause lipid peroxidation. Because of the lipid peroxidation, there is a greater utilization of lipids for new membrane biogenesis. Hence the present study was carried out to determine the alterations, if any, in the serum lipid profile of subjects with Oral submucous fibrosis (OSMF).

Materials and methods : The study included 25 patients with oral submucous fibrosis and 25 patients having tobacco habits with no OSMF. Serum lipids, including (i) Total cholesterol, (ii) LDL cholesterol (LDLC), (iii) HDL cholesterol (HDLC), (iv) VLDL cholesterol (VLDLC) and (v) Triglycerides, (vi) TC/HDL Cholesterol ratio, (vii) LDL/HDL ratio were analyzed using spectrophotometry kits.

Results : Our findings suggest that Decrease in plasma total cholesterol, triglycerides, HDL, LDL, in the subjects with the OSMF as compared to the controls was statistically significant. VLDL, TC/HDL and LDL/HDL ratio was significantly higher among the cases than controls.

Conclusion : To conclude that the lower serum lipid status may be considered as a useful indicator for initial changes occurring in the neoplastic cells.

Keywords : Oral submucous fibrosis, serum lipid profile, areca nut.

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Introduction :

Oral submucous fibrosis (OSMF) is a chronic, debilitating disease characterised by juxtaepithelial fibrosis of the oral cavity.[1] Although occasionally preceded by, or associated with, formation of vesicles, it is always associated with a juxtaepithelial inflammatory reaction followed by fibroelastic changes of the lamina propria and epithelial atrophy that leads to stiffness of the oral mucosa and causes trismus and an inability to eat.[2]

It is a chronic, progressive, scarring disease associated with the chewing of areca nut, an ingredient of betel quid and predominantly affects the people of South- East Asian origin. This condition was described first by Schwartz (1952) while examining five Indian women from Kenya, to which he gave the descriptive term 'atrophia idiopathica' tropica) mucosae oris. Later Joshi in 1953, redesignated the condition as oral sub mucous fibrosis, implying predominantly its histological nature. It is characterised by burning sensation in the mouth while consuming spicy food, appearance of vesicles in the cheek and palate and fibrosis of the oral mucosa resulting in difficulty in mouth opening. The WHO definition of an oral precancerous condition –"a generalized pathological stage of the oral mucosa associated with a significantly increased risk of cancer," describes the characteristics of OSMF.[3]

The predominant age group affected is 20-40 years.[4] The pathogenesis of the disease is not well established, but the cause of OSMF is believed to be multifactorial. Factors include areca nut chewing, ingestion of chillies, genetic and immunologic processes, nutritional deficiencies, and other factors. 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.[5]

Lipids are major cell membrane components essential for various biological functions, including cell growth and division of normal and malignant tissues. Variations in tissue/blood cholesterol levels in diagnosis and treatment of various diseases has been studied by several workers.[6,7,8] There is compelling evidence to implicate the habitual chewing of areca nut in the development of OSMF.[7]

The major alkaloid in areca nut arecoline undergoes nitrosation and gives rise to N Nitrosamine, which might have cytotoxic effect on the cells.[9] This may induce the production of free radicals and reactive oxygen species which are responsible for high rate peroxidation of polyunsaturated fatty acids, this peroxidation further releases peroxide radicals which affect essential constituents of the cell membrane and might be involved in tumorigenesis. Because of the lipid peroxidation, there is a greater utilization of lipids, including total cholesterol, lipoproteins and triglycerides for new membrane biogenesis. Cells fulfil these requirements either from circulation by synthesis through the metabolism or from degradation of major lipoprotein fractions, like VLDL, LDL or HDL.[10] Lower blood lipid levels have been associated with various cancers.[11]

However, only a few studies have been carried out on serum lipid profiles in precancerous conditions. Considering this, the present study was planned to evaluate the serum lipid profile in OSMF patients as the change in lipid levels may have a diagnostic and prognostic role in the potentially malignant lesions.

Material and Methods :

The study was conducted in the Department of Oral Medicine and Radiology, Babu Banarasi Das College of Dental Sciences, Lucknow. Total sample size of 50 subjects was chosen with study group consisting of a total of 25patients having OSMF with tobacco habit and the control group consisted of 25 age and sex matched individuals without OSMF with tobacco habit who have come for some other problem related to teeth.

Subjects with clinically diagnosed OSMF with age 25-55yrs were included in the study. Patients who have undergone or on treatment for oral submucous fibrosis, Patients with systemic disease like diabetes, cardiac problems, obesity or any known systemic disease that can alter lipid profile or patients with previous or current history of lipid lowering medications were excluded from present study.

Method of examination and confirmation of clinical diagnosis

The patients were explained in detail about the study and the procedure they were subjected to. A formal informed written consent was obtained. Examination of the patients was carried. Routine hematological examinations (to ascertain bleeding time, clotting time, fasting blood sugar levels, hemoglobin count and erythrocyte sedimentation rate) were done for all subjects to rule out any systemic diseases. A comprehensive history was obtained from the patients with reference to their habits and patients with burning sensation, difficulty in mouth opening were clinically diagnosed as OSMF according to khanna and Andrade classification (1995)

Collection of Venous blood

Under aseptic conditions, 5 ml of fasting blood sample will be obtained by venipuncture of the median cubital vein. Venous blood was withdrawn with the help of a 5 ml disposable syringe and a 24 gauge disposable needle, into plain vacuettes. These samples were allowed to clot for 30 minutes and then the serum will be separated by centrifugation at 3000 rpm for 15 minute to get a clear serum sample which is separated from the clot and transferred to a disposable vial for assay. The estimation was performed within 3 hours of receiving the samples by using an appropriate kit and (quantitated for total serum cholesterol, LDL, VLDL, HDL, triglycerides, TC/HDL Cholesterol ratio, LDL/HDL ratio using a spectrophotometric methods. Data was tabulated and subjected to Statistical analysis using Chi- square test, which was performed to compare mean values of the parameters. The SPSS 16.0 version (Chicago, Inc., USA) was used to ascertain the results. p-value < 0.05 was considered to be statistically significant.

Results :

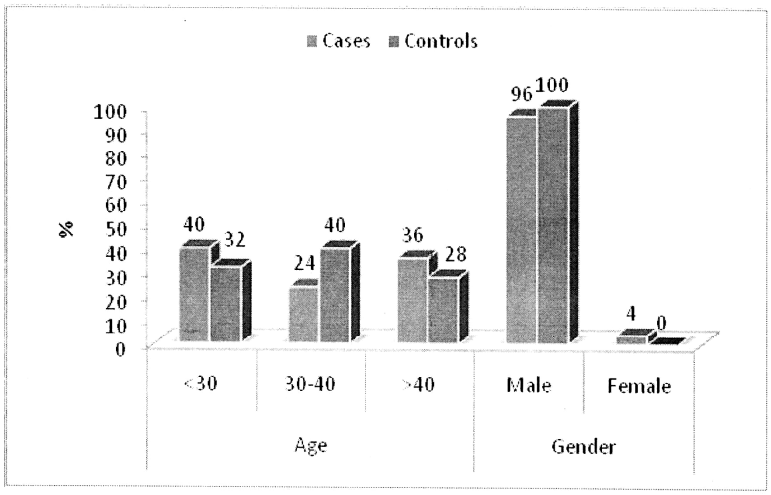
The study comprised of 50 patients who were divided into 2groups. In Group I (OSMF group), total number of patients were 25out of which 24 were male (96%) and 1 female (4%) subject. In Group II (control

group) total number of patients was 25 out of which all 25 were male subjects.

More than one third of the cases (40%) and 32% of controls were <30 years of age. However, 24% cases and 40% controls were between 30-40 years of age. The mean age of the cases and controls was 36.40 (±10.01) and 34.52 (±8.06) years respectively. The difference in the age between cases and controls was statistically insignificant (p>0.05). Majority of the cases and controls were males and the difference statistically insignificant (p>0.05).

Table-1: Age and gender distribution of cases and controls

Age and gender	Cases (n=25)		Controls (n=25)		p-value[1]
	No.	%	No.	%	
Age in years					
<30	10	40.0	8	32.0	0.47
30-40	6	24.0	10	40.0	
>40	9	36.0	7	28.0	
Mean±SD	36.40±10.01		34.52±8.06		
Gender					
Male	24	96.0	25	100.0	0.31
Female	1	4.0	0	0.0	



Graph. 1: Age and gender distribution of cases and controls

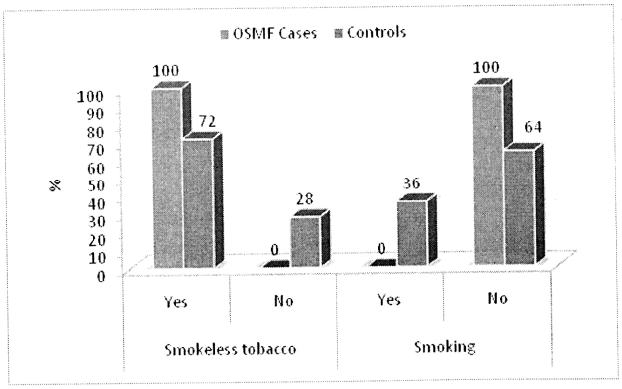
Graph. 1: 40% of the cases and 32% of controls were <30 years of age. However, 24% cases and 40% controls were between 30-40 years of age and 36% of cases and 28% of controls were above > 40yrs of age. 96% of the males had abusive habit along with the lesion present whereas 100% males were there with abusive habit with no lesion. Only 4% of females with abusive habit and lesion were present.

Table-2: Distribution of tobacco habit of OSMF cases and controls

Tobacco habit	OSMF Cases (n=25)		Controls (n=25)		p-value[1]
	No.	%	No.	%	
Smokeless tobacco					
Yes	25	100.0	18	72.0	0.004*
No	0	0.0	7	28.0	
Type	n=25		n=18		
Gutkha	25	100.0	17	94.4	0.23
Pan	0	0.0	1	5.6	
Smoking					
Yes	0	0.0	9	36.0	0.001*
No	25	100.0	16	64.0	
Type					
Cigarette	0	0.0	9	100.0	NA

[1]Chi-square test, *Significant, Not applicable

Table-2. presents the distribution of tobacco habit of the cases and controls. All OSMF cases and 72% controls were using smokeless tobacco. The Gutkha was used by all the OSMF cases and 94.4% controls. None of the OSMF cases and 36% of controls were smokers.

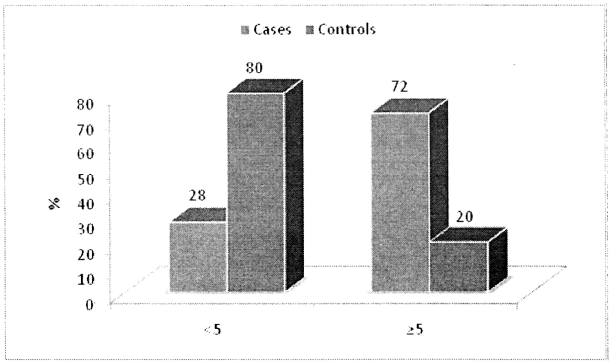


Graph. 2: Distribution of tobacco habit of OSMF cases and contro

Table-3: Duration use of tobacco habit of the cases and controls

Duration in years	Cases (n=25)		Controls (n=25)		p-value[1]
	No.	%	No.	%	
<5	7	28.0	20	80.0	0.0001*
≥5	18	72.0	5	20.0	
Mean±SD	6.40±2.87		3.08±1.44		

Table-3 depicts the duration of tobacco habit of the cases and controls. The duration of tobacco habit was <5 years in 28% of the cases and 80% of controls. However tobacco habit was >5 years was in 72% of the cases and 20% of control. The average duration of using tobacco was significantly higher among the cases than controls.



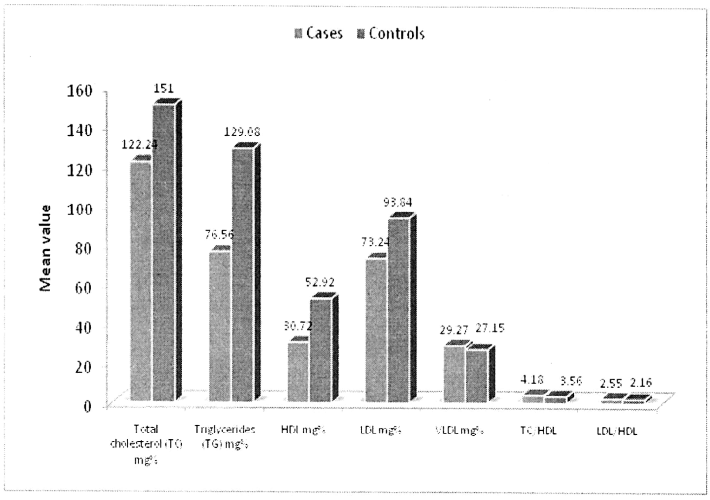
Graph. 3: Duration use of tobacco habit of the cases and controls

Graph. 3: shows the duration of tobacco habit which was <5 years in 28% of the cases and 80% of controls. However tobacco habit was >5 years was in 72% of the cases and 20% of control. The duration of using tobacco was significantly higher among the cases than controls.

Table-4: Alteration of Lipid levels of the cases and controls

Lipid levels	Cases (Mean±SD)	Controls (Mean±SD)	p-value[1]
Total cholesterol (TC) mg%	122.24±9.77	151.00±21.21	0.0001*
Triglycerides (TG) mg%	76.56±65.22	129.08±43.54	0.002*
HDL mg%	30.72±7.48	52.92±7.19	0.0001*
LDL mg%	73.24±11.64	93.84±11.85	0.0001*
VLDL mg%	29.27±11.09	27.15±9.06	0.46
TC/HDL	4.18±0.97	3.56±0.68	0.01*
LDL/HDL	2.55±0.74	2.16±0.36	0.02*

Table-4 : shows the lipid levels of the cases and controls. The TC was significantly (p=0.0001) lower among the cases (122.24±9.77) compared with controls (151.00±21.21). The decreased level of TG, HDL and LDL were also observed among the cases than controls .However VLDL level was higher among the cases than control. TC/HDL and LDL/HDL ratio was significantly higher among the cases than controls .



Graph. 4: Alteration of Lipid levels of the cases and controls

Graph 4: The decreased level of TG, HDL and LDL were also observed among the cases than controls. However VLDL level was higher among the cases than control. TC/HDL and LDL/HDL ratio was significantly higher among the cases than controls.

Discussion :

Oral submucous fibrosis is a chronic, progressive, scarring disease that predominantly affects the people of South- East Asian origin. The high incidence of OSMF is commonly associated with habit of chewing areca nut and tobacco. It is a potentially malignant disorder with multifactorial etiology.[12]

Lipids constitute a heterogenous group of biomolecules which are insoluble in water but freely soluble in organic solvents such as ether and chloroform.[13] Variation in plasma lipid profile has been associated with coronary artery disease & some malignant disease are also been reported with early changes in plasma lipid profile as oral precancerous conditions show a significant tendency to develop cancer. Free radicals and reactive oxygen species are generated from tobacco carcinogens which are responsible for high rate peroxidation of polyunsaturated fatty acids which results in more utilization of lipids for new membrane biogenesis. This further affects cell membrane resulting in tissue injury, thus damaging cellular structural blocks and thus might be involved in carcinogenesis.[14]

Cholesterol is an amphipathic lipid and as such is an essential structural component of all cell membranes It is present in tissues and in plasma lipoprotein either as free cholesterol or combined with a long-chain fatty acid, as cholesteryl ester. Lipoprotein transports free cholesterol in the circulation, where it readily equilibrates cholesterol in other lipoproteins and in membranes.[15]

Cholesterol and triglycerides, important lipid constituents of cell, are essential to carry out several vital physiological functions. It is essential for maintenance of the structural and functional integrity of cell membranes. It controls the red cells from being easily haemolyzed and helps in transportation of fat to

liver in the form of cholesterol ester for oxidation. It is also involved in the activity of membrane bound enzymes and is important for stabilization of DNA helix. Cellular uptake and regulation of cholesterol is mediated by lipoprotein receptors located on the surface of cells.[16]

As cell progresses from normal through premalignant to malignant condition their chemical character may diverge from normal. Malignant cells have distinct type of metabolism which alter the biochemical parameters which are either increased or decreased.

The alteration in circulatory cholesterol levels have been found to be associated with breast and colorectal cancer. However, only a few reports are available on plasma lipid profile in Oral precancer and cancer.[8]

As lipids may play a role in precancer and cancer, which are major cell membrane components essential for various biological functions including cell growth and division of normal and malignant tissues.[17] This study was aimed to estimate serum lipid profile in oral submucous fibrosis groups and to compare the serum lipid profiles in oral submucous fibrosis groups values with values from control groups.

In our study comparison of the serum lipid profile in OSF group and control group showed that there was significant decrease in TC0(p=.0001), HDLC(p=0.0001), LDLC & Trigycerides(p=0.002) levels in OSF group. Our results is consistent with the studies done by Patel PS et al[16] where he reported significant decrease in TC, HDL,TG,VLDL in cancer patients as well as in oral precancer conditions but LDL levels did not reveal any significant difference whereas in our study decrease in LDL(p=0.0001) levels in OSMF was seen as compared to controls

Similarly Lohe et al[18] reported a significant decrease in TC,HDL levels in oral precancerous conditions however LDL, VLDL & trigycerides did not reveal any significant difference whereas in our study VLDL(p=0.46) does not show any significant decrease.

Similarly Chalkoo et al[19] conducted a study where he reported significant decrease in TC& LDL which is consistent with our study whereas triglycerides & HDL were slightly increased in some patients with OSMF however in our study serum triglycerides & HDL showed significant decrease.

In present study VLDL do not reveal any significant decrease which is similar to a study conducted by Chawda et al.[20] Likewise in our study TC/HDL (p=0.01)& LDL/HDL(p=0.02)s ratios were significantly increase in OSMF patients which is not consistent with study conducted by Ghosh et al[21] where he found decrease in ratio in oral squamous cell carcinoma patients as compared to tobacco habituates.

Our data strengthens the evidence of an inverse relationship between serum lipid profile and oral potentially malignant disorders.

The changes in lipid profile have long been associated with cancer. Hypercholestemia has been observed in patients with cancers of various organs.

Rose et al[22] first reported the inverse relation between cholesterol level & risk of cancer. Oral malignancy serum cholesterol undergoes significant changes & low level in tissues & in blood could be due to rapidly dividing cells. In the present study our findings suggests that lower lipid levels may be mainly because of underlying disease process.

References

1. Vanaja Reddy, P.V.Wanjari, Naveen Reddy Banda, Prashanti Reddy Oral Submucous Fibrosis: Correlation of Clinical Grading to various habit factors. International Journal of Dental clinics 2011:3(1): 21-24.
2. Pindborg JJ & Sirsat SM .Oral submucous fibrosis. J. of OS OM OP. 1966 ;22: 764-79.
3. Rajendran R, Sivapathasundhram. B. Shafer's Textbook of Oral Pathology. Elseiver Publications 2009,6th edi.
4. Gopal Sharma, Deepa Das, Jaya Mukherjee, Bhagyashri Purandare. Lipid profile in oral submucous fibrosis patients in india -a pilot study. Indian Journal of Basic & Applied Medical Research; June 2013: Issue-7, Vol.-2, P. 790-796.
5. Multimodal treatment options for oral submucous fibrosis SRM University Journal of Dental Sciences Volume 1 - Issue 1 - March 2010
6. Gerber M, Richardson S, DePaulet PC, Pujol H, De Paulet AC. Relationship between vitamin E and polyunsaturated fatty acids in breast cancer:

Nutritional and metabolic aspects. Cancer 1989;64:2347-53.

7. Gerber M, Cavallo F, Marubini E, Richardson S, Barbieri A, Capitelli E. Liposoluble vitamins and lipid parameters in breast cancer. A joint study in northern Italy and southern France. Int J Cancer 1988;42:489-94.

8. Forones NM, Falcan JB, Mattos D, Barone B. Cholesterolemia in colorectal cancer.

Hepatogastroenterology 1998;45:1531-4.

9. Hoffmann D, Brunnemann KD, Prokopczyk B, Djordjevic MV. Tobacco specific N-nitrosamines and areca derived N-nitrosamines: chemistry, biochemistry, carcinogenecity, and relevance to humans. J. Toxicol Env Health 1994; 41:1-52.

10. Manoharan S, Kolanjiappan K, Suresh K, Panjamurthy K. Lipid peroxidation stautus in patients with oral squamous cell carcinoma. Indian J. Med Res 2005; 122:529- 531.

11. Alexopoulos CG, Blatsios B, Avgerinos A. Serum lipids and lipoprotein disorders in cancer patients. Cancer 1987;60:3.

12. Barakha Nayak, Sangeeta Patankar, Snehal Bansad. Comparative study of plasma lipid profile patterns in various grades of OSMF. Int. J. of Women Dentists. 2014;1(1):28-36.

13. Sandeep A Bailwad et al. Alterations in serum lipid profile patterns in oral cancer:correlation with histological grading and tobacco abuse. J. of OHDM. Sep. 2014;13(3):573-579.

14. Deepanshu garg et al. Serum lipid profile in oral precancer and cancer: a diagnostic or prognostic marker. J. of Jnternational oral health.2014;6(2):33-39.

15. Mehrotra R et al. Lipid profile in OSMF. Lipids health dis.2009;8(29):2-7.

16. Patel P.S, Shah M.H, Raval G.N et al. Alterations in plasma lipid profile pattern in head and neck cancer and oral precancer conditions. Indian Journalof cancer. 2004; 41(1):25-31.

17. Eugenia J Sherubin et al. Estimation of plasma lipids and its significance on histopathological grades in oral cancer: prognostic significance on original research. J. of Oral & Maxillofacial Pathology. April2013;17(1):4-9.

18. Lohe V.K, Dedwekar S.S, Bhowateb R.R, Kadu R.P, Dangore S.B. Evaluation of correlation of serum lipid profile in patients with oral cancer & precancer & its association with tobacco abuse. J. Oral Patho. Med. 2010;39:141-148.

19. Chalkoo H.A, Risam S.S, Farooq R. A study on alterations in plasma lipid profile patterns in OSMF patients. Journal of Indian academy of Oral Medicine and Radiology. 2011;23(1):36-38.

20. Chawda G.J, Jain S.S et al. The relionshionship between serum lipid levels and the risk of oral cancer. Indian Journal of Medical and Paediatric Oncology. 2011; 32(1):34-37.

21. Ghosh G, Jayaram M.K et al. Alterations in serum lipid profile patterns in oral squamous cell carcinoma patients. J. contemp dent pract. 2011;12(6)451-56.

22. Rose G, Shipley MJ. Plasma lipids and mortality. Lancet.1980;1:;5236.

III

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Original Article

Lycopene and intralesional betamethasone injections in the management of oral submucous fibrosis

Dipti Singh, Mathod C Shashikanth[1], Neeta Misra[2], Sudhanshu Agrawal[3]

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ABSTRACT

Aims and Objectives: This study was conducted to determine the efficacy of lycopene in the management of oral submucous fibrosis (OSMF) and to compare its efficacy with intralesional betamethasone injections. **Materials and Methods:** Forty-four patients were divided randomly into two groups. Group I subjects were treated with 10,000 mcg of lycopene (*Lyconex*) daily, in two equally divided doses, for two months. Group II subjects were given intralesional injections of betamethasone. Both the groups were assessed in terms of mouth opening and burning sensation. **Results:** A significant improvement in mouth opening was seen in both the groups and the improvement was better in Group I. The mean improvement in mouth opening in Group I was 37.62% (12 mm) at the end of the study, which was statistically highly significant and weekly evaluation revealed that this high significance was from the third week onward, and Group II patients (only intralesional steroids) showed an average improvement of 13% (3.9 mm) at the final follow-up visit. **Conclusion:** Lycopene (*Lyconex*) is better than intralesional betamethasone injections in improving mouth opening and decreasing burning sensation.

Key words: Efficacy, intralesional, lycopene, management, oral submucous fibrosis, steroids

Introduction

Oral submucous fibrosis (OSMF) is a premalignant condition of the oral mucosa first described in 1952.[1] Various factors implicated in the etiology of OSMF are environmental agents, and nutritional, genetic, and autoimmune factors. Environmental agents, such as, the allied preparations of betel nut and betel quid have been observed to be associated with OSMF.[2] The potential for malignant transformation in OSMF is high.[3]

In the biosynthesis of many carotenoids, lycopene is an important intermediate. Lycopene has been seen to have many anti-carcinogenic and antioxidant properties. It also plays a role in the improvement of precancerous lesions. Studies also suggest that as lycopene inhibits hepatic fibrosis in rats and human fibroblast activity *in vitro*, it appears to be a promising agent in the management of OSMF.[4] So far, studies on the management of OSMF using lycopene are only few.[4] Therefore, a need was felt to conduct a study to determine the efficacy of lycopene in the management of OSMF and to compare this treatment with intralesional betamethasone injections.

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	DOI: 10.4103/0972-1363.145000

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Received: 16-06-2014 **Accepted:** 09-11-2014 **Published:** 19-11-2014

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Materials and Methods

The patients for the study were those who visited the Department of Oral Medicine and Radiology. In the present study, 44 patients who presented with signs and symptoms suggestive of OSMF were enrolled. The sample size was determined based on the number of patients with OSMF attending the department in a month. Ethical clearance for the study was obtained from the Institutional Ethical Committee. Each patient was informed about the protocol and was given appropriate instructions after obtaining a written consent. The inclusion and exclusion criteria for the study were as follows:

Inclusion criteria

- Patients who were healthy and well-oriented to place, person, and time
- Patients of either sex aged between 15 and 60 years
- Patients with a history of areca nut chewing and burning sensation on eating spicy food
- Restricted mouth opening and presence of palpable vertical fibrous bands, stiffness, and blanching

Exclusion criteria

- Subjects who had undergone any treatment for OSMF
- Those with TMJ disorders
- Those with any systemic disease
- Those who were allergic to drugs

Only clinically diagnosed cases of OSMF were considered for this study, as a biopsy could further decrease mouth opening, due to secondary contractures, which might affect the patient follow-up findings. However, a brush biopsy was done for the patients. Following the establishment of the diagnosis, each patient was educated about the nature of the condition and its precancerous potential, and they were motivated to discontinue the use of areca nut, tobacco or any other abusive habit in any form. The study included patients with OSMF encompassing all the groups based on the Lai DR classification (group A, B, C, and D). Patients with any other pre-malignant disorder, such as leukoplakia, were not included. All the patients of the study were advised oral prophylaxis to remove extrinsic stains. This was done to motivate the patient toward recovery and to know if the patient resumed the habit.

The patients were divided randomly into two groups:

Group I: Subjects were treated with 10,000 mcg of lycopene daily in two equally divided doses for two months. Lycopene used in the study was in the form of *Lyconex soft gels* (lycopene-5000 mcg, vitamin A acetate-5000 I.U., vitamin C-75 mg, vitamin E acetate-15 mg, vitamin B1-10 mg, vitamin B2-10 mg, vitamin B6-3 mg, vitamin B12-5 mcg, vitamin D3 granules equivalent (eq.) to cholecalciferol-500 I.U., sodium selenite eq. to elemental selenium-70 mcg, chromium chloride eq. to elemental chromium-100 mcg, zinc sulfate monohydrate eq. to elemental zinc-15 mg, manganese sulfate eq. to elemental manganese-1.4 mg, folic acid-1.5 mg, niacinamide-50 mg, calcium pantothenate-12.5 mg) manufactured by Sicare Pharma Pvt. Ltd., Delhi, India.

Group II: Subjects were given intralesional injections of betamethasone (1 mL ampule of 4 mg each) where the fibrous bands were palpable, twice weekly for two months.

Both the groups were assessed in terms of mouth opening and burning sensation before the treatment, weekly during the treatment period of two months, and the final evaluation was done two months after the completion of the treatment. The complete period of the study was four months.

This was a prospective, randomized, and blinded controlled study, in which the patients were selected through a chit system. Chits of Group I and Group II were placed in a box and patient was asked to blindly pick a chit according to which the patients were divided into Group I or II.

Mouth opening

This was assessed as the interincisal distance measured from the mesioincisal edge of the upper left central incisor tooth to the mesioincisal edge of the lower left central incisor tooth. The measurement was made using a vernier caliper and was recorded in millimeters.

Burning sensation

The intensity of the burning sensation was determined using a Visual Analog Scale (VAS) of 0-100, where 0 indicated no burning sensation and 100 indicated the worst possible burning sensation. This was recorded at baseline and weekly intervals. It was recorded based on the patient's response.

Statistical analysis

All the relevant data were entered in a proforma. It was then sorted, tabulated, and statistically analyzed to draw a conclusion. All quantified variables in the study, that is, mouth-opening measurements, age, quantity, and duration of habit, were subjected to statistical analysis. All these values were analyzed for mean (or median as applicable), standard deviation, errors, and range. The unpaired *t*-test was used for evaluation of the statistical significance of mouth-opening values between groups.

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The paired *t*-test was used for evaluation of the statistical significance of mouth-opening values between weeks in the same group. The *P*-value was set at 0.05 and was considered highly significant at <0.01 and very highly significant at <0.001.

Results

Age and sex distribution

In the present study 44 OSMF patients were included and were randomly divided into two groups. The mean age of subjects in Group I was 29.41 ± 9.11 years, while the mean age of Group II subjects was 25.59 ± 6.98 years. In Group I, 54.5% of the patients were above 25 years of age, while in Group II, 59.1% of the subjects were below 25 years of age. Statistically, there was no significant difference in terms of age between the two groups ($P = 0.365$). The majority of subjects in both the groups were males. Statistically, there was no significant difference in terms of gender between the two groups ($P = 0.680$, Table 1).

Table 1: Age-group distribution

Age group (years)	Group I ($n = 22$)		Group II ($n = 22$)	
	No.	(%)	No.	(%)
<25	10	45.5	13	59.1
>25	12	54.5	9	40.9
Mean age ± SD	29.41 ± 9.11		25.59 ± 6.98	

Habits

All patients of this study had a positive history of chewing areca nut or any of its commercial preparations. The most common form of areca nut used was found to be *gutkha* in both the groups, with 72.7% in Group I and 90.9% patients in Group II using it. The mean period of addiction in Group I was 7.08 ± 6.78 years, while in Group II it was 5.68 ± 3.73 years. In Group I, nine (40.9%) and in Group II, ten (45.5%) subjects used to consume >5 packs per day. The mean packs consumed in a day were 8.14 ± 7.83 in Group I and 7.59 ± 5.13 in Group II.

Mouth opening

Pretreatment

Group I had a more consistent range of mouth opening (range 2.4-4.5 mm) with a mean of 3.19 ± 0.55 mm, while Group II had a relatively wide range (1.3-4.6 mm) with a mean of 3.00 ± 0.82 mm. Comparison of data in the two groups did not reveal a statistically significant intergroup difference ($P = 0.382$).

Post-treatment

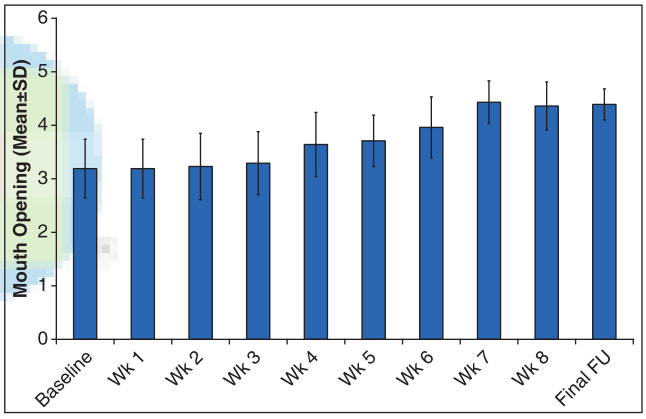
The average mouth opening in Group I was 4.39 + 0.29 mm and in Group II it was 3.39 ± 0.63 mm.

Change in mouth opening and statistical analysis

Group I: At baseline (pretreatment), the mean mouth opening was 3.19 ± 0.55 mm, which remained unchanged at week one, but thereafter, it started showing a gradual increase. At the end of the treatment, there was a 37.62% increase in mouth opening, which was very highly significant as compared to the baseline ($P < 0.001$, Table 2, Graph 1).

Table 2: Post-treatment assessment of mouth opening in Group I

Period	Mean mouth opening		Change from baseline		Statistical significance	
	Mean	SD	Mean	SD	*t*	*P*
Baseline	3.19	0.55				
Week 1	3.19	0.55	0	0	–	–
Week 2	3.23	0.62	0.05	0.28	0.755	0.459
Week 3	3.29	0.59	0.10	0.25	1.979	0.061
Week 4	3.64	0.60	0.45	0.54	3.964	<0.001
Week 5	3.71	0.48	0.53	0.44	5.595	<0.001
Week 6	3.96	0.57	0.78	0.73	4.992	<0.001
Week 7	4.43	0.40	1.25	0.58	10.122	<0.001
Week 8	4.36	0.45	1.17	0.61	8.949	<0.001
Final FU	4.39	0.29	1.20	0.57	9.966	<0.001



Graph 1: Post-treatment assessment of mouth opening in Group I

Group II: At baseline (pretreatment), the mean mouth opening was 3.00 ± 0.82 mm, which remained unchanged at week one, but thereafter, started showing an increase. At the end of the treatment, a 13% improvement in mouth opening was seen showing a statistically highly significant difference from baseline ($P = 0.018$, Table 3, Graph 2).

Burning sensation

Group I: At baseline (pretreatment), the burning sensation was 51.82 ± 24.08. At the end of treatment a 94.5% reduction in burning sensation was seen, which was statistically very highly significant ($P < 0.001$).

Group II: At baseline (pretreatment), the burning sensation was 49.55 ± 24.15. At the end of treatment a 54.1% reduction in burning sensation was seen and it was very highly significant ($P < 0.001$).

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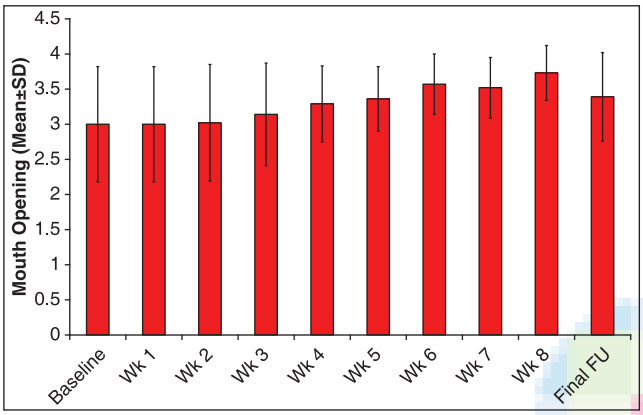
Table 3: Post-treatment assessment of mouth opening in Group II

Period	Mean mouth opening		Change from baseline		Statistical Significance	
	Mean	SD	Mean	SD	*t*	*P*
Baseline	3.00	0.82				
Week 1	3.00	0.82				
Week 2	3.02	0.83	0.02	0.07	1.283	0.213
Week 3	3.14	0.73	0.14	0.38	1.689	0.106
Week 4	3.29	0.54	0.29	0.51	2.667	0.014
Week 5	3.36	0.46	0.36	0.51	3.324	0.003
Week 6	3.57	0.43	0.57	0.79	3.353	0.003
Week 7	3.52	0.43	0.52	0.83	2.967	0.007
Week 8	3.73	0.39	0.73	0.90	3.800	0.001
Final FU	3.39	0.63	0.39	0.71	2.557	0.018



Graph 2: Post-treatment assessment of mouth opening in Group II

No significant association between the percentage reduction in burning sensation, percentage increase in mouth opening, and duration of symptom of burning sensation as well as mouth opening was observed in either group, independently or collectively.

Discussion

Lycopene is a powerful antioxidant obtained from tomatoes. Studies also suggest that lycopene inhibits hepatic fibrosis in rats and human fibroblast activity *in vitro*. Therefore, it can be beneficial in the management of OSMF. The present study was designed to evaluate the efficacy of lycopene-10,000 mcg (*Lyconex*) in the management of OSMF and to compare this treatment with intralesional betamethasone injections (1 mL ampule of 4 mg each). In the present study 44 subjects with OSMF were in the age range of 17-50 years, with a mean age of 28 years. This was comparable to the mean age of 28 years observed by Kumar *et al.*[3] and 28.8 years observed by Hazarey *et al.*[5]

Among the 44 OSMF subjects, 37 (84%) were male and seven (16%) were female, thus showing extreme male predominance, with a ratio of 5.25:1. A similar male predominance was reported by Sinor *et al.* (58 out of 60 were men, 29:1) and Pindborg (81 out of 118 were male, 2.2:1). The male predominance could be due to easy accessibility for males to use these products more frequently than females in our society.[6,7]

Restriction of mouth opening is a major disability associated with OSMF. An improvement of a few millimeters has been reported. In our study a significant improvement was seen in both the groups. The mean improvement in mouth opening in Group I (lycopene group) was 37.62% (12 mm) at the end of the treatment and it was very highly significant ($P < 0.001$). There are very few published studies on evaluating the effectiveness of lycopene in OSMF. Kumar *et al.* also conducted a similar study in 2007, on efficacy of lycopene in the management of OSMF. They reported that the mouth opening was increased by 3.4 mm in patients receiving 16 mg of lycopene and 4.6 mm in patients receiving 16 mg of lycopene along with biweekly intralesional steroid injections.[3] In our study there was more improvement in mouth opening as compared to the study by Kumar *et al.*, which could be due to the drug *Lyconex* (which was used in our study) also containing Vitamin A, C, E, and B. These vitamins help to enhance the immune system, encourage cell growth and division, and boost the metabolism. Studies have also been done using antioxidant supplements and have shown good results. Gupta *et al.*[8] reported a 50% improvement in mouth opening in six of their patients treated with *Antoxid*.

The mean improvement in mouth opening in Group II (only intralesional steroids) was 13% (3.9 mm) at the end of the treatment, which was a statistically highly significant difference from the baseline ($P = 0.018$). Canniff *et al.*, in 1986, found that only intralesional steroids were not very useful in the management of OSMF.[3] In another study done by Borle and Borle, in which intralesional injections of triamcinolone were combined with hyaluronidase, there was no improvement in mouth opening.[9] In our study there was 13% improvement with only intralesional steroids.

A burning sensation when eating spicy food or even normal food is common among OSMF patients, due to which they switch over to a bland diet, which is generally not nutritionally adequate. Although the exact mechanism causing the burning sensation is not clear, intolerance to spices could be due to the atrophic and permeable epithelium. In our study, patients treated with lycopene (Group I) had a reduction in burning sensation by 94.2% at the end of the therapy and it was very highly significant ($P < 0.001$). Similar findings were reported in a study done by Kumar *et al.*, in which burning sensation was reduced effectively in patients taking lycopene and lycopene with intralesional steroids.[3]

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Patients treated with intralesional steroid injections (Group II) had a reduction in burning sensation by 54.1% at the end of the treatment. The change in burning sensation was very highly significant ($P < 0.001$). Borle and Borle found that treatment of OSMF with intralesional injections of hyaluronidase and corticosteroids (triamcinolone acetonide) reduced burning sensation by 86.84%.[9] Katharia *et al*. observed that in the treatment of OSMF, with intralesional placental extracts the burning sensation improved by 40.2% and Haque *et al*. noticed that the burning sensation reduced by 54-60% with IFN γ.[10] When the results of both the groups were compared the mean burning sensation in Group I was very highly significant as compared to Group II ($P < 0.001$).

The results of the present study indicate that lycopene (*Lyconex*) is more effective than intralesional steroid injections in improving the mouth opening and burning sensation in patients with OSMF. The reasons for this efficacy may be:

- Lycopene prevents free radical damage to cells. Studies have shown that it reduces the susceptibility of lymphocyte DNA to oxidative damage, inactivates hydrogen peroxide (H_2O_2) and nitrogen oxide (NO), and protects cells from NO-induced membrane damage and cell death. Lycopene has both physical and chemical antioxidant properties, in which physical quenching in much better than chemical quenching.[11]
- Lycopene inhibits hepatic fibrosis in rats and human fibroblast activity *in vitro*. Thus, it can be beneficial in the management of OSMF (Kitade *et al*.[12]). This also suggests inhibition of the stellate cell activity.

There were no associated side effects with the use of lycopene (*Lyconex*). There were a few limitations to this study. The duration of follow up was only two months; a longer follow-up study should be done to rule out any long-term effects of lycopene. The *Lyconex* capsule that was used in the study also contained vitamin A, B, C, and E, in addition to lycopene, so the improvement observed in the study could be the combined effect of all these and it could not be attributed to lycopene alone. From our study, it could be inferred that lycopene in combination was better than intralesional betamethasone injections for improving mouth opening and decreasing the burning sensation in patients with OSMF. Further studies, with a larger sample size, and with longer follow-up periods are required to determine the long-term effect of lycopene (*Lyconex*) in OSMF.

Conclusion

A positive response was seen in Group I in our study when compared with Group II. Group I patients were given capsules of *Lyconex*, which also contained Vitamins A, B, C, and E, in addition to lycopene. Thus, the improvement observed in the study could be the combined effect of all these, which was better than betamethasone. Lycopene in combination was efficacious as well as a safe and reliable drug in the management of OSMF. It was a noninvasive option for the management of OSMF, which helped in the improvement of the signs and symptoms of the condition. This combination could, therefore, be used as a first-line drug of treatment in patients with this debilitating disease. Further trials in this regard should be carried out, to investigate the probable mechanism by which this combination exerts the beneficial effect.

References

1. Prabhu SR, Wilson DF, Daftary DK, Johnson MN. Oral Diseases in the Tropics. 1st ed. New Delhi: Oxford University Press; 1993. p. 417-22.
2. Raina C, Raizada RM, Chaturvedi VN, Harinath BC, Puttewar MP, Kennedy AK. Clinical profile and serum beta-carotene levels in oral submucous fibrosis. Indian J Otolaryngol Head Neck Surg 2005;57:191-5.
3. Maher R, Lee AJ, Warnakulasuriya KA, Lewis JA, Johnson NW. Role of areca nut in the causation of oral submucous fibrosis: A case-control study in Pakistan. J Oral Pathol Med 1994;23:65-9.
4. Kumar A, Bagewadi A, Keluskar V, Singh M. Efficacy of lycopene in the management of oral submucous fibrosis. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2007;103:207-13.
5. Hazarey VK, Erlewad DM, Mundhe KA, Ughade SN. Oral submucous fibrosis: Study of 1000 cases from central India. J Oral Pathol Med 2007;36:12-7.
6. Sinor PN, Gupta PC, Murti PR, Bhonsle RB, Daftary DK, Mehta FS, *et al*. A case-control study of oral submucous fibrosis with special reference to the etiologic role of areca nut. J Oral Pathol Med 1990;19:94-8.
7. Pindborg JJ, Murti PR, Bhonsle RB, Gupta PC, Daftary DK, Mehta FS. Oral submucous fibrosis as a precancerous condition. Scand J Dent Res 1984;92:224-9.
8. Gupta S, Reddy MV, Harinath BC. Role of oxidative stress and antioxidants in aetiopathogenesis and management of oral submucous fibrosis. Indian J Clin Biochem 2004;19:138-41.
9. Borle RM, Borle SR. Management of oral submucous fibrosis: A conservative approach. J Oral Maxillofac Surg1991;49:788-91.
10. Katharia SK, Singh SP, Kulshreshtha VK. The effects of placental extract in management of oral-submucous fibrosis. Indian J Pharm 1992;24:181-3.
11. Anshumalee N, Shashikanth MC, Shambulingappa P, Deepak U. Lycopenne: A promising antioxidant. J Indian Acad Oral Med Radiol 2007;19:458-63.
12. Kitade Y, Watanabe S, Masaki T, Nishioka M, Nishino H. Inhibition of liver fibrosis in LEC rats by a carotenoid, lycopene, or a herbal medicine, Sho-saiko-to. Hepatol Res 2002;22:196-205.

How to cite this article: Singh D, Shashikanth MC, Misra N, Agrawal S. Lycopene and intralesional betamethasone injections in the management of oral submucous fibrosis. J Indian Acad Oral Med Radiol 2014;26:264-8.

Source of Support: Nil, **Conflict of Interest:** None declared.

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Original Article

Estimation of serum zinc, copper, and iron in the patients of oral submucous fibrosis

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ABSTRACT

Introduction: The role of trace elements in various diseases has been a matter of controversy with various authors reporting on conflicting data. They are receiving much attention in the detection of oral cancer and precancer as they are found to be significantly altered and have an important role in carcinogenesis. Trace elements have been extensively studied in the recent years to assess whether they have any modifying effect in the etiology of oral malignant conditions. **Materials and Methods:** A study was conducted on fifty subjects with clinically diagnosed oral submucous fibrosis (OSMF) and fifty controls with no apparent lesions of the oral mucosa and without any areca nut-related oral habit. **Results:** The level of serum zinc was significantly ($P < 0.0001$) lower among cases (73.48 ± 24.21) compared with controls (119.48 ± 52.78). However, the serum copper level was significantly ($P < 0.0001$) higher among cases (155.50 ± 40.13) than controls (100.40 ± 24.52). The level of serum iron was observed to be lower among the cases (66.57 ± 27.76) as compared to controls (94.19 ± 35.70), and the difference was statistically significant. **Conclusion:** It can be concluded from this study that serum zinc, copper, and iron levels could be used as a potential prognostic and diagnostic markers in OSMF patients.

Key words: Oral submucous fibrosis, precancer, trace elements

Introduction

Oral submucous fibrosis (OSMF) is a chronic condition of the oral mucosa, first described by Schwartz in 1952 among 5 East African women of Indian origin under the term, "atrophia idiopathica (tropica) mucosae oris."[1] Trace elements have been studied in the recent years to assess whether they have any modifying effect in the etiology of oral malignant conditions, but relatively less scientific work has been performed in the area of oral premalignant conditions. These can be used as an auxiliary test to clinicopathological diagnosis and/or in combination with other biochemical tests in the diagnosis and prognosis of OSMF. The aim of the study was to determine the serum levels of zinc, copper, and iron in different stages of OSMF and normal healthy subjects and to compare serum levels of zinc, copper, and iron in subjects of OSMF and normal healthy subjects.

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DOI: 10.4103/0975-5950.183851

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How to cite this article: Yadav A, Kumar L, Misra N, Deepak U, Shiv Kumar GC. Estimation of serum zinc, copper, and iron in the patients of oral submucous fibrosis. Natl J Maxillofac Surg 2015;6:190-3.

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Materials and Methods

The study was conducted in the Department of Oral Medicine and Radiology of Babu Banarasi Das College of Dental Sciences, Lucknow. Ethical clearance for the study was obtained from the Institutional Ethical Committee. In this study, fifty patients who presented with signs and symptoms suggestive of OSMF and fifty controls were enrolled. Patients who were healthy and well oriented to place, person, and time, either sex aged between 18 and 60 years, a positive history of chewing of areca nut or one of its commercial preparation, burning sensation on eating spicy foods, restricted mouth opening, and the presence of palpable vertical fibrous band, stiffness, and blanching were included in the study. Subjects having any systemic disorder, previous history of treatment for the same condition, history of drug intake containing zinc, copper, and iron, pregnant women were excluded from the study. Khanna and Andrade classification system of OSMF[2] based on interincisal opening was used for the study.

Under aseptic conditions, 5 ml of venous blood was obtained by venipuncture of the median cubital vein, kept standing for 30 min at room temperature. Then, the serum was separated by centrifugation at 3000 rpm for 15 min and preserved in a frozen state at 2–8°C for 5 days until analysis. Serum sample used for the estimation was mixed in appropriate proportion with buffer and color reagents supplied in the estimation kits in the clean glass tubes as per the manufacturer's instructions. The absorbance of these samples was compared with the standard solution provided in the kit using a colorimeter. The data obtained from the procedures will be tabulated and analyzed using statistical methods.

Results

Age distribution

The age distribution of the patients and controls is depicted in Table 1 and Figure 1. More than half of the cases (58%) and controls (84%) were below 30 years. The mean age was almost similar ($P > 0.05$) in both the groups; thus, both groups were comparable in terms of age.

Sex distribution

The sex distribution of the patients and controls is presented in Table 2 and Figure 2. More than half of the cases (84%) and controls (78%) were males. The percentage of male/female ratio was almost similar ($P > 0.05$) in both the groups; thus, both groups were comparable in terms of sex.

Comparison of trace elements

The level of serum zinc was significantly ($P < 0.0001$) lower among cases (73.48 ± 24.21) compared with controls (119.48 ± 52.78). However, the serum copper level was significantly ($P < 0.0001$) higher among the cases (155.50 ± 40.13) than controls (100.40 ± 24.52). The level of serum iron was observed to be lower among the cases (66.57 ± 27.76) as compared to controls (94.19 ± 35.70), and the difference was statistically significant [Table 3 and Figure 3].

Comparison of trace elements according to grade

The comparison of trace elements according to grade among the cases is given in Table 4 and Figure 4. Analysis of variance revealed that there was a significant

Table 1: Age distribution of the patients and controls

Age in years	Cases ($n = 50$) n (%)	Controls ($n = 50$) n (%)	P[a]
< 30	29 (58.0)	42 (84.0)	0.12
≥ 30	21 (42.0)	8 (16.0)	
Mean ± SD	28.64 ± 9.76	26.12 ± 6.16	

[a]Unpaired *t*-test, SD: Standard deviation

Table 2: Sex distribution of the patients and controls

Sex	Cases ($n = 50$) n (%)	Controls ($n = 50$) n (%)	P[a]
Male	42 (84.0)	39 (78.0)	0.44
Female	8 (16.0)	11 (22.0)	

[a]Chi-square test

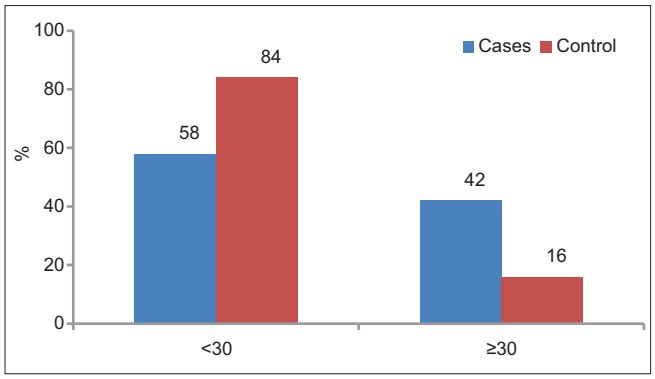

Figure 1: Age distribution of the cases and controls

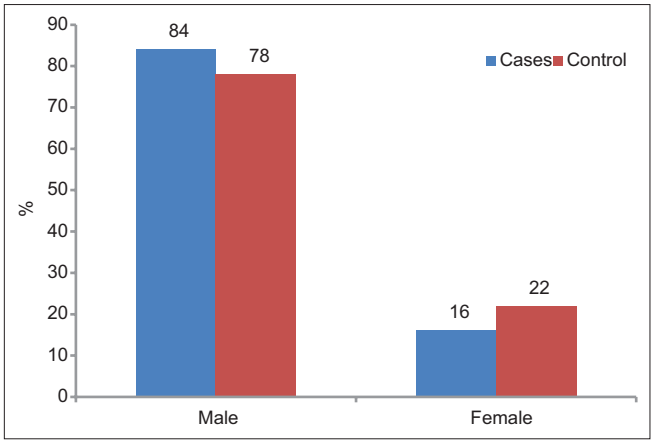

Figure 2: Sex distribution of the cases and controls

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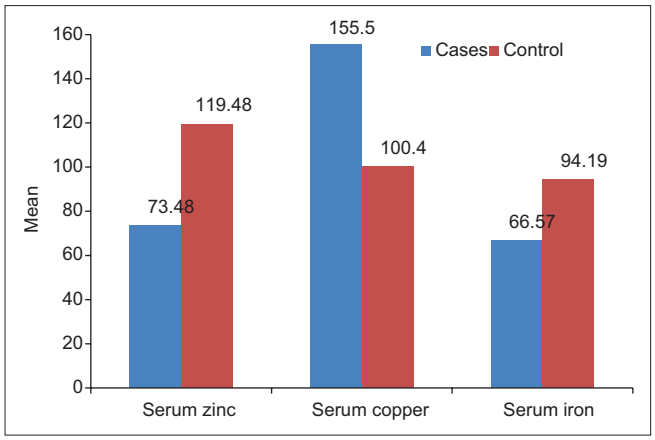

Figure 3: Comparison of trace elements between cases and controls

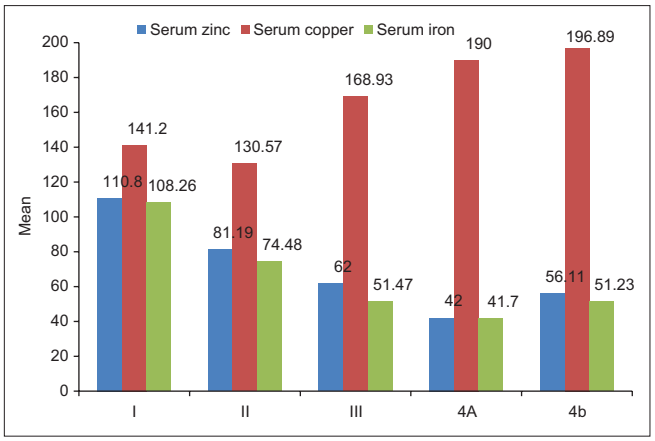

Figure 4: Comparison of trace elements according to grade among the cases

Table 3: Comparison of trace elements between cases and controls

Trace element	Mean ± SD		*P*[a]
	Cases (*n* = 50)	Controls (*n* = 50)	
Serum zinc	73.48 ± 24.21	119.48 ± 52.78	< 0.0001
Serum copper	155.50 ± 40.13	100.40 ± 24.52	< 0.0001
Serum iron	66.57 ± 27.76	94.19 ± 35.70	< 0.0001

[a]Unpaired *t*-test, SD: Standard deviation

Table 4: Comparison of trace elements according to grade among the cases

	Number of patients	Serum zinc	Serum copper	Serum iron
I	5	110.80 ± 10.18	141.20 ± 40.30	108.26 ± 15.32
II	21	81.19 ± 19.65	130.57 ± 33.94	74.48 ± 25.96
III	14	62.00 ± 17.94	168.93 ± 26.07	51.47 ± 18.44
IVa	1	42.00	190.00	41.70
IVb	9	56.11 ± 18.12	196.89 ± 30.26	51.23 ± 19.38
ANOVA *P*		0.001*	0.001*	0.001*

*Significant. ANOVA: Analysis of variance

difference in all the trace elements among all the grades. Since there is only one case in the Grade IVa, the *post hoc* multiple comparison test could not be done to compare the grades.

Discussion

OSMF is a well-recognized, potentially malignant condition of the oral cavity. Controlling the devastating, widespread consequences of OSMF requires interventions in at-risk persons ideally before the disease becomes invasive. Detection of the premalignancies and preventing them from malignant transformation seem to be the best available tool in the fight against oral cancer. Very few studies have been conducted to find out the role of different trace elements in oral precancer and cancer. Hence, a comprehensive study has been carried out to estimate levels of serum zinc, copper, and iron in patients with OSMF in the population of Lucknow District.

Age

In the present study, fifty subjects with OSMF were in the age range of 17–55 years with a mean age of 28.64 years. This is comparable to mean age of 28 years observed by Kumar *et al.*,[3] 28.8 years by Hazarey *et al.*,[4] Maher *et al.*,[5] and Borle and Borle.[6]

The age range seen in our study can be attributed to changing lifestyle of youngsters and increased in the usage of Gutkha/pan masala [Table 1 and Figure 1].

Sex

Among the fifty OSMF subjects, 42 were male and 8 were female patients, thus showing an extreme male predominance over female with the ratio of 5.25:1. A similar male predominance was reported by Sinor *et al.*,[7] Pindborg *et al.*,[8] Ahmad *et al.*,[9] and Hazarey *et al.*[4] [Table 2 and Figure 2].

Male predominance can be related to easy accessibility for males to use these products more frequently than females in our society.

Comparison of trace elements between cases and controls

Serum copper levels in oral submucous fibrosis patients

In our study, the serum copper level was significantly ($P < 0.0001$) higher among the cases (155.50 ± 40.13) than controls (100.40 ± 24.52). It was similar to the study by Balpande *et al.*[10] and Shetty *et al.*[11] [Tables 3, 4 and Figures 3, 4]

Increased serum copper in OSMF can be correlated to copper present in areca nut increases the collagen production in oral fibroblasts by upregulating lysyl oxidase leading to crosslinking of collagen and elastin[11] Trivedy *et al.* has also reported on the copper-induced mutagenesis through the p53 aberrations in OSMF, which may be critical in the

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progression of the potentially malignant lesions to squamous cell carcinoma.[12]

Serum zinc levels in oral submucous fibrosis patients
The level of serum zinc was significantly ($P < 0.0001$) lower among cases (73.48 ± 24.21) compared with controls (119.48 ± 52.78). It was similar to the study done by Paul *et al*.,[13] Nayak *et al*.,[14] Varghese *et al*.,[15] Balpande *et al*.,[10] Kode and Karjodkar,[16] and Shettar.[17]

This could be because the malignant cells probably require more zinc which is taken up from the serum causing low levels of zinc in it. As there is negative interaction between copper and zinc, an increase in copper level may cause subsequent reduction in zinc level as well.[10]

Serum iron levels in oral submucous fibrosis patients
The serum iron level was observed to lower among the cases (66.57 ± 27.76) as compared to controls (94.19 ± 35.70), and the difference was statistically significant. It was similar to the done by Paul *et al*.,[13] Balpande *et al*.,[10] Karthik *et al*., and[18] Shetty *et al*.[11] Decreased iron levels in OSMF patients might be due to utilization of iron in collagen synthesis.[10] It has been stated that the decrease in iron content leads to decrease in epithelial vascularity which results in increased penetration of arecoline which leads to fibrosis.

Cytochrome oxidase is an iron-dependent enzyme which is required for the normal maturation of the epithelium. In iron deficiency state, the levels of cytochrome oxidase are low, consequently leading to epithelial atrophy. An atrophic epithelium makes the oral mucosa vulnerable to the soluble irritants.[3]

Summary and Conclusion

In the recent times, although there has been a consistent rise in the number of patients with OSMF, there is still no detailed understanding of the mechanism directly delineating the etiopathogenetic mechanism involved in the formation of the condition. It can be concluded from this study that serum zinc, copper, and iron levels could be used as a potential prognostic and diagnostic markers in OSMF patients. However, as there are controversial reports on the association of OSMF and these trace elements, future studies are anticipated on a larger heterogeneous population to confirm the hypothesis.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

References

1. Prabhu SR, Wilson DF, Daftary DK, Johnson NW. Oral Diseases in the Tropics. 1st ed. New Delhi: Oxford; 1993.
2. Khanna JN, Andrade NN. Oral submucous fibrosis: A new concept in surgical management. Report of 100 cases. Int J Oral Maxillofac Surg 1995;24:433-9.
3. Kumar A, Sharma SC, Sharma P, Chandra OM, Singhal KC, Nagar A. Beneficial effect of oral zinc in the treatment of oral submucous fibrosis. Indian J Pharmacol 1991;23:236-41.
4. Hazarey VK, Erlewad DM, Mundhe KA, Ughade SN. Oral submucous fibrosis: Study of 1000 cases from central India. J Oral Pathol Med 2007;36:12-7.
5. Maher R, Lee AJ, Warnakulasuriya KA, Lewis JA, Johnson NW. Role of areca nut in the causation of oral submucous fibrosis: A case-control study in Pakistan. J Oral Pathol Med 1994;23:65-9.
6. Borle RM, Borle SR. Management of oral submucous fibrosis: A conservative approach. J Oral Maxillofac Surg 1991;49:788-91.
7. Sinor PN, Gupta PC, Murti PR, Bhonsle RB, Daftary DK, Mehta FS, *et al.* A case-control study of oral submucous fibrosis with special reference to the etiologic role of areca nut. J Oral Pathol Med 1990;19:94-8.
8. Pindborg JJ, Murti PR, Bhonsle RB, Gupta PC, Daftary DK, Mehta FS. Oral submucous fibrosis as a precancerous condition. Scand J Dent Res 1984;92:224-9.
9. Ahmad MS, Ali SA, Ali AS, Chaubey KK. Epidemiological and etiological study of oral submucous fibrosis among gutkha chewers of Patna, Bihar, India. J Indian Soc Pedod Prev Dent 2006;24:84-9.
10. Balpande AR, Sathawane RS. Estimation and comparative evaluation of serum iron, copper, zinc and copper/zinc ratio in oral leukoplakia, submucous fibrosis and squamous cell carcinoma. J Indian Acad Oral Med Radiol 2010;22:73-6.
11. Shetty SR, Babu S, Kumari S, Shetty P, Hegde S, Karikal A. Role of serum trace elements in oral precancer and oral cancer – A biochemical study. J Cancer Res Treat 2013;1:1-3.
12. Trivedy CR, Warnakulasuriya KA, Peters TJ, Senkus R, Hazarey VK, Johnson NW. Raised tissue copper levels in oral submucous fibrosis. J Oral Pathol Med 2000;29:241-8.
13. Paul RR, Chatterjee J, Das AK, Dutta SK, Roy D. Zinc and iron as bioindicators of precancerous nature of oral submucous fibrosis. Biol Trace Elem Res 1996;54:213-30.
14. Nayak AG, Chatra L, Shenai KP. Analysis of copper and zinc levels in the mucosal tissue and serum of oral submucous fibrosis patients. World J Dent 2010;1:75-80.
15. Varghese I, Sugathan CK, Balasubramoniyan G, Vijayakumar T. Serum copper and zinc levels in premalignant and malignant lesions of the oral cavity. Oncology 1987;44:224-7.
16. Kode MA, Karjodkar FR. Estimation of the serum and the salivary trace elements in OSMF patients. J Clin Diagn Res 2013;7:1215-8.
17. Shettar SS. Estimation of serum copper and zinc levels in patients with oral submucous fibrosis. J Indian Acad Oral Med Radiol 2010;22:193.
18. Karthik H, Nair P, Gharote HP, Agarwal K, Ramamurthy Bhat G, Kalyanpur Rajaram D. Role of hemoglobin and serum iron in oral submucous fibrosis: A clinical study. ScientificWorldJournal 2012;2012:254013.