ESTIMATION OF SERUM BETA CAROTENE IN PATIENTS WITH ORAL SUB MUCOUS FIBROSIS, ORAL SQUAMOUS CELL CARCINOMA

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CERTIFICATE

This is to certify that **Dr.P.VIJAYACHANDAR**, Post graduate student (2010 – 2013) in the Department of Oral Medicine and Radiology branch IX, Tamil Nadu Government Dental College and Hospital, Chennai – 600 003 has done this dissertation titled **"ESTIMATION OF SERUM BETA CAROTENE IN PATIENTS WITH ORAL SUB MUCOUS FIBROSIS, ORAL SQUAMOUS CELL CARCINOMA"** under my direct guidance and supervision for partial fulfillment of the M.D.S degree examination in April 2013 as per the regulations laid down by Tamil Nadu Dr.M.G.R. Medical University, Chennai -600 032 for **M.D.S., Oral Medicine and Radiology (Branch – IX)** degree examination.

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CONTENTS

ABSTRACT

Title: ESTIMATION OF SERUM BETA CAROTENE IN PATIENTS WITH ORAL SUBMUCOUS FIBROSIS, ORAL SQUAMOUS CELL CARCINOMA

Background: Oral submucous fibrosis is a chronic, debilitating premalignant condition of the oral mucosa which is associated with chewing of areca nut. The etiology and pathogenesis has been studied in detail over the past few decades, but many controversies still persist. Increased oxidative stress associated with disturbances in antioxidant defense system have been implicated in the pathogenesis of several diseases, most notably oral cancer.

Aim: To estimate the serum beta carotene level in patients with oral sub mucous fibrosis and oral squamous cell carcinoma.

Materials and Methods: In the present study, totally 180 cases were selected from the Dept. of Oral Medicine and Radiology, Tamilnadu Government Dental College & Hospital. They were divided into three groups. Group I consisted of 60 patients with clinically diagnosed oral sub mucous fibrosis of different stages. Group II consisted of 60 patients with clinically diagnosed and histopathologically proven oral squamous cell carcinoma. Group III was the control group which consisted of 60 normal patients. The age of the patients ranged from 20 to 60 years. The concentration of Beta Carotene present in the serum samples was determined by Bradley and Hornbeck method in Dept. of Biochemistry, Madras Medical College & Hospital.

Results: Results were analyzed using one way ANOVA test for comparing the concentration of serum beta carotene level among group I, II and III. The results of our study revealed a significant decrease in the concentration of serum β-carotene level in group II patients (56.46μg/dl) when compared with group I patients (77.54 μg/dl) and group III (120.21μg/dl).In group I, patients were compared using clinical staging wherein stage II showed the highest serum beta carotene level (79.51 μg/dl) and stage III showed a level of 66.32 μg/dl. But when functional staging was compared, stage A had the highest level of serum beta carotene level 82.57 μg/dl, while stage C had the lowest level of 53.30μg/dl and stage B in between the two (67.14μg/dl).In group II patients were compared based on TNM staging, stage II showed the highest value (72.88μg/dl) and was lowest for stage IV (42.31μg/dl). When same patients were compared using histological staging, well differentiated group had the highest level of serum β-carotene 58.63μg/dl with lowest level of 34.29μg/dl for poorly differentiated group.

Conclusion: From the present study, we conclude that oral submucous fibrosis and oral squamous cell carcinoma cause a significant reduction in level of serum β-carotene and this decrease correlates well with the disease progression. So Beta carotene can be used for the prevention and to limit the progression of these diseases. Further longitudinal studies with increased sample size are required to substantiate the role of Beta carotene level in precancerous condition and malignancy.

Keywords: Oral submucous fibrosis, Oral squamous cell carcinoma, antioxidants, β-carotene.

INTRODUCTION

Oxygen is essential for the survival of human life. Yet, paradoxically, Oxygen is also involved in toxic reactions and is therefore a constant threat to the well being of all living things. Most of the potentially harmful effects of oxygen are believed to be due to formation and activity of reactive oxygen species. The reactive oxygen and nitrogen species, mainly free radicals, are found in normal physiological conditions and can be beneficial when produced at low levels. At abnormal levels, they are known to be involved in the process of development of pre-cancer and cancer.

All forms of life maintain a reducing environment within the cells. The maintenance of this status is achieved possibly through the antioxidant defense system, which is in action to protect cellular homeostasis against harmful ROS (reactive oxygen species) produced during normal cellular metabolism, as well as in the pathophysiological states. The antioxidant system is preserved by antioxidant substances that maintain the reduced state by a constant input of metabolic energy.[1] Antioxidant substances are small molecules that can scavenge free radicals by accepting or donating an electron to eliminate the unpaired condition. Typically, this means that the antioxidant molecule becomes a free radical in the process of scavenging a ROS to a more stable and less reactive molecule.

When the endogenous antioxidant defense systems are overwhelmed (oxidative stress), the free radicals reach high concentrations, what has been implicated in disease states. This paradigm has been widely documented for many settings, and a causal relationship has been suggested for oxidative stress and pathologic alterations.[2]

Oxidative stress is implicated in the causation and progression of different diseases including atherosclerosis, *carcinogenesis*, neuro degenerative diseases, chronic inflammatory diseases, radiation damage, ageing and various other patho biological effects.[3]

Antioxidant molecules can be produced endogenously or provided exogenously through diet or antioxidant supplements. Many studies show that dietary antioxidants, which include vitamin C (ascorbic acid), vitamin E (α-tocopherol), β-carotene (a carotenoid), and flavonoids (a subgroup of the phytochemicals), when used within experimental in vitro biological systems, act as effective antioxidants protecting plasma components and cells from damage. Carotenoids are important as they convert into vitamin A when needed and are theorized to have possible antioxidant activity.[4]

Beta-carotene is one of literally hundreds of carotenoids found in nature, particularly in dark green, deep orange and yellow fruits and vegetables. β-carotene is perhaps best known for its role as a precursor to vitamin A, or pro-vitamin A, meaning that the human body converts β-carotene to vitamin A as needed. Compared to its many carotenoid companions, β-carotene is the most potent source of vitamin A.[5]

Oral submucous fibrosis (OSMF) is a chronic insidious and progressive disease involving oral mucosa. Overall prevalence of this precancerous condition in India is about 0.5%, with a range of 0.2-1.2% in different regions of the country.[6] However, recent epidemiological surveys show an increasing prevalence of this malady in different states of India.

Oral cancer is a major health concern in many parts of the world. Oral and pharyngeal cancer, grouped together, is the sixth most common cancer in the world. The annual incidence is about 275,000.[7] South and South East Asia, including India, fall under areas of high incidence rate for oral cancer.[7]

In India amongst all malignancies, it is the most common malignancy in males and third most common malignancy in females.[8] Worldwide, oral cancer has one of the lowest survival rates and remains unaffected despite recent therapeutic advances.[7] For those who develop Oral Squamous cell carcinoma (OSCC), the overall 5-year survival rate is approximately 50%, unchanged over the last 30 years.[9]

Oxidants and antioxidants may play a role in the later stages of cancer development. There is increased evidence that oxidative process contributes to the promotion stages of carcinogenesis, at this stage the level of antioxidants are very crucial in prevention and progression of carcinogenesis.[10] Numerous epidemiological studies based on blood measurement and dietary intake (Peto et al 1981, Willett 1990, Ziegler 1988) support the hypothesis that high intake of β-carotene may reduce the incidence of cancer.

Individuals with oral precancer such as oral submucous fibrosis and oral leukoplakia run a risk that is 69 times higher for them to develop oral cancer compared to tobacco users who do not have pre cancer.[11] It has also been reported that leukoplakia, an oral premalignant lesion can be successfully treated by antioxidant supplementation.[12] Studies have revealed that serum β-carotene levels were below the normal range in all cases of OSMF and β-carotene level estimation can be useful to monitor the oxidative stress in OSMF.[6,13]

Oral submucous fibrosis being a premalignant condition and associated with carcinogens was thought to have a definite association with reactive oxygen species and antioxidant levels which is very crucial in the prevention and progression to oral cancer and there is increased evidence that antioxidant vitamin levels may alter quantitatively in Oral submucous fibrosis and Oral cancer.

Epidemiological studies have suggested that high endogenous levels of prooxidants and deficiencies in antioxidants level are likely to be important risk in the progression of pre cancer to cancer.[10] Free radicals and other reactive oxygen species are difficult to measure quantitatively.[14] Antioxidant nutrients which play a crucial role against defense of prooxidants can be measured. If they are reduced from the normal level they can be supplemented.[15]

This clinical study is planned to estimate the serum β-carotene level in Oral Submucous fibrosis, Oral Squamous cell carcinoma patients and correlate it with the clinical profile and also compare the values with those of healthy individuals.

AIM AND OBJECTIVES

AIM OF THE STUDY:

To estimate the serum beta carotene level in patient with Oral sub mucous fibrosis and Oral squamous cell carcinoma.

OBJECTIVES:

- To analyze the serum beta carotene level between patients with oral sub mucous fibrosis and oral squamous cell carcinoma.
- To compare the serum beta carotene level among different stages of oral sub mucous fibrosis.
- To compare the serum beta carotene level among different stages of oral squamous cell carcinoma.

REVIEW OF LITERATURE

PRECANCEROUS LESION AND CONDITION:

Working group of the World Health Organization proposed in 1978 that clinical presentations of the oral cavity that are recognized as precancerous (hereafter referred to as potentially malignant disorders) be classified into two broad groups, as lesions and conditions

(**WHO 1978**)[16], with the following definitions:

•A **precancerous lesion** is: a morphologically altered tissue in which oral cancer is more likely to occur than in its apparently normal counterpart;

• A **precancerous condition** is: a generalized state associated with a significantly increased risk of cancer

Classification of precancerous lesions and conditions [WHO (1978)] [17]

Precancerous lesions

- Leukoplakia
- Erythroplakia
- Palatal lesions in reverse smokers

Precancerous conditions

- Oral Submucous fibrosis
- Actinic keratosis
- Lichen planus
- Discoid lupus erythematosus

POTENTIALLY MALIGNANT DISORDERS:

According to **Warnakulasuriya (2007)**[18] the distinction between a precancerous lesion and a precancerous condition was considered not just academic. At the time these terms were coined, it was considered that the origin of a malignancy in the mouth of a patient known to have a precancerous lesion would correspond with the site of precancer. On the other hand, in precancerous conditions, cancer may arise in any anatomical site of the mouth or pharynx. Therefore he did not favour subdividing precancer to lesions and conditions and the consensus view was to refer to all clinical presentations that carry a risk of cancer under the term 'potentially malignant disorders' to reflect their widespread anatomical distribution.

Oral submucous fibrosis:

Definition:

Pindborg J.J. and Sirsat S.M. (1966):[19]

"Oral submucous fibrosis is an insidious chronic disease affecting any part of the oral cavity and sometimes the pharynx. Although occasionally preceded by and/or associated with vesicle formation, it is always associated with juxta-epithelial inflammatory reaction followed by a fibro-elastic change of the lamina propria with epithelial atrophy leading to stiffness of mucosa and causing trismus and inability to eat."

Historical Review:

In ancient Indian Medical Manuscripts, around 400 B.C. Sushruta described a condition "Vidari" under the mouth and throat diseases causing progressive narrowing of the mouth, depigmentation of oral mucosa and pain on taking food. These are the

typical features of the entity presently known as “oral submucous fibrosis” (cited by Mukherjee and Biswas 1972).[20]

In 1952, Schwartz described 5 Indian women from Kenya with a condition of oral mucosa involving the palate and pillars of the fauces to which he ascribed the descriptive term “atrophia idiopathica (tropica) mucosae oris.[21]

The term “submucous fibrosis of the palate and pillars” was first introduced in India by Joshi, an ENT surgeon in 1953.[22] The first report among non-Indians was from Taiwan by Su in 1954.[23] The wide variation in the terminology of this condition is suggestive of the uncertainty regarding the exact etiology, which also reflects on the successful management of the case. The term “**oral submucous fibrosis”** has been widely accepted over the years as it implies the nature of the condition in a simplified form.

Incidence and Prevalence:

OSMF is a chronic oral mucosal condition that occurs predominantly among Indians, people of Indian origin living outside India, and occasionally in other Asians.[24] However, sporadic cases have been reported in a female of Caucasian descent[25], a Greek female, and among other Europeans[24]. An ethnic basis has been indicated for OSMF because it is found most commonly in Asians[21].

Schwartz in 1952 first described OSMF in 5 Indian women from Kenya and East Africa[26].Since then numerous reports and studies on OSMF have been published in the literature. A sharp spurt in the incidence of OSMF was noted after various areca and tobacco products came into the market.

A survey was conducted by **Angadi PV et al (2011)[24]** in a teaching hospital in southern India over a period of 16 years, which included 205 cases of OSMF. OSMF

amounted to almost 4.38% of the total cases. It was also observed areca nut/betel nut was associated with almost 78% of the patients with OSMF, while smoking alone leading to OSMF was seen in a very small percentage of patients (2.43%) and was especially associated with beedi smoking. Multiple habits, i.e., areca nut, smoking, alcohol was observed in 6.82% people.

Variations in prevalence figures are common between different studies, probably because of difference in clinical criteria for diagnosis. While some authors adhered to earlier signs and symptoms such as pain, history of vesicles and ulcers, and blanching of mucosa for the diagnosis of OSMF, others looked for fibrous bands as the diagnostic criterion[21].

Age:

The age of the patients affected in OSMF is highly variable as reported by various studies.

Raina C et al (2005)[13] studied 100 cases of OSMF in Maharashtra, India and found that 45% of the patients were in the age group of 21 – 30 years of age. The patients ranged from 12 – 78 years, with the mean age of 29.09 years.

An etiological and epidemiological study of OSMF was carried out in Patna, Bihar, India by **Ahmad MS et al (2006)**[27] including 157 cases of OSMF in the period 2002 – 2004. Maximum number of cases belonged to 21 – 40 years with the youngest recorded case in a 11 year old and oldest one being of 54 years of age.

Hayes ML et al (2006)[28] reported 2 cases of OSMF in 11 and 13 year old patients associated with betel chewing habit.

Kumar KK et al (2007)[29] studied 75 cases of OSMF in Chennai, South India and found that half of the study population belonged to the age group of 20 – 29 years.

Pandya S et al (2009)[30] in their study on OSMF in Allahabad, India, studied 239 patients and found that maximum patients were in the 21 – 30 years age group.

A hospital based case-control study conducted by **Bathi RJ et al (2009)**[31] included 220 patients with OSMF. Majority of the patients included were under 35 years of age (85.5%).

Shirzai M (2011)[32] reported a case of OSMF in a 15 year old Iranian boy with the habit of chewing supari since the age of 10 years about 15 times per day.

A review was done by **Angadi PV et al (2011)**[24] on 205 cases in southern India diagnosed between January 1989 and June 2005. The age range was 14 - 78 years, with mean age being 46 years. OSMF was most frequent in the age range of 21 - 30 years (47.8%). Another interesting finding was that the females affected were at a slightly later age range of 3^{rd} -5^{th} decade.

High occurrence of OSMF in the younger age groups has given rise to the notion that there will be a parallel increase in the incidence rates of oral cancer in this group.[33]

Gender:

Pindborg JJ et al (1984)[34] in their study of 89 patients of OSMF in Ernakulam, Kerala, India, found 29 males and 60 females with OSMF.

In 1986, a house-to-house survey of tobacco habits was conducted among 30,544 villagers of all ages in 373 villages in three areas of Kolar District, Karnataka, to gather baseline information for an intervention study. About 8-16% of men and 29-39% of women had chewing habits. While the content of the substances chewed was not defined in this study, a case-control study carried out in Karnataka by one of the authors identified the chewing habits as including tobacco, betel leaf, areca nut and slaked lime and as being the only tobacco habit of women.[35]

Various reports have suggested an increased female predominance which is in accordance with the increased prevalence of the habits in women. The general female preponderance may also be related to the deficiency of iron and vitamin B complex among many Indian women.[21]

Various investigators have also found a male predominance contradictory to the earlier studies. Males were found to be dominating, as they were using gutka and other related products more because of easy availability in all the places, whereas females being more conscious about their health and esthetic value, probably felt uncomfortable to ask the vendors in getting the gutka products. This is one of the reasons, which may be responsible for a high male to female ratio.[27]

Investigator	Location	No. of patients studied	Male: female ratio
Rao ABN (1962)	Hyderabad	46	17 : 29
Wahi PN (1966)	Agra	104	2 : 1
Pindborg JJ et al (1968)	India	63	1 : 3
Shiau YY (1979)	Taiwan	35	34 : 1
Caniff JP et al (1986)	London	44 (43 Indians + 1 Pakistani)	1 : 4.5
Bailoor DN (1993)	Mangalore	12	1 : 1.4
Maher et al (1994)	Pakistan	157	1 : 2.3
Shah N et al (1998)	New Delhi	236	1.8 : 1
Ranganathan K et al (2004)	Chennai	185	9.9 : 1
Raina C (2005)	Maharashtra	100	3.3 : 1
Ahmad MS et al (2006)	Bihar	157	2.7 : 1
Kumar KK (2007)	Chennai	75	6 : 1
Pandya S et al (2009)	Allahabad	239	6.8 : 1

A study was conducted by **Seedat et al (1988)**[36] in Durban, South Africa, where Indians constituted 46% of the population. Women chewers predominated in the ratio of 13:1. **Haider SM et al (2000)**[37] found in their study on 325 patients of OSMF that 52% were females and 48% were males.

A hospital-based cross-sectional study on various habit patterns associated with OSMF was performed in Nagpur, Central India, by **Hazarey et al (2007)**[38] over a 5-year period. A total of 1,000 OSMF cases were included. The severity of OSMF was more prevalent in women than men even though the male: female ratio was 4.9:1. They found that, an underprivileged socio-economic status and poor education was significantly higher in women than men. These factors may have contributed to the increased severity of OSMF in women compared with men.

A hospital based case-control study conducted by **Bathi RJ et al (2009)**[31] included 220 patients with OSMF. Majority of the patients included were males (96.4%).

A review was done by **Angadi PV et al (2011)**[24] on 205 cases in southern India diagnosed between January 1989 and June 2005. The overall male to female ratio was around 11:1 with a general trend towards male preponderance.

From the literature reviewed, the question "Does gender have any influence on oral mucosal disorders among gutka chewers?" seems yet to be answered.[39] However, the recent studies have shown a male predominance.

ORAL CARCINOMA:

Oral carcinoma is a global health problem with increasing prevalence and mortality rates. Among these, the majority are squamous cell carcinoma.

Epidemiology:

Oral carcinoma is a global health problem with increasing prevalence and mortality rates. It is the sixth most common cancer in the world.[7] Worldwide, the annual incidence exceeds 3,000,000 new cases.[40] More than 90% of oral malignancies are squamous cell carcinomas. There is a wide geographical variation (approximately 20-fold) in the incidence of this cancer. The areas characterized by high incidence rates for oral cancer (excluding lip) are found in the South and Southeast Asia (e.g. Sri Lanka, India, Pakistan and Taiwan), parts of Western (e.g. France) and Eastern Europe (e.g. Hungary, Slovakia and Slovenia), parts of Latin America and the Caribbean (e.g. Brazil, Uruguay and Puerto Rico) and in Pacific regions (e.g. Papua New Guinea and Melanesia).[7] In high-risk countries such as Sri Lanka, India,

Pakistan and Bangladesh, oral cancer is the most common cancer in men, and may contribute up to 25% of all new cases of cancer.

Age and sex distribution:

In most countries around the world, oral cancer is more common in men than in women. The reported sex differences are attributable to heavier indulgence in risk habits by men and exposure to sunlight (for lip cancer) as a part of outdoor occupations. The ratio of males to females diagnosed with oral cancer, however, has declined over the decades and is now about 1.5:1 for the mouth. The risk of developing oral cancer increases with age and the majority of cases occur in people aged 50 or over. About 6% of oral cancers occur in young people under the age of 45 years. In high-incidence countries of the world, many cases are reported before the age of 40.[7]

Anatomic sites:

Cancer of the buccal mucosa is more common among Asian populations due to betel quid/tobacco chewing habits. In Sri Lanka, 40% of oral cavity cancers are found on buccal mucosa.[7] Tongue is the most common site for intraoral cancer among European and the US populations, amounting to 40–50% of oral cancers.[7] Other intraoral sites that can be involved are floor of the mouth, palate and alveolus.

Oral cancer prevalence in India:

Oral cancer constitutes the largest group of malignancies in the Indian subcontinent with an incidence rate as high as 30-40%.[41] Data from National Cancer Registry programme of the Indian Council of Medical Research has confirmed the fact that oral cancer is indeed a common form of cancer in India.[40] It is the most

prevalent cancer in males as well as third most common in females.[8] According to Kalyani R et al., among adolescent and young adults, oral cancer was the most prevalent cancer in both the genders.[42] The spectrum of oral malignancy varies from place to place within the country with a marked increase in occurrence in many parts of the country like Uttar Pradesh, Madhya Pradesh, Gujarat, Bihar and Maharashtra.[7]

ETIOLOGY AND RISK FACTORS:

The aetiology of oral cancer is multifactorial. Based on available global evidence, **Warnakulasuriya, 2009**[7] grouped the risk factors as established, strongly suggestive, possible and speculative factors.

RISK FACTORS FOR ORAL CANCER AND PRECANCER

(Warnakulasuriya, 2009[7])

Established	**Strongly suggestive**	**Possible**	**Speculative**
Smoking	Sunlight (lip)	Viruses	Mouthwashes
Chewing tobacco	Radiation	Immune deficiency	Mate drinking
Snuff dipping		Dentition	Periodontal disease
Alcohol misuse		Ethnicity	Familial
Betel quid, syphilis			

The most important etiological factors are tobacco, excess consumption of alcohol [43] and betel quid usage, these factors act separately or synergistically.[44] Attributable risk of oral cancer due to both tobacco and alcohol is estimated to be more than 80%.[7] A diet deficient in antioxidants is a further factor that predisposes towards the development of oral cancer.[45] Other factors such as HPV infection may also be involved.[46]

MORBIDITY AND MORTALITY:

Many patients who are successfully treated for oral cancer have to cope with the devastating consequences of their treatment. These may affect the patient's appearance and function, e.g. eating, drinking, swallowing and speaking. These residual defects may lead to other problems such as depression and nutritional deficiency.[7]

For most countries, five-year survival rates for cancers of the tongue, oral cavity and oropharynx are around 50%. The best outcome is for the cancer of the lip, with over 90% of patients surviving for five year period.[7] In general, prognosis decreases with advanced disease and increasing inaccessibility of the tumour. For cancers of both the tongue and the oral cavity, women had higher survival rates than men. TNM stage at presentation significantly affects five-year survival. For mobile tongue, five-year survival for stage disease is 80%, while for stage 1V survival drops to 15%.[7]

For most countries age-adjusted death rates from oral cancer have been estimated at 3–4 per 100,000 men and 1.5–2.0 per 100,000 for women.

BETA-CAROTENE:

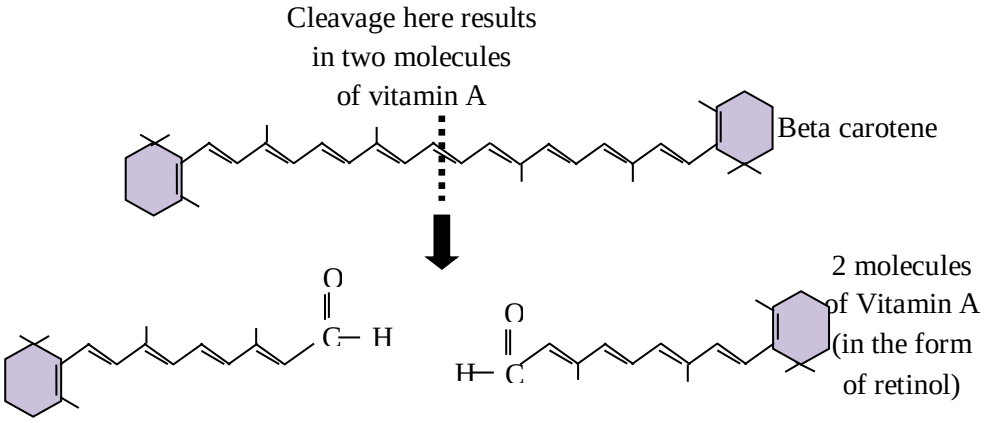
Beta-carotene is a phytochemical classified as a **carotenoid.** Although there are more than 600 carotenoids found in nature, only about 50 are found in the typical human diet. The six most common carotenoids found in human blood are alpha-carotene, β-carotene, β-cryptoxanthin, lutein, lycopene, and zeaxanthin. Of these, alpha-carotene, β-carotene, and β-cryptoxanthin are referred to as provitamin A carotenoids.[5]

Provitamins are inactive forms of vitamins that the body cannot use until they are converted to their active form. Our bodies convert β-carotene to an active form of vitamin A, or retinol; thus, beta-carotene is a precursor of retinol.[5]

Chemical structure:

Beta-carotene is a fat soluble member of the carotenoids. Much of the natural β-carotene is composed of "all-trans" isomers.[47]

Levin G et al (1997)[48] have shown that 9-cis β-carotene is a better antioxidant than its all-trans counterpart.



Units:[49]

One *Retinol Activity Equivalent (RAE)* (US):

= 1 μg all-trans-Retinol

= 2 μg β-carotene in oil

= 12 μg β-carotene in foods

= 24 μg β-carotene in foods

= 24 μg β-cryptoxanthin in foods

One *Retinol Equivalent (RE)* (Europe):

= 1 μg all-trans-retinol

= 6 μg β-carotene

= 12 μg other provitamin A carotenoids

Food sources of β-carotene:[5,50]

Fruits	**Vegetables**
Apricots	Carrots
Cantaloupe	Pumpkin
Mango	Sweet potatoes
Plantain	Winter squash
Peaches	Broccoli
Prunes	Dark green lettuces and spinach
Watermelon	Red peppers

Recommended dietary intake:

The National Nutrition Societies of Germany, Austria and Switzerland (DACH 2000) recommend a daily β-carotene intake of 2-4 mg.[49]

Mechanism of action:

Two principal mechanisms of action have been proposed for any antioxidant:[51]

1. The first is a chain-breaking mechanism by which the primary antioxidant donates an electron to the free radical present in the system.
2. The second mechanism involves removal of ROS/RNS initiators (secondary antioxidants) by quenching chain-initiating catalysts.

The antioxidant action of β-carotene is found to be due to the suppression of singlet oxygen formation and the reaction with peroxynitrite.[1] Beta-carotene exacerbates DNA oxidative damage and modifies p53-related pathways of cell proliferation and apoptosis in cultured cells exposed to tobacco smoke but has no significant effect in cells unexposed to tobacco.[52]

BETA-CAROTENE IN ORAL SUBMUCOUS FIBROSIS AND ORAL SQUAMOUS CELL CARCINOMA:

It was probably Bruce Ames who first drew general attention to the importance of oxidative damage in human cancer aetiology and the likely importance of antioxidant defences.[53]

A study conducted by **Babu S et al (1996)**[54] in 50 patients of OSMF found that habitual chewing of pan masala/gutka is associated with earlier presentation of OSMF than betelquid use. The absence of the betel leaf and its carotenes such as hydroxychavicol was considered to be one of the factors responsible for this difference.

Uikey AK et al (2003)[55] conducted a study to estimate the serum status of antioxidants, superoxide dismutase (SOD) and glutathione peroxidase (GPX) in OSMF. A total of 60 subjects were included in the study comprising of 30 cases of OSMF and 30 healthy controls. In OSMF, serum antioxidant enzyme levels were found to be decreased (mean SOD 86.63 ± 20.36 and mean GPX 1.50 ± 0.30 U/ml) compared to the control group (mean SOD 127.1 ± 18.14 and mean GPX 2.71 ± 0.43 U/ml).

The results suggested that low values of SOD and GPX may be associated with the development of carcinoma in OSMF.

An investigation was undertaken by **Subapriya R et al (2003)**[56] to examine the blood levels of lipid peroxides and antioxidants in oral precancer, preoperative, postoperative, and recurrent oral cancer patients, with age- and sex-matched normal healthy subjects as controls. In patients with oral precancer and cancer, enhanced lipid peroxidation was accompanied by antioxidant depletion. These changes were more pronounced in patients with recurrent oral cancer.

Twelve weeks after surgery, decreased lipid peroxidation was accompanied by increased antioxidant levels. The results of this study indicated that the imbalance in redox status of oral precancer and cancer patients might be due to enhanced lipid peroxidation and compromised antioxidant defences.

Another clinical follow up study was undertaken on 34 patients by **Gupta S et al (2004)**[6] to assess the blood levels of lipid peroxidation product - MDA and antioxidant defense system in OSMF cases and to re-evaluate the patients after antioxidant supplementation. Plasma MDA level was increased in OSMF; β-carotene level was found to be decreased in all grades of OSMF cases (81.7+14.3 µg/dl) compared to healthy controls (110+20.8 µg/dl), the decrease being more in grade II and grade III cases with subsequent increase in the levels on β-carotene supplementation. Hence it was concluded that MDA and β-carotene level estimation can be useful to monitor the oxidative stress in OSMF cases for better management.

A cross-sectional study was conducted by **Raina C et al (2005)**[13] on 100 patients with OSMF aged 12-78 years to know the prevalence, predisposing factors and clinical profile of OSMF and to estimate serum β-carotene levels in OSMF. Mean serum β-carotene levels were 101.8 $\pm$ 10.7 µg% in controls, 87.7 µg% in grade I, 76.3

µg% in grade II, and 69.9 µg% in grade III. Hence, it was concluded that serum β-carotene levels were below the normal range in all cases of OSMF, but were lower in grade II as compared to grade I and lowest in grade III cases.

Metkari SB et al (2007)[57] conducted a study on 40 OSMF patients and 40 controls to correlate the serum levels of lipid peroxidation product MDA, antioxidants superoxide dismutase and vitamin A in relation to clinical and histopathological grading of OSMF. The mean vitamin A level gradually decreased from clinical grade I to grade IV as compared to controls.

Results indicated positive correlation of increased lipid peroxidation and decreased antioxidants with clinical grades of OSMF. The study concluded that estimation of lipid peroxidation and antioxidants in circulation of OSMF patients could help in assessing the degree of oxidative damage of the disease. Further, correcting the underlying deficiency of antioxidants could improve the treatment planning for OSMF.

'Antoxid' is an antioxidant formulation that contains β-carotene, zinc, copper, manganese and selenium. It has been found that these micronutrients have antioxidant properties and enhance cellular immunity. A study was conducted by **Jirge V et al (2008)**[58] to evaluate the effects of levamisole and antoxid. Out of 45 patients of OSMF, 15 were administered two capsules of antoxid per day, each day for 6 consecutive weeks. There was significant improvement in mouth opening and reduction in burning sensation.

Another study on 96 cases of head and neck malignancy was conducted by **Shariff AK et al (2009)**[59] to ascertain the variations in the serum levels of MDA and

total antioxidant status in different stages of head and neck malignancies and to validate the protective effects of antioxidant supplementation during radiotherapy. Pre-treatment serum total antioxidant status was significantly declined in all the stages of head and neck malignancies (628.75 ± 76.72 μmoles/L) when compared with the values of healthy controls (997.13 ± 82.25 μmoles/L), with increased values after radiotherapy and antioxidant supplementation.

The study findings concluded that the estimation of serum MDA and total antioxidant status in head and neck malignancy cases served as a good indicator of oxidative stress in different stages correlating with the severity and staging of head and neck malignancies, also suggesting oral antioxidant supplementation to be an adjuvant to radiotherapy.

Ching S et al (2002)[60] also observed that increased serum β-carotene levels were associated with reduction in breast cancer risk.

Bathi RJ et al (2009)[61] conducted a study to detect the gene polymorphism of detoxification enzymes and estimate the antioxidant enzyme status in patients with oral cancer, oral leukoplakia and OSMF. The mean values of glutathione were significantly raised in all groups. The mean values of ceruloplasmin and MDA was statistically significant among cancer and OSMF patients but was insignificant in smokers and cases with leukoplakia.

They concluded that the level of antioxidant enzymes correlate with the degree of oxidative damage. Epidemiological evidence consistently relates low antioxidant intake or low blood levels of antioxidants with increased cancer risk. Antioxidant levels have thus been used as an indicator for the oxidative stress in such conditions.[4,62]

Factors affecting serum β-carotene levels:

Beta-carotene has been known to be affected by a multitude of factors, because of which the serum β-carotene levels in individuals are quite varied.

Metabolic situations in which radicals are formed at a higher rate such as stress after an accident, surgery under anesthetic, smoking, alcoholism, handling of carcinogens at work, physical exertion, intensive sun exposure etc. greatly reduce the concentration of β-carotene in plasma.[49]

A study was conducted by **Tang G et al (1996)**[63] to investigate the effect of gastric acidity on β-carotene using omeprazole. The authors found that serum β-carotene concentrations were significantly lower at a higher gastric pH of 6.4 $\pm$ 0.3 (with omeprazole) than at a gastric pH of 1.3 $\pm$ 0.1 (without omeprazole). Lipid micelles containing carotenoids formed in the duodenum as a result of fat digestion, release carotenoids into mucosal cells of the duodenum by passive diffusion, determined by the concentration gradients between the two. The authors theorized that a higher gastric pH increased the negative surface charges of the carotenoid-containing micelle and the intestinal lumen, inhibiting passive diffusion. Thus, high gastric pH is known to decrease the absorption of beta carotene.

A study conducted by **Berg G et al (1997)**[64] investigated the influence of oral contraceptives on the serum concentration of β-carotene. It was concluded that the use of oral contraceptives was strongly related to serum β-carotene levels, particularly among women above the age of 35 years. This was attributed to the age dependent use of oral contraceptives with higher estrogen content (ethylestradiol).

Dietary fat is also a factor in carotenoid absorption. High-fat diets (18-24 g fat with breakfast, 45 g fat with mid-day meal) produced better β-carotene absorption than low-fat diets (no fat for breakfast and 6 g for midday meal) in test meals when subjects were given 45 mg β-carotene for five days.[65]

The association of lifestyle factors including alcohol use, physical activity and dietary habits with serum levels of carotenoids (lycopene, lutein, cryptoxanthin and β-carotene), retinol and α-tocopherol were studied in 194 healthy males aged 24 – 60 years who smoked more than 15 cigarettes/ day by **Kitamura Y et al (1997)**.[66] Of the dietary items studied, total vegetable intake was significantly, positively associated with β-carotene levels, as was fruit intake with serum levels of each carotenoid. Alcohol consumption was most strongly and inversely associated with β-carotene.

Another study by **Galan P et al (2005)**[67] assessed the relationships between energy, nutrient and food intakes, alcohol consumption, smoking status and body mass index, and serum concentrations of β-carotene, α-tocopherol, vitamin C, selenium and zinc. Women had higher baseline serum β-carotene than men. In women, younger age was associated with lowered mean concentration of serum β-carotene. Alcohol consumers and smokers had lower concentrations of serum β-carotene. Serum β-carotene concentrations were lower in obese subjects. They concluded that serum β-carotene concentrations are primarily influenced by sex, age, obesity, tobacco smoking, alcohol consumption and especially dietary intake.

Individuals with higher body mass index are found to have a lower serum β-carotene concentration. β-carotene is distributed differently between plasma and adipose tissue, the former being the dominant storage tissue of β-carotene in humans. Accordingly, a person with high fat mass would have a larger proportion of ingested

β-carotene absorbed by fat tissue than would a lean person if all other metabolic factors were equal. A second explanation could be that the estimates of β-carotene and fibre intakes among obese individuals fail to detect a lower consumption of foods that would increase serum β-carotene concentrations.[67]

Cigarette smoking, a major risk factor for oropharyngeal cancer, is reported to alter oral levels of carotenoids and tocopherols. A study was conducted by **Gabriel HE et al (2006)**[68] to determine whether chronic smoking is associated with altered concentrations of these nutrients in serum and buccal mucosa. It was found that chronic cigarette smokers have lower concentrations of many dietary antioxidants in serum and buccal mucosal cells compared with non-smokers.

The lower levels of antioxidants found in smokers may partly be a consequence of a greater antioxidant depletion due to a sustained smoke-related oxidant load. Components of cigarette smoke in the presence of high oxygen tension combine to induce oxidation of the nutrient, resulting in a pro-oxidant effect.[52]

Serum β-carotene is also found to be affected by the levels of triglycerides, total cholesterol levels[64] and cardiovascular drugs [69,70] because of which serum β-carotene has been found to be lower in older individuals.[70]

MATERIALS AND METHODS

The study was conducted at

- Department of Oral Medicine and Radiology,

  Tamil Nadu Government Dental College and Hospital,

  Chennai – 600 003.

- Department of Microbiology,

  Madras Medical College,

  Chennai – 600 003.

The study protocol was approved by the Institutional Ethical Committee.

DURATION OF THE STUDY: From February 2012 to November 2012.

SAMPLE DESIGN:

Totally 180 cases were included under the study. The cases were selected from the Department of Oral Medicine and Radiology, Tamil Nadu Government Dental College and Hospital, Chennai – 600 003 between February 2012 and November 2012. All were in the age group of 20 – 60 years. They were divided into three groups consisting of 60 cases each.

- **Group I (Oral sub mucous fibrosis):** Patients with clinically diagnosed oral sub mucous fibrosis of different stages- 60 cases.

- **Group II (Oral squamous cell carcinoma):** Patients with clinically different TNM staging and histopathologically proven oral squamous cell carcinoma – 60 cases.
- **Group III (Control)):** Healthy individuals– 60 cases.

INCLUSION CRITERIA:

The following patients were included under the study.

- Age range: 20 – 60 years
- Gender: Both males and females

- Group I:
    - Patients with mucosal blanching, burning, hardening and presence of characteristic fibrous bands, and decreased mouth opening.
    - None of the lesions should have been treated in any manner prior to sample collection.
- Group II:
    - Patients with clinically different TNM staging and histopathologically proven oral squamous cell carcinoma.
    - None of the lesions should have been treated in any manner prior to sample collection.
- Group III:
    - Healthy controls both the gender, age ranges from 20-60 years and free of any habits and systemic illness.

EXCLUSION CRITERIA:

- Patients with chronic systemic diseases like diabetes mellitus, hypertension, pregnancy, liver disorders and patient under antioxidant therapy.
- Patients not willing to participate in the study.

METHODOLOGY:

Following the selection of cases, written informed consent was obtained from all the patients selected for the study. They were requested to sign the information sheet after explaining the study procedure. Complete medical history and clinical findings of all the cases were recorded in the structured proforma prepared for the study.

The first appointment included the complete medical history and clinical findings of all the cases were recorded in the proforma for the study. Diagnosis of all the cases of OSMF was done on clinical grounds (presence of burning sensation, restricted mouth opening, mucosal blanching, restricted tongue protrusion and presence of palpable fibrous bands). The mouth opening was measured from the mesioincisal angle of the maxillary central incisor to mesioincisal angle of mandibular central incisor and recorded in mm for dentulous patients. In patients missing maxillary and / or mandibular central incisor, the distance between the highest point in maxillary and mandibular alveolar crest midline was recorded[71]. The cases were classified into three stages based on mouth opening according to the functional staging of OSMF given by Haider SM et al (2000).[37]

Stage A : Mouth opening $\geq$ 20 mm

Stage B : Mouth opening = 11-19 mm

Stage C : Mouth opening $\leq$ 10 mm

The patients were also classified into three stages based on the site of involvement according to the clinical staging of OSMF given by Haider SM et al (2000).[37]

Stage I: Faucial bands

Stage II: Faucial and buccal bands

Stage III: Faucial, buccal and labial bands

Patients with non-healing ulcer/ ulcero-proliferative growth with or without Lymphadenopathy (oral cancer).Incisional biopsy is to be performed for clinically suspected oral squamous cell carcinoma with different TNM staging and diagnosis to be established based on clinical and histopathological findings. Once the clinical diagnosis was established, the patients were sent for routine blood investigations.

Armamentarium:

Examination of the patient:

- Electrically operated dental chair
- Patient's apron
- Disposable mouth mask
- A pair of disposable latex examination gloves
- Stainless steel kidney trays
- mouth mirror
- Stainless steel probe

- Tweezer
- Divider
- Metallic scale
- Tongue depressor

SERUM SAMLPE COLLECTION AND STORAGE:

Collection of serum:

- A pair of disposable latex surgical gloves (No. 6½)
- Tourniquet
- Sterile cotton rolls
- Surgical spirit (70% ethyl alcohol)
- 5 ml sterile disposable syringe
- 24 gauge needle of 1" length
- Vaccutainer test tube

Armamentarium required in Microbiology Department:

- Sterile glass test-tube
- Centrifuging machine used at 2500 rpm
- Automatic pipette with disposable plastic pipette tips
- Disposable plastic Eppendroff tube
- Deep freezer

Collection of blood sample:

- The patient was then asked to attend the next morning after an overnight fast, to avoid any dietary influence on the serum beta carotene level. Upon returning, the patient was asked to sit comfortably in a dental chair in a

reclining position. A tourniquet was then applied above the left cubital fossa, and the needle of a disposable 2-ml, 23-gauge syringe was inserted into the vein. About 2 ml of venous blood was withdrawn and then transferred to a plain 10ml vaccutainer test tube.

- Then the samples were transferred to the Department of Microbiology, Madras Medical College on the same day. There, after the blood had coagulated, the test tube containing the blood was subjected to centrifugation for about 4-5 min at 2500 rpm. The test tube was then removed from the centrifuge, and the serum layer was pipetted into a vial. Serum transferred to Eppendroff tube and stored at -20 degree.

Beta-carotene estimation:

- 95% Ethanol
- Beta-carotene powder
- Hexane
- Chemical balance
- Patient serum
- 100 ml glass beakers (2 in no.)
- Conical flask
- Sterile glass test-tubes with a wooden test-tube stand
- Glass pipettes (2 ml, 10 ml)
- Cuvette
- Centrifuging machine used at 2500 rpm
- Spectrophotometer used at 450 nm

Estimation of serum beta-carotene:

The concentration of Beta Carotene present in the serum samples was determined by Bradley and Hornbeck method using a beta-carotene stock standard[72] (Sigma-Aldrich Corp. St. Louis, MO, USA). The analysis was performed at the Department of Microbiology, Madras Medical College.

Beta-carotene:

- Stock β-carotene standard was prepared by dissolving 1 mg of β-carotene in 10 ml hexane.

Estimation procedure:

The estimation procedure was carried out in dim lighting conditions. Serum (0.2 ml), 0.2 ml ethanol, and 0.5 ml hexane were pipetted into a small test tube. The solvent system was mixed on a vortex mixer 1 min and then centrifuged for 4-5 min at 2500 rpm. The top hexane layer was carefully removed and recentrifuged in a small polyethylene microtube for 2 min in an IEC centrifuge.

A 0.35 ml aliquot was carefully pipetted out and transferred to a 0.4 ml Zeiss microcell. Carotene content was determined by reading the solution optical density (OD) at 450 nm.

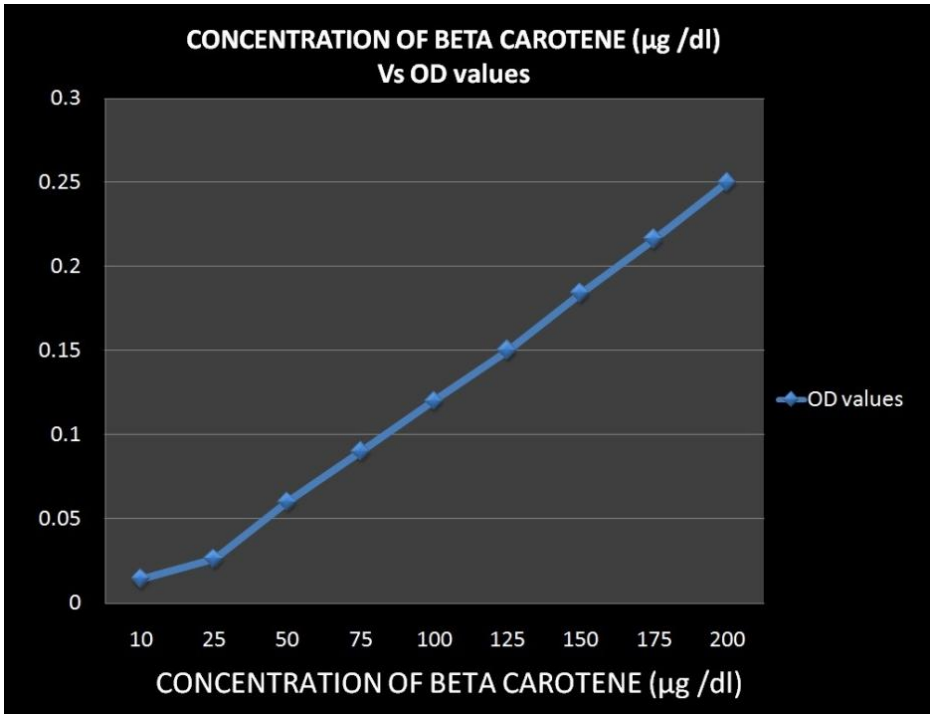
Calculation of concentration of serum Beta Carotene sample:

A linear standard curve was generated by plotting the average absorbance of each Standard on the vertical axis versus the corresponding Beta Carotene standard concentration on the horizontal axis. The amount of Beta Carotene in each sample

was determined by extrapolating OD values against Beta Carotene standard concentrations using the standard curve.

CONCENTRATION OF BETA CAROTENE (µg /dl)	OPTICAL DENSITY
10	0.014
25	0.026
50	0.060
75	0.090
100	0.120
125	0.150
150	0.184
175	0.216
200	0.250

BETA CAROTENE STANDARD CURVE



PHOTOGRAPHS

I-ARMAMENTARIUM

FIG 1: DIAGNOSTIC INSTRUMENTS

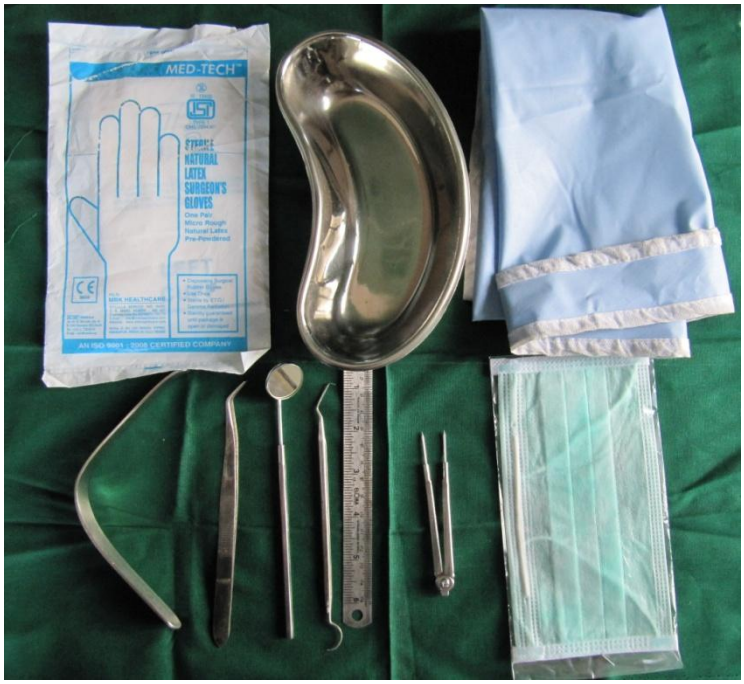


FIG 2: INSTRUMENTS FOR COLLECTION OF BLOOD

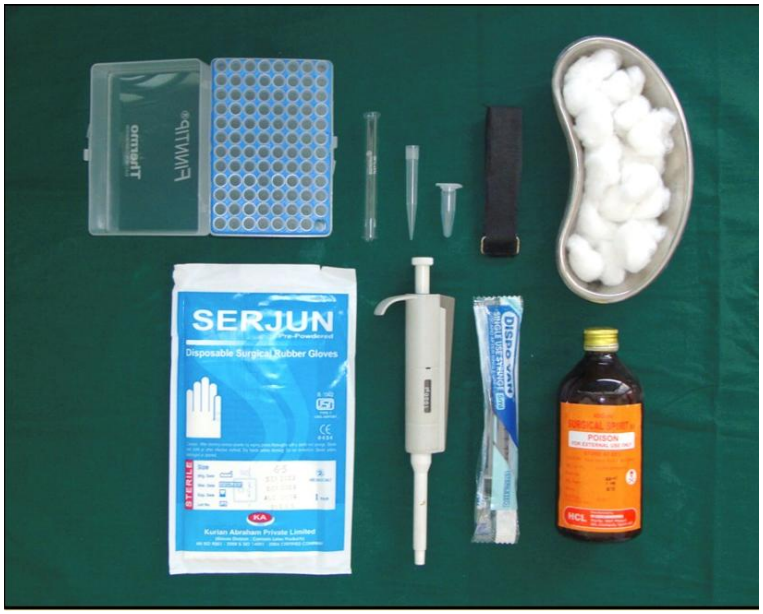


FIG 3: COLLECTION OF BLOOD SAMPLE

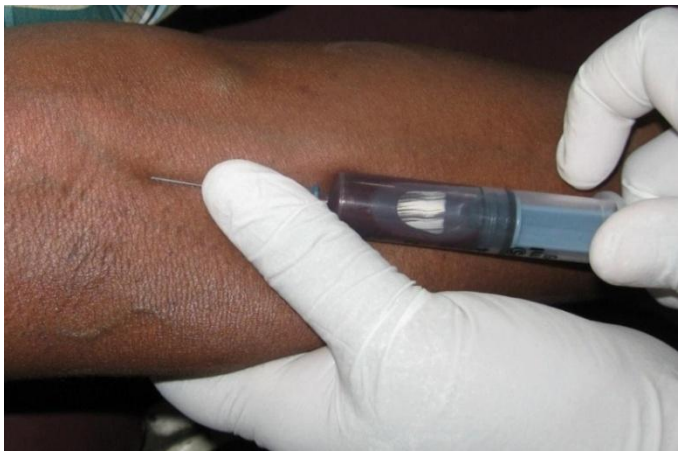


FIG 4: CENTRIFUGING MACHINE

FIG 5: TRANSPORTING KIT

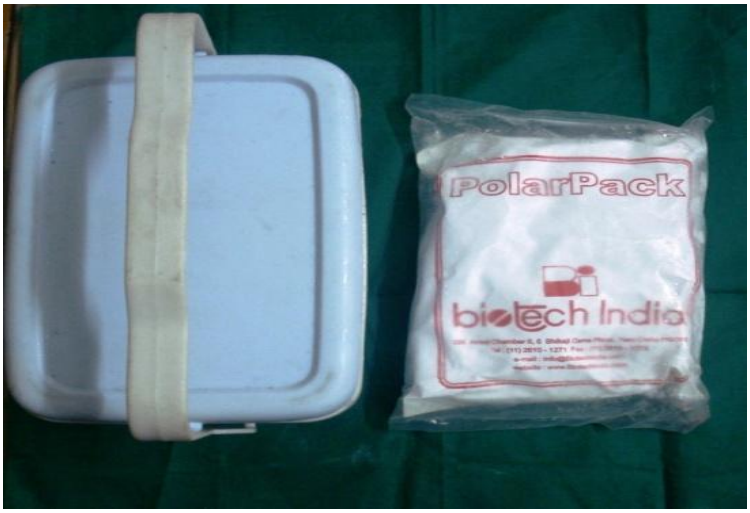


FIG 6: SERUM SAMPLES

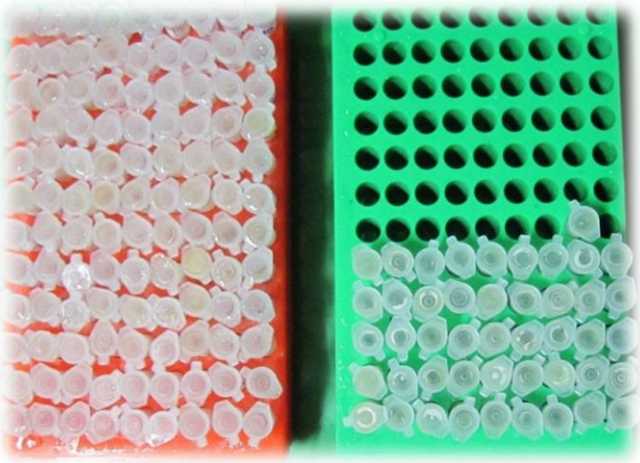
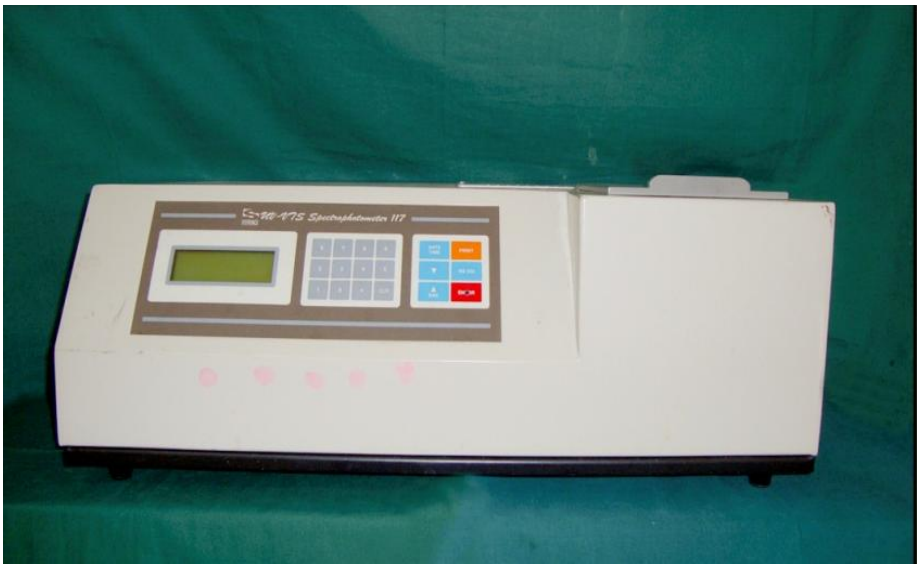


FIG 7: DEEP FREEZER

FIG 8: INSTRUMENTS FOR SERUM β-CAROTENE

FIG 9: REAGENTS FOR SERUM BETA CAROTENE ESTIMATION

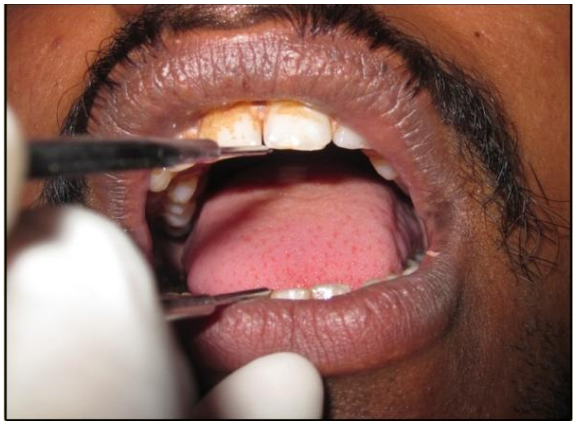
FIG 10: SPECTROPHOTOMETER



II: CLINICAL CASES

ORAL SUBMUCOUS FIBROSIS

FIG 11: MEASUREMENT OF MOUTH OPENING



CLINICAL STAGE II

FIG 12: BLANCHING OF THE RIGHT BUCCAL MUCOSA AND RETROMOLAR AREA

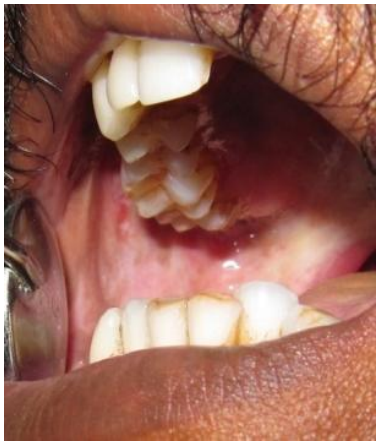
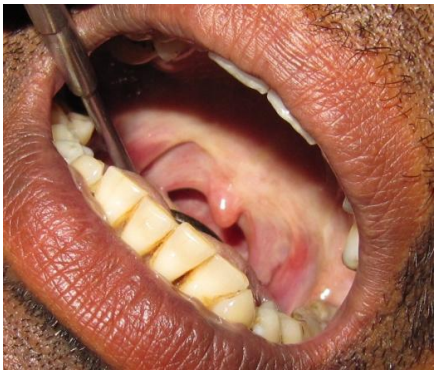


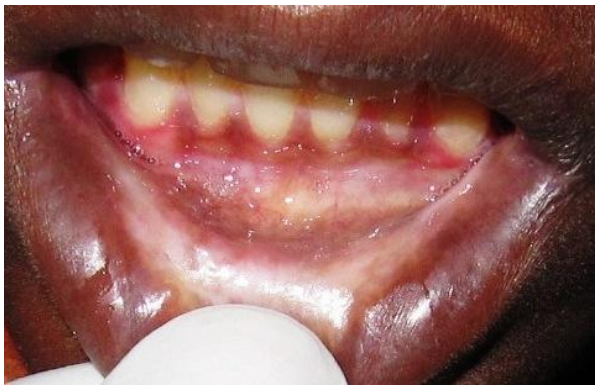
FIG 13: BLANCHING IN SOFT PALATE DEVIATION UVULA TOWARDS LEFT SIDE



CLINICAL STAGE III

FIGURE 14

BLANCHING IN LOWER LABIAL MUCOSA



BLANCHING IN RIGHT BUCCAL MUCOSA AND RETROMOLAR AREA

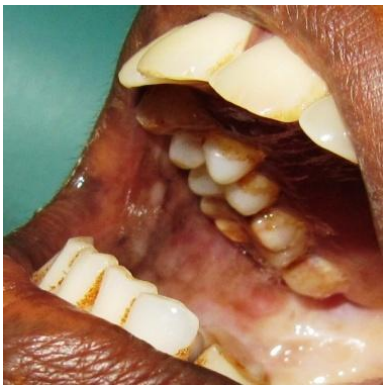
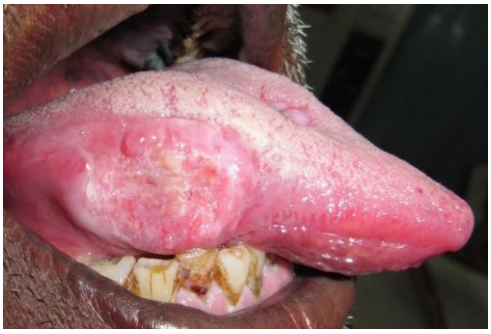


FIG 15: ORAL SQUAMOUS CELL CARCINOMA (TNM STAGE III, WELL DIFFERENTIATED TYPE)



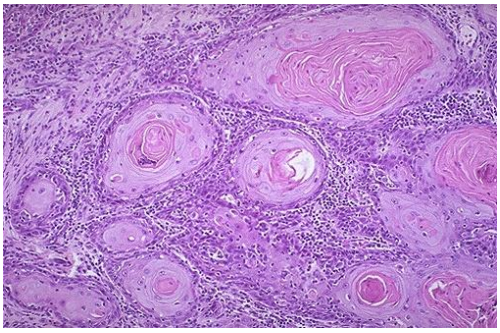
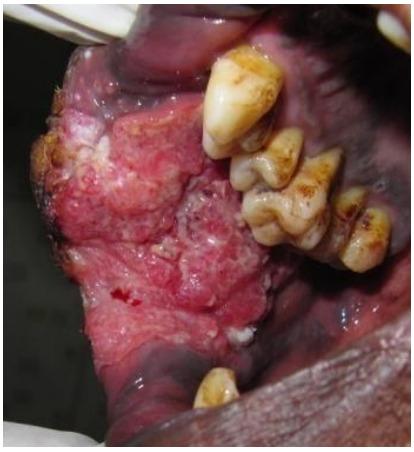
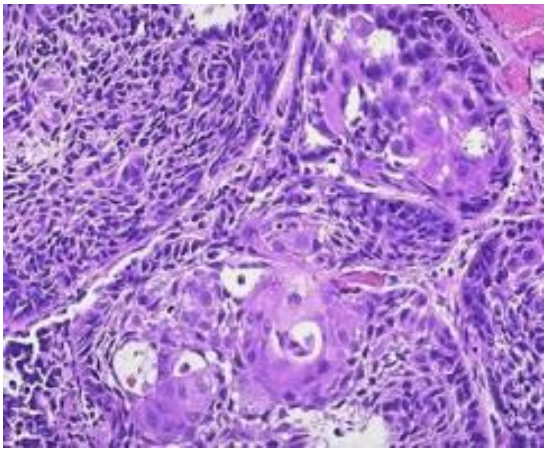


FIG 16: ORAL SQUAMOUS CELL CARCINOMA (TNM STAGE IV, MODERATELY DIFFERENTIATED TYPE)





MASTER CHART I – GROUP I (ORAL SUBMUCOUS FIBROSIS)

S.NO	C.NO	AGE (YEARS)	SEX	CH	SH	AH	CD (/YEARS)	CF (/DAYS)	SD (/YEARS)	SF (/DAYS)	AD (/YEARS)	AF (/MONTHS)	MOUTH OPENING (MM)	Clinical staging	Functional staging	Serum beta carotene level (microgram/dl)
1	1	41	M	C	0	A	6	4	0	0	10	2	15	II	B	69.01
2	2	53	F	C	0	0	30	5	0	0	0	0	24	II	A	67.5
3	3	46	M	C	S	0	20	4	20	3	0	0	22	II	A	76.5
4	4	45	M	C	S	0	10	4	20	6	0	0	24	II	A	67.32
5	5	21	M	C	S	A	3	5	3	7	2	2	18	II	B	59.01
6	6	28	M	C	S	0	5	3	6	6	0	0	26	II	A	80.23
7	7	27	M	C	0	0	10	6	0	0	0	0	14	II	B	85.01
8	8	42	F	C	0	0	5	3	0	0	0	0	26	II	A	72.36
9	9	30	M	C	0	0	7	3	0	0	0	0	21	II	A	77.46
10	10	23	M	C	S	0	5	4	5	6	0	0	9	III	C	58.16
11	11	22	M	C	0	A	3	4	0	0	3	3	27	II	A	74.32
12	12	21	M	C	0	0	1	2	0	0	0	0	22	II	A	104.4
13	13	54	M	C	0	A	30	5	0	0	30	3	17	II	B	68.32
14	14	32	M	C	S	0	5	3	4	6	0	0	25	II	A	89.39
15	15	37	M	C	S	A	6	4	3	7	2	2	23	II	A	71.37
16	16	24	M	C	0	0	4	3	0	0	0	0	19	II	B	65.49
17	17	32	M	C	0	A	7	3	0	0	6	4	21	II	A	68.94
18	18	35	M	C	0	0	10	6	0	0	0	0	17	II	B	67.11
19	19	21	M	C	S	0	4	3	2	3	0	0	29	II	A	73.21
20	20	48	M	C	0	A	20	3	0	0	10	2	34	II	A	70.04
21	21	32	M	C	0	0	3	4	0	0	0	0	29	II	A	92.41
22	22	27	M	C	S	0	4	2	4	2	0	0	32	II	A	87.32
23	23	23	M	C	0	0	1	2	0	0	0	0	24	III	A	112.36
24	24	37	M	C	S	0	2	4	10	6	0	0	27	II	A	79.64
25	25	56	M	C	S	A	30	4	30	6	10	3	23	III	A	68.82
26	26	37	M	0	S	0	0	0	12	8	0	0	27	II	A	87.14
27	27	40	F	C	0	0	6	4	0	0	0	0	19	II	B	73.29
28	28	22	M	C	0	0	4	6	0	0	0	0	27	II	A	79.91
29	29	31	M	C	0	A	4	6	0	0	10	2	24	II	A	84.62

30	30	30	M	C	0	0	10	4	0	0	0	0	14	II	B	71.42
31	31	55	F	C	0	0	20	4	0	0	0	0	27	II	A	76.5
32	32	50	M	C	0	0	35	6	0	0	0	0	22	III	A	81.16
33	33	48	M	C	0	A	10	4	0	0	20	3	24	II	A	72
34	34	30	M	C	0	0	4	2	0	0	0	0	26	II	A	78.13
35	35	48	F	C	0	0	4	3	0	0	0	0	21	II	A	100.46
36	36	37	M	C	S	A	12	6	10	2	10	1	17	III	B	54.31
37	37	60	M	C	S	0	10	3	40	6	0	0	32	II	A	84.61
38	38	31	F	C	0	0	5	4	0	0	0	0	18	II	B	73.37
39	39	55	M	C	0	0	30	4	0	0	0	0	36	II	A	94.37
40	40	43	M	0	S	0	0	0	15	6	0	0	29	II	A	89.49
41	41	32	M	C	0	A	10	3	0	0	10	1	13	III	B	58.39
42	42	23	M	C	S	0	6	8	6	7	0	0	9	III	C	45.32
43	43	43	M	C	0	0	10	3	0	0	0	0	29	II	A	84.89
44	44	26	M	C	0	A	6	4	0	0	6	3	22	II	A	93.17
45	45	59	M	C	S	0	10	4	30	6	0	0	26	II	A	84.01
46	46	21	M	C	0	0	3	6	0	0	0	0	21	II	A	95.46
47	47	28	M	C	0	0	6	4	0	0	0	0	27	II	A	79.39
48	48	35	M	C	0	0	10	6	0	0	0	0	24	II	A	84.14
49	49	28	M	C	0	A	10	6	10	7	8	2	9	III	C	57.42
50	50	30	M	C	0	0	4	6	0	0	0	0	26	II	A	88.71
51	51	32	M	C	0	A	6	3	0	0	10	3	23	II	A	80.36
52	52	47	M	C	S	0	12	3	14	6	0	0	32	II	A	92.16
53	53	54	M	C	S	0	30	6	29	6	0	0	8	II	C	52.31
54	54	30	M	C	0	0	4	3	0	0	0	0	31	II	A	112.11
55	55	47	M	C	0	0	20	4	0	0	0	0	21	II	A	79.26
56	56	28	M	C	S	0	4	3	6	3	0	0	27	II	A	77.16
57	57	41	M	C	0	0	20	6	0	0	0	0	17	III	B	61.01
58	58	32	M	C	0	A	6	4	0	0	6	3	26	II	A	73.24
59	59	30	M	C	0	0	40	6	0	0	0	0	33	II	A	68.32
60	60	50	M	C	S	0	30	3	30	6	0	0	22	II	A	79.04

M – Male **F** – Female **CH** – Chewing Habit **SH** – Smoking Habit **AH**- Alcohol Habit **CD**- Chewing Duration **SD** – Smoking Duration **AD**- Alcohol Duration **CF** – Chewing Frequency **SF** – Smoking Frequency **AF** - Alcohol Frequency

MASTER CHART II – GROUP II (ORAL SQUAMOUS CELL CARCINOMA)

S.No	C. No	Age (years)	SEX	CH	SH	AH	CD (/years)	CF (/days)	SD (/years)	SF (/days)	AD (/years)	AF (/months)	TNM staging	Histological Grading	Serum Beta Carotene Level (microgram/dl)
61	1	60	M	0	S	A	0	0	30	7	20	3	III	MD	53.12
62	2	47	F	C	0	0	10	6	0	0	0	0	IV	WD	49.39
63	3	38	M	0	S	0	0	0	15	6	0	0	II	WD	96.4
64	4	59	M	0	S	0	0	0	30	7	0	0	III	WD	53.34
65	5	57	M	C	S	0	30	3	25	6	0	0	II	WD	70.31
66	6	38	F	C	0	0	5	3	0	0	0	0	IV	MD	49.17
67	7	58	F	C	0	0	25	6	0	0	0	0	III	WD	61.31
68	8	38	M	C	S	0	15	3	18	6	0	0	I	MD	85.16
69	9	48	M	C	S	0	20	4	15	6	0	0	III	WD	50.36
70	10	49	M	0	S	0	0	0	19	7	0	0	II	WD	67.6
71	11	45	F	C	0	0	12	3	0	0	0	0	III	MD	57.21
72	12	60	M	0	S	0	0	0	40	6	0	0	III	WD	59.32
73	13	38	M	0	S	A	0	0	15	6	15	3	I	WD	83.6
74	14	53	M	C	S	0	24	4	25	6	0	0	III	WD	55.26
75	15	51	M	C	S	A	20	3	20	6	15	2	III	WD	47.24
76	16	56	F	C	0	0	30	6	0	0	0	0	III	WD	54.15
77	17	53	M	0	S	A	0	0	30	6	30	3	II	WD	58.17
78	18	57	F	C	0	0	24	5	0	0	0	0	III	WD	51.32
79	19	53	M	C	S	0	30	6	30	7	0	0	IV	WD	57.12
80	20	40	M	0	S	0	0	0	20	6	0	0	II	MD	61.2
81	21	53	F	C	0	0	17	6	0	0	0	0	III	WD	48.44
82	22	60	M	0	S	A	0	0	40	7	40	2	III	WD	49.17
83	23	48	M	0	S	A	0	0	22	6	20	2	IV	WD	39.29
84	24	60	F	C	0	0	24	7	0	0	0	0	IV	PD	37.16
85	25	60	M	C	S	A	25	6	30	7	23	2	III	WD	51.01
86	26	59	F	C	0	0	20	4	0	0	0	0	IV	WD	40.01
87	27	50	F	C	0	0	11	3	0	0	0	0	IV	WD	37.16
88	28	43	M	0	S	0	0	0	20	7	0	0	II	WD	59.32
89	29	42	F	C	0	0	6	4	0	0	0	0	III	MD	48.16
90	30	50	M	0	S	0	0	0	20	6	0	0	III	WD	53.14

91	31	50	F	C	0	0	12	3	0	0	0	0	III	WD	52.62
92	32	45	M	C	S	0	14	4	20	6	0	0	II	WD	57.17
93	33	58	M	C	S	A	20	4	20	6	15	2	I	WD	40.17
94	34	57	M	0	S	0	0	0	30	7	0	0	III	WD	89.36
95	35	50	M	0	S	A	0	0	30	6	30	3	IV	PD	31.42
96	36	55	F	C	0	0	20	7	0	0	0	0	III	WD	57.16
97	37	32	M	0	S	A	0	0	10	6	10	2	III	WD	52.12
98	38	39	M	0	S	A	0	0	5	6	10	2	II	WD	112.16
99	39	43	M	C	S	0	14	3	25	5	0	0	III	MD	48.23
100	40	54	M	0	S	0	0	0	33	6	0	0	I	WD	64.15
101	41	38	M	C	S	A	10	4	10	6	10	1	III	MD	45.01
102	42	59	F	C	0	0	31	7	0	0	0	0	III	MD	54.11
103	43	44	F	C	0	0	17	4	0	0	0	0	III	MD	48.29
104	44	38	M	C	S	A	10	4	10	3	10	3	III	WD	58.31
105	45	48	F	C	0	0	10	4	0	0	0	0	III	MD	46.59
106	46	46	M	C	S	0	20	6	20	4	0	0	III	WD	60.04
107	47	60	M	0	S	0	0	0	30	6	0	0	IV	MD	32.17
108	48	40	F	C	0	0	7	3	0	0	0	0	III	WD	55.24
109	49	57	F	C	0	0	35	4	0	0	0	0	II	MD	69.41
110	50	55	F	C	0	0	12	3	0	0	0	0	III	MD	48.41
111	51	56	F	C	0	0	18	6	0	0	0	0	III	MD	51.32
112	52	47	M	C	S	0	20	4	20	6	0	0	III	WD	66.17
113	53	40	M	C	S	A	15	6	15	4	15	2	II	WD	77.15
114	54	57	M	0	S	A	0	0	35	6	35	3	III	WD	61.332
115	55	55	F	C	0	0	13	4	0	0	0	0	IV	WD	39.42
116	56	43	M	0	S	A	0	0	21	7	21	3	III	WD	52.11
117	57	37	M	0	S	A	0	0	11	5	11	2	III	WD	71.39
118	58	55	M	C	S	A	25	6	25	3	25	3	I	WD	44.32
119	59	39	M	C	S	0	11	4	12	6	0	0	IV	WD	53.14
120	60	52	M	0	S	0	0	0	30	7	0	0	III	WD	65.36

M – Male **F** – Female **CH** – Chewing Habit **SH** – Smoking Habit **AH**- Alcohol Habit **CD**- Chewing Duration **SD** – Smoking Duration **AD**- Alcohol Duration **CF** – Chewing Frequency **SF** – Smoking Frequency **AF** - Alcohol Frequency **MD** – Moderately Differentiated **WD** – Well Differentiated

PD – Poorly Differentiated

MASTER CHART III – GROUP III (CONTROL GROUP)

S.NO.	CASE NO.	AGE(Years)	SEX	Serum Beta Carotene Level (micro gram/dl)
121	1	56	M	119.68
122	2	51	M	93.52
123	3	42	M	133.12
124	4	53	F	97.26
125	5	33	M	116.26
126	6	46	F	140.11
127	7	45	M	133
128	8	51	F	128
129	9	27	M	117.12
130	10	32	M	177.22
131	11	42	M	99.01
132	12	31	M	91.46
133	13	36	F	84.31
134	14	54	M	113.21
135	15	53	M	101.23
136	16	24	M	144.12
137	17	32	M	126
138	18	37	M	165.72
139	19	41	M	88.53
140	20	33	M	97.12
141	21	26	M	103.1
142	22	42	F	117
143	23	27	M	129.09
144	24	24	M	121.4
145	25	55	M	130.9
146	26	51	M	104.1
147	27	50	M	111.08
148	28	28	F	84.71
149	29	42	M	188.4
150	30	31	M	143.7
151	31	33	M	106.16
152	32	42	M	121.23
153	33	22	M	142.12
154	34	57	M	70.31
155	35	52	F	62.5
156	36	26	M	223.01
157	37	42	M	100
158	38	31	M	97.03
159	39	55	F	116.12
160	40	24	M	141.25

161	41	26	M	135.11
162	42	41	M	157.27
163	43	46	M	142.36
164	44	32	F	81.01
165	45	27	M	132.12
166	46	29	M	114.26
167	47	37	M	118.16
168	48	31	M	89.06
169	49	42	M	99.27
170	50	44	F	143.21
171	51	21	M	100.59
172	52	44	M	101.23
173	53	36	M	127.12
174	54	56	M	61.05
175	55	43	F	136.21
176	56	29	M	167.11
177	57	27	M	207.46
178	58	44	M	90.66
179	59	34	F	124.12
180	60	53	M	106.26

M – Male F – Female

STATISTICAL ANALYSIS

The statistical analysis was done using the computer software program SPSS version17.

The percentage of distribution of various age groups, and of male and female were calculated within each study group. The percentage of distribution of various clinical staging and functional staging were calculated within the study group I. Also, the percentage of distribution of various TNM stages and histological grades were calculated within the study group II.

Arithmetic Mean and Standard Deviation were estimated for different variables in each study group.

One way ANOVA was used,

- for comparing the concentration of serum beta carotene level among group I, II and III,
- for comparing the concentration of serum beta carotene level among various clinical staging and functional staging were calculated within the study group I, respectively and
- for comparing the concentration of serum beta carotene level among various TNM stages and histological grades were calculated within the study group II.

In the present study, *P-value <0.05* was considered as the level of significance.

TABLES

TABLE 1: AGE DISTRIBUTION

Age (years)	Group			TOTAL (n) (%)
	I (n) (%)	II (n) (%)	III (n) (%)	
20-30	23 38.34%	0 0	15 25%	**38** **21.11%**
31-40	15 25%	13 21.67%	15 25%	**43** **23.90%**
41-50	14 23.33%	18 30%	17 28.33%	**49** **27.22%**
51-60	8 13.33%	29 48.33%	13 21.67%	**50** **27.77%**
Total	**60** **100%**	**60** **100%**	**60** **100%**	**180** **100%**

TABLE 2: SEX DISTRIBUTION

Group	SEX		Total(n) (%)
	Male (n) (%)	Female (n) (%)	
I	48 80.0%	12 20.0%	**60** **100.0%**
II	39 65.0%	21 35.0%	**60** **100.0%**
III	54 90.0%	6 10.0%	**60** **100.0%**
Total	**141** **78.3%**	**39** **21.7%**	**180** **100%**

TABLE 3: COMPARISON OF SERUM β-CAROTENE LEVEL AMONG GROUP I, II & III

Group	No. of patients	Mean serum β-carotene level (µg/dl)	Standard deviation	P -value
I	60	77.54	13.64	
II	60	56.46	14.88	**0.000**
III	60	120.21	31.74	

P-value – 0.000 i.e. significant at 1% level

TABLE 4: COMPARISON OF SERUM β-CAROTENE LEVEL WITH CLINICAL STAGING IN GROUP I

Functional Staging	No. of patients (n=60)	Mean serum β-carotene level (µg/dl)	Standard deviation	P-Value
Stage II	51	79.51	11.38	**0.006**
Stage III	9	66.32	19.90	

P-value –0.006 i.e. significant at 1% level

TABLE 5: COMPARISON OF SERUM β-CAROTENE LEVEL WITH FUNCTIONAL STAGING IN GROUP I

Functional Staging	No. of patients (n=60)	Mean serum β-carotene level (µg/dl)	Standard deviation	p-value
Stage A	44	82.57	11.15	**0.000**
Stage B	12	67.14	8.34	
Stage C	4	53.30	5.92	

P-value – 0.000 i.e. significant at 1% level

TABLE 6: COMPARISON OF SERUM β-CAROTENE LEVEL AMONG VARIOUS TNM STAGING IN GROUP II

TNM Staging	No. of patients (n=60)	Mean serum β-carotene level (µg/dl)	Standard deviation	P -value
I	5	63.48	9.44	**0.000**
II	10	72.88	5.71	
III	34	55.16	1.46	
IV	11	42.31	2.57	

P-value – 0.000 i.e. significant at 1% level

TABLE 7: COMPARISON OF SERUM β-CAROTENE LEVEL AMONG VARIOUS HISTOLOGICAL GRADINGS IN GROUP II

Histological Grading	No. of patients (n=60)	Mean serum β-carotene level (µg/dl)	Standard deviation	P -value
Well differentiated	43	58.63	15.17	**0.045**
Moderately differentiated	15	53.17	12.06	
Poorly differentiated	2	34.29	4.06	

P-value – 0.045 i.e. significant at 5% level

TABLE 8: DISTRIBUTION OF SERUM β-CAROTENE LEVEL WITH CLINICAL STAGING IN GROUP I

Functional Staging	No. of patients (n=60)	Percentage %
Stage II	51	85
Stage III	9	15
Total	**60**	**100**

TABLE 9: DISTRIBUTION OF SERUM β-CAROTENE LEVEL WITH FUNCTIONAL STAGING IN GROUP I

Functional Staging	No. of patients (n=60)	Percentage %
Stage A	44	73.33
Stage B	12	20.00
Stage C	4	6.67
Total	**60**	**100.0**

TABLE 10: DISTRIBUTION OF VARIOUS TNM STAGING AMONG GROUP II

TNM Staging	No. of patients (n=60)	Percentage %
Stage I	5	8.3
Stage II	10	16.7
Stage III	34	56.7
Stage IV	11	18.3
Total	**60**	**100**

TABLE 11: DISTRIBUTION OF VARIOUS HISTOLOGICAL GRADES AMONG GROUP II

Histological grading	No. of patients (n=60)	Percentage %
Well differentiated	43	71.67
Moderately differentiated	15	25.0
Poorly differentiated	2	3.33
Total	60	100

TABLE 12: COMPARISON OF SERUM β-CAROTENE LEVEL WITH AGE IN GROUP I

Age (yrs)	No. of patients (n=60)	Mean serum β-carotene level (µg/d)l	Std. Deviation	P-value
20-30	23	79.28	17.02	**0.805**
31-40	15	75.84	11.01	
41-50	14	78.19	10.79	
51-60	8	74.55	13.11	

P-value – 0.805 i.e. not significant.

TABLE 13: COMPARISON OF SERUM β-CAROTENE LEVEL WITH AGE IN GROUP II

Age (yrs)	No. of patients (n=60)	Mean serum β-carotene level (µg/dl)	Std. Deviation	P-value
30-40	13	69.23	20.47	**0.001**
41-50	18	51.34	9.38	
51-60	29	53.90	11.60	

P-value –0.001 i.e. significant at 1% level

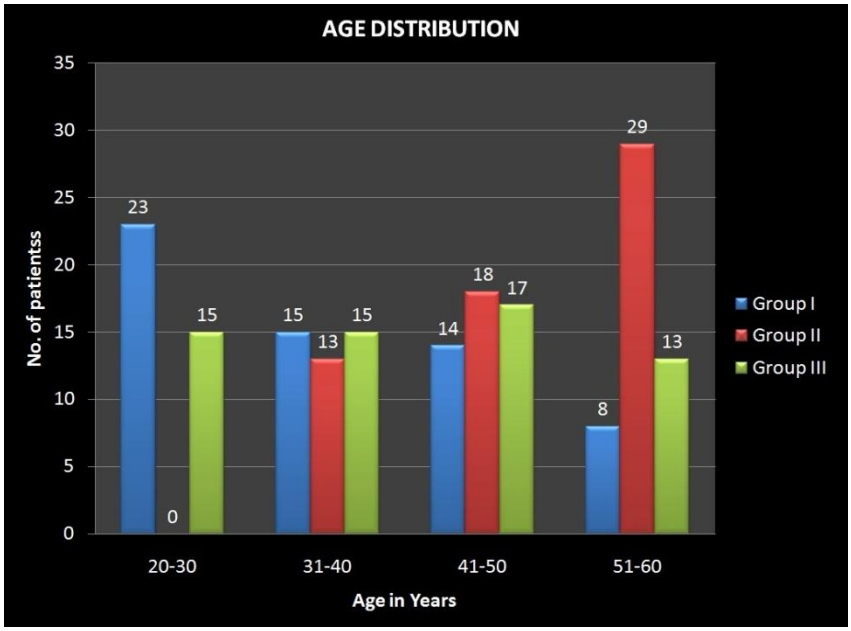
TABLE 14: COMPARISON OF SERUM β-CAROTENE LEVEL WITH AGE IN GROUP III

Age (yrs)	No. of patients (n=60)	Mean serum β-carotene level (µg/dl)	Std. Deviation	P-value
20-30	15	137.50	37.62	**0.015**
31-40	15	116.29	28.84	
41-50	17	123.62	26.75	
51-60	13	100.31	23.22	

P-value –0.015 i.e. significant at 5% level

CHARTS

CHART 1



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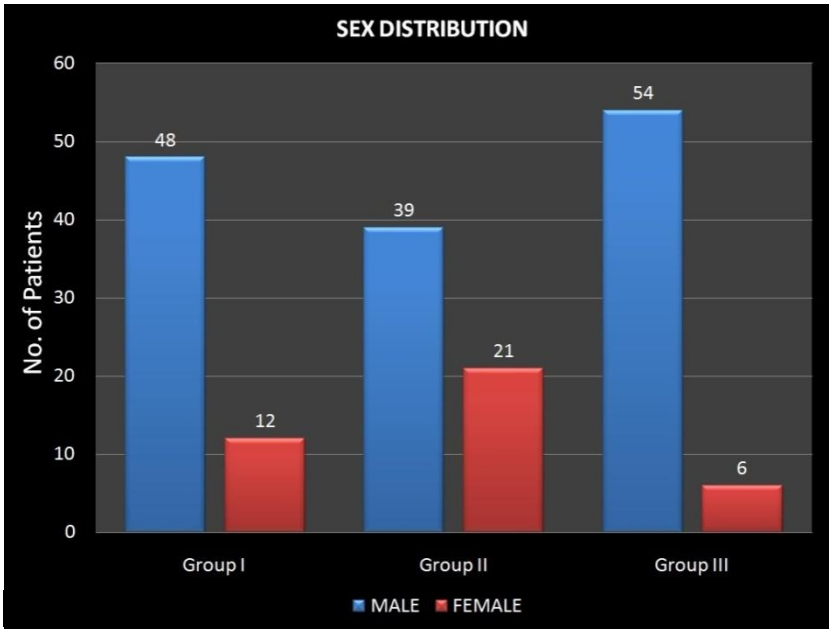

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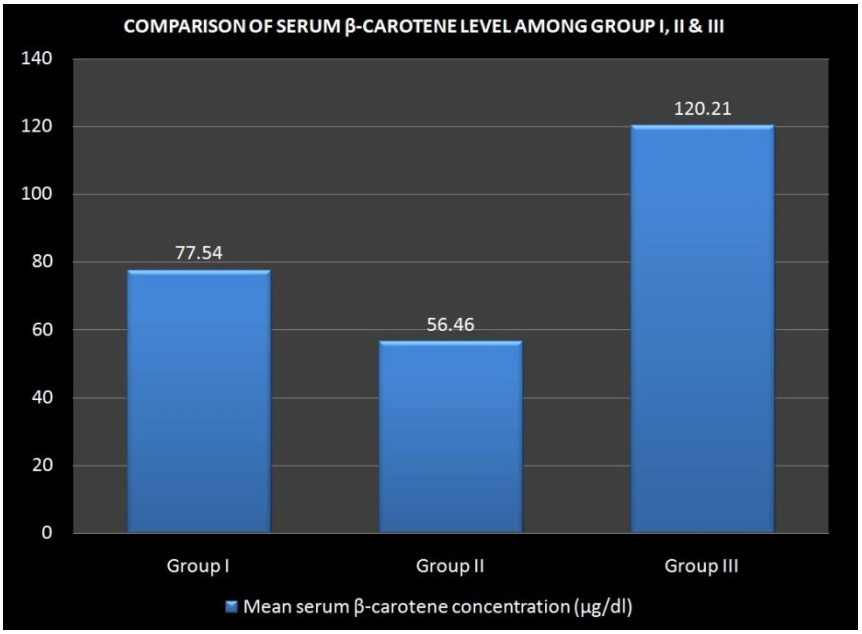

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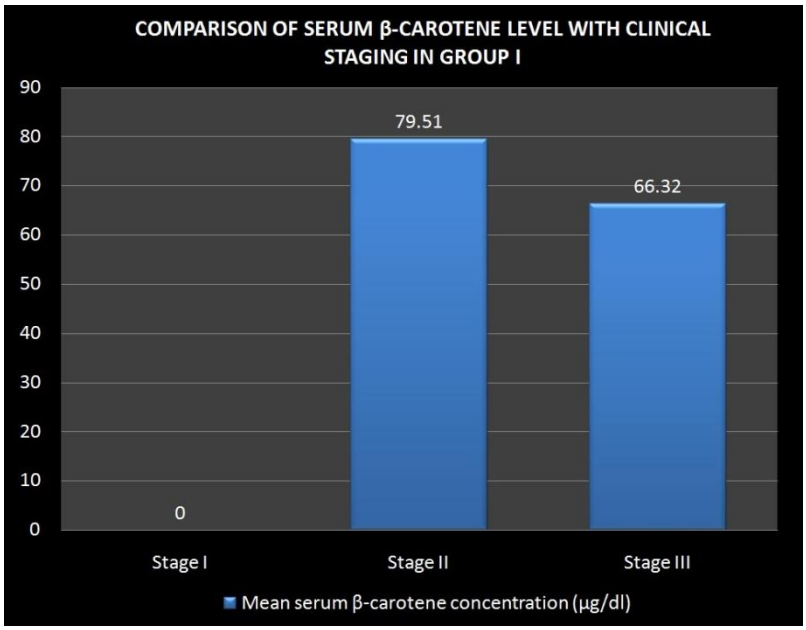

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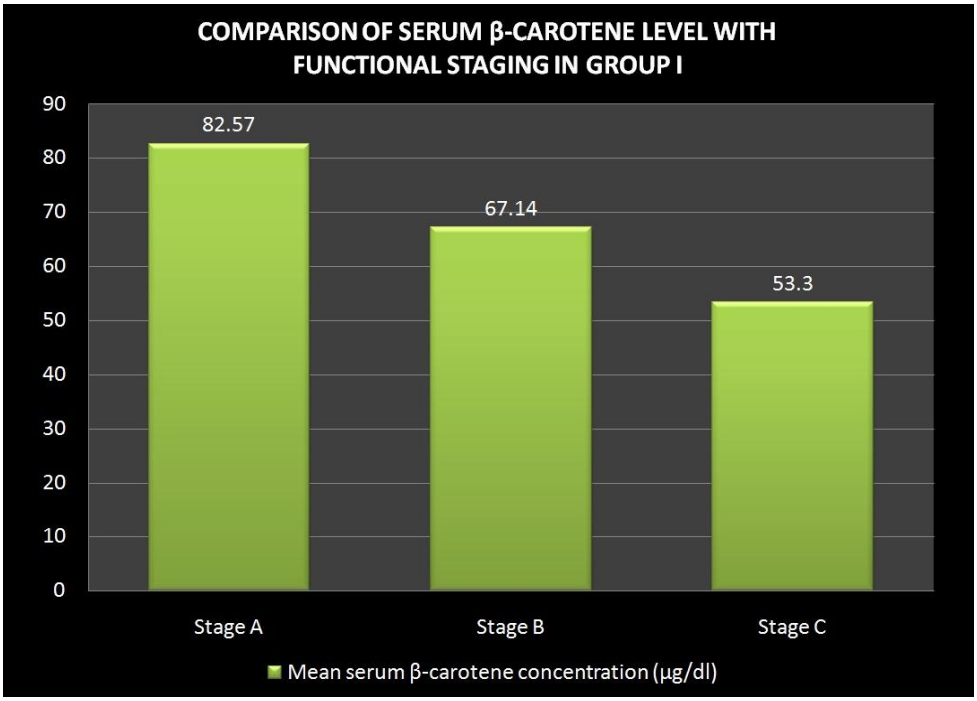

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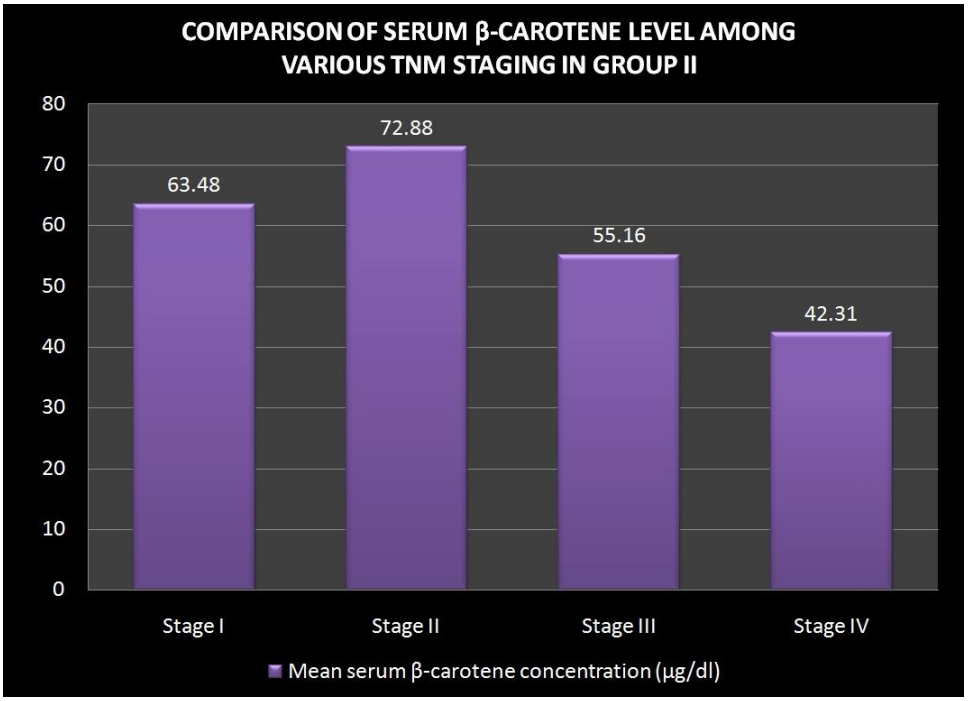

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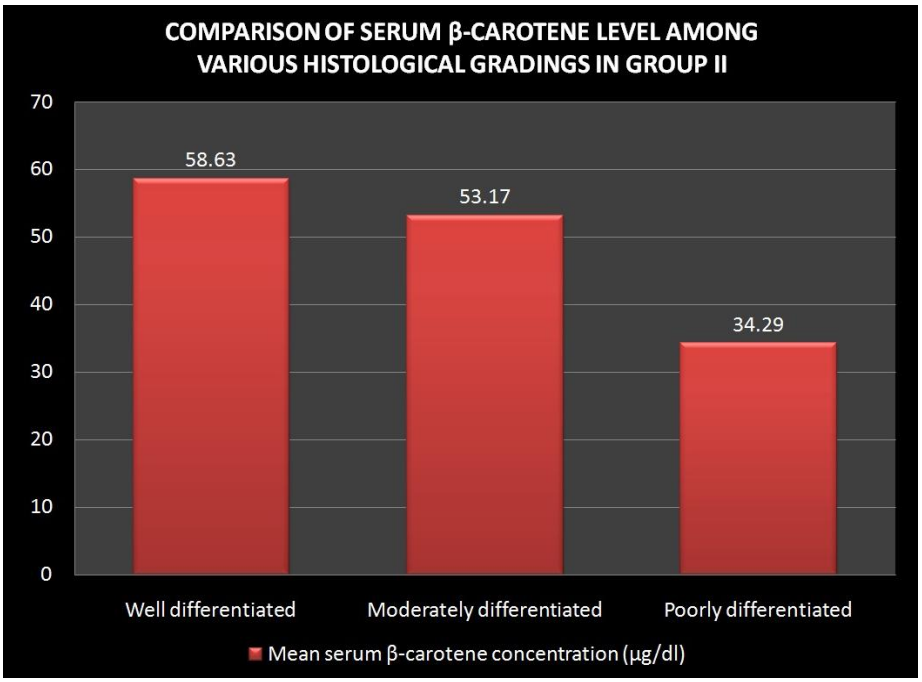

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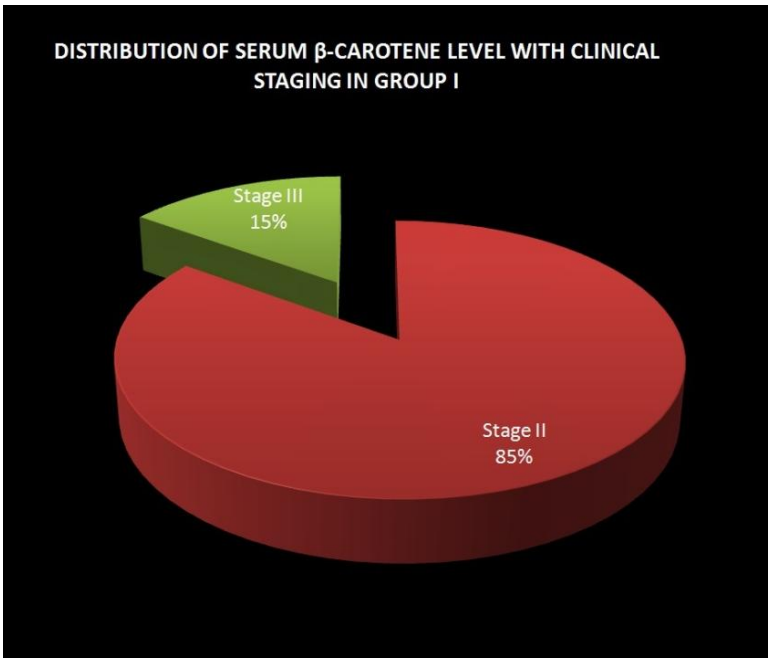

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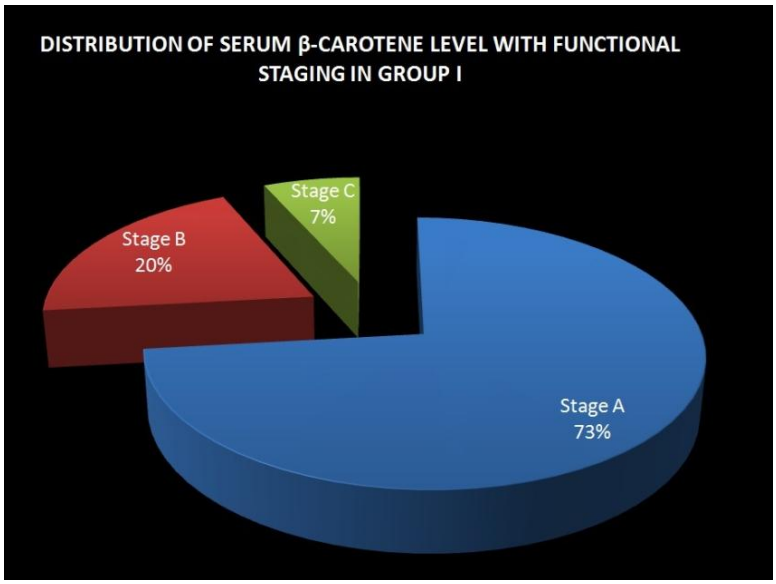

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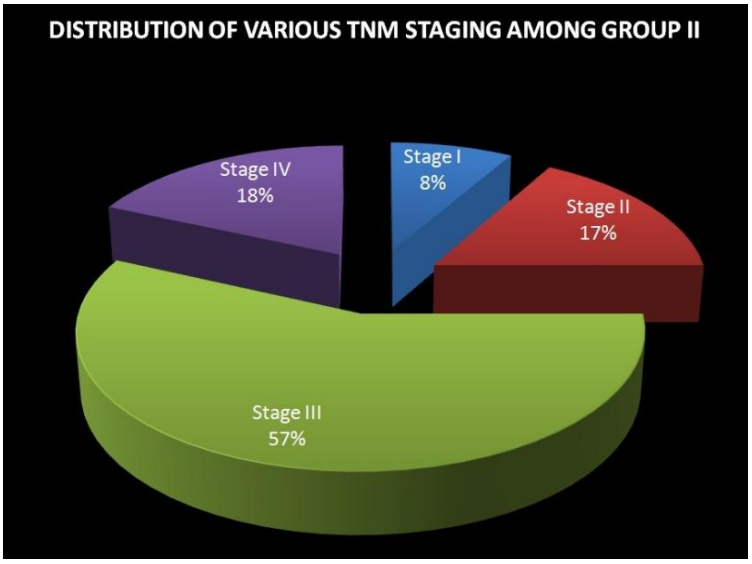

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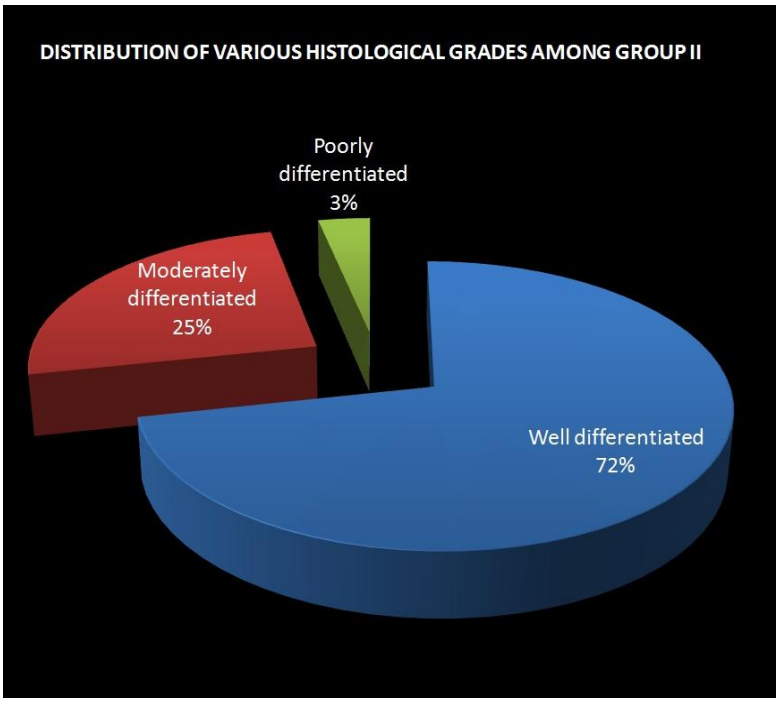

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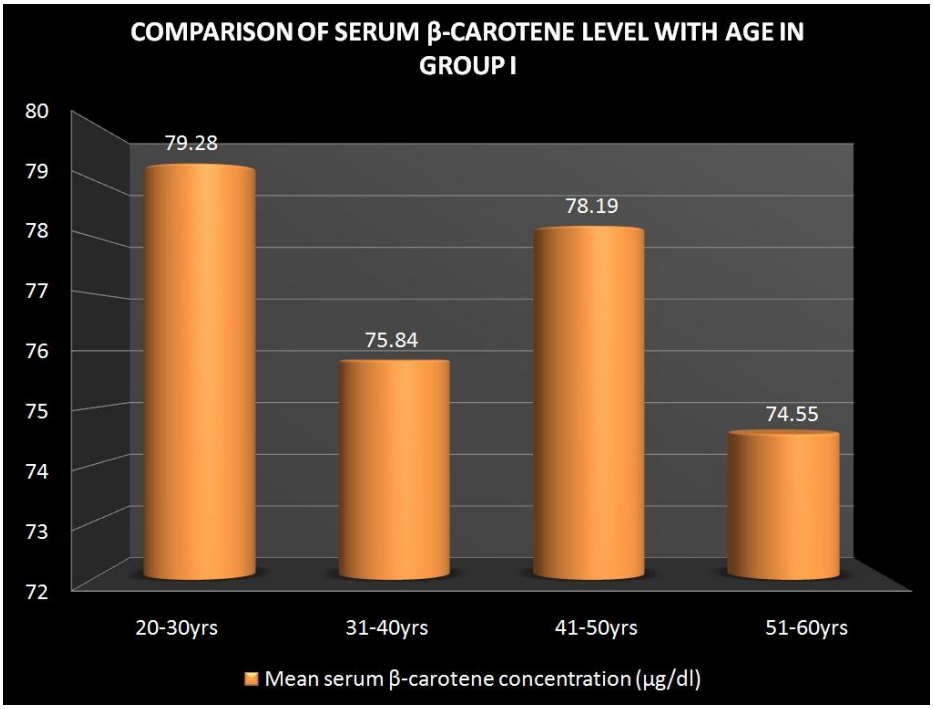

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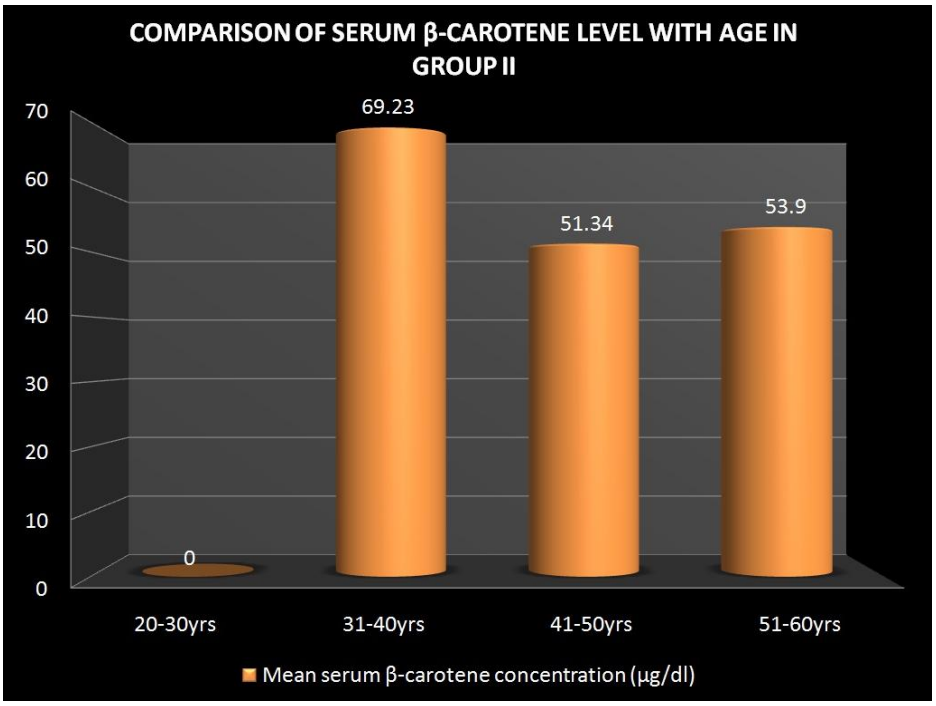

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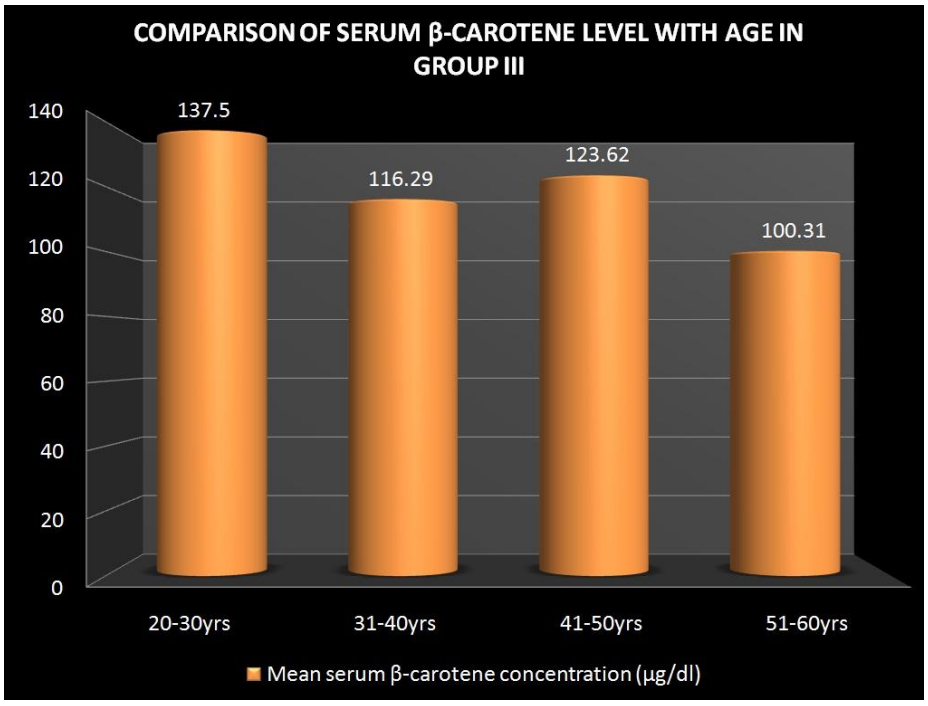

RESULTS

This clinical study was conducted among the patients attending the Department of Oral Medicine and Radiology, Tamilnadu Government Dental College and Hospital. In the present study, totally 180 cases were included. They were divided into three groups - I, II and III. Group I consisted of 60 patients with clinically diagnosed oral sub mucous fibrosis of different stages. Group II consisted of 60 patients with clinically diagnosed and histopathologically proven oral squamous cell carcinoma. Group III is the control group which consisted of 60.

Blood was collected from all the patients and Beta carotene levels were estimated by the Bradley and Hornbeck method.[72]

AGE DISTRIBUTION:

The subjects were divided into four age groups which are as follows: 20-30 years, 31-40 years, 41-50 years, 51-60 years. Among the 60 in group I, 23(39%) were between 20-30 years, 15(25%) between 31-40 and 14(23%) between 41-50 years and 8(13%) between (51-60) years. The group II comprised of 13(22%) were between 31-40 years, 18(30%) were between 41-50 years and 29(48%) were between (51-60) years. In group III, 15(25%) belonged to 20-30 years, 15(25%) belonged to 31-40years, 17(28%) belonged to 41-50 years, 13(22%) belonged to 51-60 years. [Table 1, chart 1].

SEX DISTRIBUTION:

Out of 180 patients included in this study, 141 (78.3%) were males and 39 (21.7%) were females. In Group I, 48 (80%) were males and 12 (20%) were females.

In Group II, 39 (65%) were males and 21(35%) females and in Group III, 54 (90%) were males and 6 (10%) were females. [Table 2, chart 2].

COMPARISON OF SERUM β-CAROTENE LEVEL AMONG GROUP I, II & III:

The mean concentration of serum β-carotene level among groups I, II and III were 77.54 µg/dl, 56.46 µg/dl and 120.21 µg/dl respectively. On comparing the concentration of serum β-carotene level among groups I, II and III, the results were statistically significant between all the three groups ('p' value: 0.000 – significant at 1% level).The mean serum beta carotene level of group II was lower than that of group I and group III. The mean serum beta carotene level of group I was lower than that of group III. [Table 3, chart 3].

COMPARISON OF SERUM β-CAROTENE LEVEL WITH CLINICAL STAGING IN GROUP I:

Out of the 60 OSMF patients included in the group I, 51 patients in clinical Stage II were found to have a mean serum β-carotene level of 79.51 + 11.38 µg/dl. 9 patients in Stage III OSMF had a mean serum β-carotene level of 66.32 + 19.90 µg/dl. There was a decrease in the mean serum β-carotene level when OSMF progressed from clinical Stage II to Stage III. This was found to be statistically significant ('p' value: 0.006 – significant at 1% level). [Table 4, chart 4].

COMPARISON OF SERUM β-CAROTENE LEVEL WITH FUNCTIONAL STAGING IN GROUP I:

When the mean serum β-carotene levels in different functional stages of OSMF was assessed, it was found to decrease progressively with the advancement of functional stage. The 44 patients in Stage A had a mean serum β-carotene level of 82.57 + 11.15

µg/dl. This was significantly higher than the mean levels in 12 patients in Stage B (67.14 + 8.34 µg/dl) and 4 patients in Stage C (53.30 ± 5.92 µg/dl). [Table 5, chart 5].

The difference in mean was found to be highly significant statistically between Stage A and B, and C, ('p' value: 0.000 – significant at 1% level).

The serum β-carotene level in patients under Group A also correlated with the mouth opening using Pearson's correlation co-efficient. Results showed an r value of 0.576 and p value of 0.000 which was statistically highly significant. This signifies that the serum β-carotene level in patients with OSMF varies directly with regard to the mouth opening; i.e. as the mouth opening decreased, the serum β-carotene level also decreased simultaneously. Hence, the progression of the disease process was associated with further decrease in serum β-carotene level

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COMPARISON OF SERUM β-CAROTENE LEVEL AMONG VARIOUS TNM STAGING IN GROUP II:

The mean concentration of serum β-carotene level among stage I, II, III and IV of oral squamous cell carcinoma are 63.48 ± 9.44 µg/dl, 72.88 ± 5.71 µg/dl , 55.16 ± 1.46 µg/dl and 42.31 ± 2.57 µg/dl respectively. The difference in mean was found to be highly significant statistically between Stage I and II, III and IV, the p value was 0.000. [Table 6, chart 6].

COMPARISON OF SERUM β-CAROTENE LEVEL AMONG VARIOUS HISTOLOGICAL GRADINGS IN GROUP II:

The mean concentration of serum β-carotene among well differentiated, moderately differentiated and poorly differentiated oral squamous cell carcinoma are 58.63 ± 15.17 µg/dl,53.17 ± 12.06 µg/dl and 34.29 ± 4.06 µg/dl respectively.When

the concentration of serum beta carotene among them were compared, the results were significant. ('p' value – 0.045) . [Table 7, chart 7].

DISTRIBUTION OF SERUM β-CAROTENE LEVEL WITH CLINICAL STAGING IN GROUP I:

All the three clinical stages were included under Group I (60 cases): Stage II – 51 cases (85%), Stage III – 9 cases (15%). [Table 8, chart 8].

DISTRIBUTION OF SERUM β-CAROTENE LEVEL WITH FUNCTIONAL STAGING IN GROUP I:

All the three functional stages were included under Group I (60 cases): Stage A – 44 cases (73.33%), Stage B – 12cases (20.00%) and Stage C – 4 cases (6.67). [Table 9, chart 9].

DISTRIBUTION OF VARIOUS TNM STAGING AMONG GROUP II

All the four TNM stages were included under Group II (60 cases): Stage I – 5 cases (8.3%), Stage II – 10 cases (16.7%), Stage III – 34 cases (56.7%) and Stage IV – 11 cases (18.3%).[Table 10, chart 10].

DISTRIBUTION OF VARIOUS HISTOLOGICAL GRADES AMONG GROUP II

In the present study, the three different histological grades of oral squamous cell carcinoma under group II (60 cases) were distributed as follows: Well differentiated squamous cell carcinoma - 43 cases (71.67%), Moderately differentiated squamous cell carcinoma – 15 cases (25%) and Poorly differentiated squamous cell carcinoma – 2 case (3.33%).[Table 11, chart 11].

COMPARISON OF SERUM β-CAROTENE LEVELS WITH AGE IN GROUP I

In group I subjects, the mean concentration of serum β-carotene level among four age groups (20-30, 31-40, 41-50, 51-60) were 79.28µg/dl, 75.84µg/dl ,78.19µg/dl and 74.55 µg/dl respectively. On comparing the concentration of serum β-carotene level among these age groups, the results were not statistically significant ('p' value: 0.805).[Table 12, chart 12].

COMPARISON OF SERUM β-CAROTENE LEVEL WITH AGE IN GROUP II

The mean concentration of serum β-carotene level among three age groups (31-40, 41-50, 51-60) were 69.23 µg/dl , 51.34 µg/dl and 53.90 µg/dl respectively for patients with group II situation . But no patients with group II condition belonging to age group 20-30 were reported during the study. On comparing the concentration of serum β-carotene level among these age groups, the results were statistically significant ('p' value : 0.001). The 31-40 age group showed highest mean serum β-carotene level while 41-50 group had lowest mean serum β-carotene level.[Table 13, chart 13].

COMPARISON OF SERUM β-CAROTENE LEVEL WITH AGE IN GROUP III

When serum β-carotene level were calculated among age groups, (20-30, 31-40, 41-50, 51-60) the mean value got were 137.50 µg/dl, 116.29 µg/dl, 123.62 µg/dl, 100.31 µg/dl respectively. The comparison of these mean values demonstrated a statistically significant difference among these four groups (p value: 0.015). The mean value was lowest for 51-60 age group, with highest value for 20-30 age group.[Table 14, chart 14].

DISCUSSION

Over the last few decades, a new concept involving the biological effects of highly reactive oxygen and nitrogen species in the mechanisms causing disease has filled the scientific literature. Reactive oxygen species are highly reactive molecules and have been implicated in the pathophysiology of many diseases including precancerous conditions and cancer. The protective effect in the body against these oxygen species is provided by an array of protective antioxidant mechanisms, one of which is β-carotene that prevents the production of free radicals and repairs oxidative damage. However, when the free radicals are produced in excess, there is a depletion of these antioxidants and predisposing the patient to develop disease.[1, 4]

Beta-carotene is known to act in a similar manner and help in maintaining the integrity of oral epithelium. Many studies have shown a decreased serum β-carotene in patients with pre-cancerous or cancerous condition. It has been reported that there was decreased serum β-carotene level in OSMF patients in many of the previous studies.[6,13]Beta-carotene is a micronutrient in the blood whose level seems to be affected by a multiple factors like the age, sex, lifestyle, socio-economic status, physical activity, smoking, alcohol consumption, gastric pH, dietary intake and body mass index.[63,66,67]

Oral submucous fibrosis has been regarded as a premalignant condition with a malignant transformation rate of 7.6% over a period of 17 years.[73]Worldwide estimates in 1996 indicate that 2.5 million people were affected by oral submucous fibrosis. In 2002, the statistics for OSMF from the Indian continent alone was about 5 million people (0.5% of the population of India). This indicates that the worldwide estimate will be much higher in recent times.[74]The role of the constituents of areca nut

in the pathogenesis of OSMF has been studied in detail over many years. Thechemicals in the areca nut appear to interfere with the molecular processes of deposition and/or degradation of extracellular matrix molecules such as collagen, causing imbalance in the normal process.[75]

Oral squamous cell cancer has been ranked as the sixth most common cancer in the world. It is the most common form of cancer affecting males and account for 50-70% of all cancer diagnosed in India.[76]Thenumber of patients with oral cancer is increasing gradually, especially in younger age group. The purpose of the study was to evaluate the level of serum β-carotene in patients with oral submucous fibrosis and oral squamous cell carcinoma.

Total of 180 cases which met inclusive criteria were divided into three groups - I, II and III. Group I consisted of 60 patients with clinically diagnosed oral sub mucous fibrosis. Group II consisted of 60 patients withclinically diagnosed and histopathologically proven oral squamous cell carcinoma. Group III is the control group of 60normal people. The age of the patients ranged from 20 to 60 years.The concentration of Beta Carotene present in the serum samples was determined by Bradley and Hornbeck method using a beta-carotene stock standard[72]

In this study, the serum β-carotene level was decreased in all OSMF patients as compared to thehealthy controls and also there was decreased serum β-carotene level in patients with oral squamous cell carcinoma as compared to the healthy controls. The difference in the serum β-carotene level was highly significant in these two comparisons.When compared with OSMF group, patients with Oral Squamous cell carcinoma had a significant decrease inserum β-carotene level. ($P < 0.001$).

In this study,Group Ipatients within the age range of 20 – 60 years wereincluded. More than one third of the study population (38.34%) belonged to the age group of 20 – 30 years. As the age advanced, the number of patients with OSMF was comparatively fewer. This is in agreement with the studies done by **Kumar KK et al(2007)[29], Angadi PV et al (2011)[24]**, where a major subgroup of patients were in the age range of 21-30 yrs.

The faster development of OSMF in younger age group may also be attributed to the increased mitotic potential of fibroblasts in these patients. In the younger people, the fibroblast undergoes approximately 50 cell divisions as compared to the older people where the mitotic division is restricted to only about 20[77].

Out of 180 patients included in this study, 141 (78.3%) were males and 39 (21.7%) were females. In Group I, 48 (80%) were males and 12 (20%) were females. These gender distributions in this study were consistent with the gender distributions found in the study conducted by **Pandya S et al (2009)[30], Bathi RJ et al (2009)[31], Reddy V et al (2011)[78] and Angadi PV et al (2011)[24]**, who have reported the same increased prevalence of OSMF in men.

Trismus in OSMF occurs due to the increased deposition of collagen fibers in the submucosal tissue as the name 'oral submucous fibrosis' suggests. This sign is characteristically present in all cases of OSMF included in this study and has been selected under the diagnostic criteria for OSMF. Mouth-opening has also been used as a predictor of the severity and extent of OSMF. Various authors have suggested different staging systems based on mouth openingfor OSMF[37, 29, 79].Functional staging by **Haider SM et al (2000)[37]**were followed to categorizethe patients in this study.

Results showed that out of 60 OSMF 44 patients, majority were in functional Stage A (73.33%). 12 patients (20.00%) had Stage B OSMF and only 4 patients (6.67%) were in Stage C. These findings are in accordance with the study carried out in this area of the country by **Ceena DE et al (2009)[80]** who found that 72.5% of the OSMF patients were in Stage A, 22.5% were in Stage B and only 5% of the cases were in Stage C out of 40 OSMF patients. The mild variation in the results can be attributed to the varied sample size in both the studies.

It was observed that a substantial proportion of the patients were in functional stage A. The onset of restriction of mouth opening along with burning sensation caused inability to eat food and hence was the driving factor for the patients to consult the oral medicine specialists for treatment, as bothare thecommon symptoms present in the early functional stages of the disease.

On intra oral examination, all the 60 patients in Group I presented with visible blanching and palpable fibrous bands of oral mucosa. This is consistent with the findings of other studies on OSMF by **Raina C et al (2005)[13], Haider SM et al (2000)[37],Pandya S et al (2009)[30] andAngadi PV et al (2011).[24]**

Blanching in OSMF occurs due to impairment of the local vascularity subsequent to the deposition of fibrous bands.[81]Palpable fibrous bands of the buccal mucosa could be appreciated in all the patients of OSMF in this study, which is in accordance with the studies by **Ceena DE et al (2006)[73],Pandya S et al (2009)[30] andAngadi PV et al (2011)[24]** who supported the view that buccal mucosa was the most frequently affected site.

Evidence to the contrary was presented by authors like **Pindborg JJ et al (1964)[82] and Haider SM et al (2000)[37]**who were of the view that faucial pillar is the

most commonly affected site. This could be attributed to the swallowing of the quid.[83,84]

Though buccal mucosa was the most common site involved in this study as evidenced in all 60 OSMF patients, there was no patient in clinical stage I in our study and all the patients who had buccal bands also had faucial bands. 9 patients (15%) exhibited palpable fibrous bands of labial mucosa as well. This is in accordance with the findings of **Haider SM et al (2000)**[37]who found that all those with buccal bands also had bands in the fauces.

It was observed that the fibrous bands were palpated only in the posterior part of the buccal mucosa and in faucial pillars in the initial stages of the disease process. As the condition advanced, there was involvement of the anterior part of the buccal and labial mucosa and in later stages, there was involvement of the floor of the mouth and atrophy of the uvula in 2 cases.

The fibrosis seems to begin around the pterygomandibular raphe causing varying degrees of trismus in the patient and then simultaneously progressing to the faucial pillars posteriorly and buccal mucosa and labial mucosa anteriorly. This may be attributed to the placement of gutka in the buccal vestibule, where the disease process is more likely to begin. This is in agreement with the findings of **Lemmer J et al (1967)**[25]**, Chiang CP et al (2002)**[85], who stated both buccal mucosa and faucial pillars were the most common site.

As noted in this study, majority of the patients (85%) were in Stage II OSMF, with the remaining 15% patients in Stage III. This showed that more than 75% of the study population reported for treatment only after the disease had advanced to clinical

stage II probably because of the low socioeconomic status and lack of awareness about the condition and its implications.

Supplementation with β-carotene has also been tried by investigators like **Jirge V et al (2008)**[58] who observed significant improvement in mouth opening and reduction in burning sensation after administration of antoxid tablets containing β-carotene along with other antioxidants. **Gupta S et al (2004)**found decreased serum β-carotene levels in all grades of OSMF cases (81.7+14.3 µg/dl) compared to healthy controls (110+20.8 µg/dl), with subsequent increase in the levels on β-carotene supplementation. These findings along with the observations in this study is strongly suggestive of increased risk of OSMF in association with low serum β-carotene levels.

Beta-carotene is known to be a potent antioxidant. It has been put forth that the imbalance in redox status in precancer and cancer might be due to enhanced lipid peroxidation and compromised antioxidant defenses.**Uikey AK et al (2003)**[55]found a declined antioxidant level in OSMF patients. **Subapriya R et al (2003)**[56]supported the hypothesis that enhanced lipid peroxidation was accompanied by antioxidant depletion in precancer and cancer. As Oral submucous fibrosis and oral squamous cell carcinoma has been well recognised, the findings in this study substantiates the above hypothesis.

In this study, the decrease in mean serum β-carotene in OSMF from clinical Stage II (79.51 + 11.38 µg/dl) to Stage III (66.32 + 19.90 µg/dl) was found to be statistically significant ($p = 0.006$). Comparison with Stage I could not be carried out as there were no patients in this study. This is in accordance with the findings of **Ashish et al (2011).**[86]

The correlation of serum beta-carotene level with the mouth opening showed high statistical significance indicating the progression of OSMF with decrease in mouth opening.

The subject who had the lowest serum β-carotene level of 45.32 µg/dl seemed to have an advanced OSMF condition with a mouth opening of only 9 mm. This also suggests that the serum β-carotene level decreases as OSMF progresses.

The results of this study also showed that the mean serum β-carotene level in OSMF patients decreased progressively with the advancement from functional stage A (82.57+11.15µg/dl) to Stage B (67.14+ 8.34 µg/dl) and further decreased in Stage C (53.30 +5.92 µg/dl). This decrease was statistically significant ($p < 0.001$) among three stages. However, the decrease in serum β-carotene level from Stage B to Stage C was not statistically significant (p value > 0.05). This could be attributed to the small sample size as evidenced by Stage C comprising of only four patients.

The study included 60 patient of oral squamous cell carcinoma. In group II patients were in the age range of 20- 60 years. Almost half of the study population (48.33%) was found to be in the age group of 51- 60 years. In group III, 28.33% were in the age range of 41-50.

In group II 39(65%) were males and 21 (35%) were females. Males were found to be more prevalent. In group III 54(90%) were males and 6(10%) were females.

In Group II, majority of patient 34(56.7%) were in stage III TNM staging. Further there was a significant decrease in the concentration of serum beta carotene

among patients with TNM stage II, III, IV with highest mean value for stage II(72.88 µg/dl) and lowest for stage IV(42.31 µg/dl) (Significant at 1% level). TNM stage I (63.48 µg/dl)and stage III (55.16 µg/dl) had serum beta carotene level values in between. But there was no significant difference between stage I and stage II. (P value is 0.475) This could be due to reduced sample size and other variables such as lifestyle, dietary intake, socio economic status, alcohol consumption and smoking status.

In the present study 43 (72%) were in well differentiated squamous cell carcinoma based on histopathology. Further, we noted a significant decrease in the concentration of serum beta carotene among patients with histological grading in the advanced stages. Well differentiated had highest mean value of 58.63 µg/dl, poorly differentiated showed lowest mean value of 34.29 µg/dl and moderately differentiated group had a mean value in between (53.17µg/dl).There was a significant difference among these groups at 5% level.

An antioxidant [87] is a molecule capable of slowing or preventing the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals, which start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions by being oxidized themselves. As a result, antioxidants are often reducing agents such as thiols, ascorbic acid or polyphenols.

Although oxidation reactions are crucial for life, they can also be damaging; hence, plants and animals maintain complex systems of multiple types of antioxidants, such as glutathione, vitamin C, and vitamin E as well as enzymes such

as catalase, superoxide dismutase and various peroxidases. Low levels of antioxidants, or inhibition of the antioxidant enzymes, causes oxidative stress and may damage or kill cells.

As oxidative stress might be an important part of many human diseases, the use of antioxidants in pharmacology is intensively studied, particularly as treatments for stroke and neurodegenerative diseases. However, it is unknown whether oxidative stress is the cause or the consequence of disease. Antioxidants are also widely used as ingredients in dietary supplements in the hope of maintaining health and preventing diseases such as cancer and coronary heart disease. Epidemiologists studying both diet and serum levels of beta-carotene,observed that high levels of beta-carotene, a precursor to vitamin A, were associated with a protective effect, reducing the risk of cancer.

The search for treatments that can cause reversal of suppression of pre malignancy constitutes an important strategy for overall prevention of cancer. Antioxidant micronutrients found in diet have been shown in numerous studies to be protective against oral cancer. There is limited information on the relationship between blood antioxidant micronutrient levels and pre cancer, oral cancer.

Carotenoids are natural compounds with lipophilic properties, greater than 500 different compounds have been identified and β-carotene is the most prominent among them. β-carotene contains an extended system of conjugated double bonds which is responsible for its antioxidant activity. Epidemiological studies in humans have suggested that β-carotene aids in cancer prevention. β-carotene also helps to reverse the field cancerization defect in the epithelium at risk for oral cancer.

β-carotene suppresses micronuclei in exfoliated oral mucosal cells for subjects at risk for Oral cancer. Studies showed that regular intake of β-carotene considerably decreases the risk of malignant transformation of oral premalignant conditions and lesions.[88-92]

The control group was selected with the inclusion of individuals without any systemic diseases because wide range of systemic disease is thought to contribute to the development of oxidative stress. The antioxidant levels will be reduced during oxidative stress, the systemic diseases include Alzheimer's disease as stated by **Christen (2000)**[93], Parkinson's disease as stated by **Wood et al (2006)**[94], diabetes as stated by **Davi et al (2005)**[95], motor neuron diseases as stated by **Cookson (1999)**[96], cardiovascular disease as stated by **Aviram (2000)**[97]and general tissue damage as stated by **Rhee (2006)**[98]**.**

Serge Hereberg in 2005[99]stated that a combination of antioxidants including β-carotene, vitamin C & vitamin E, at doses achievable through the diet, may have protective effects on mortality rates and on the total number of cancers among apparently healthy men, with no evident increase in cancer risk.

The analysis of thisstudy suggested that the level of β-carotene decreased asprecancerous and cancerous conditionsprogress into the advanced stages and its estimation can be useful to monitor the oxidative stress in these cases for better management. Increased dietary intake of beta carotene can probably provide a productive effect against OSMF and prevent further progress into cancer. Also,pharmacological supplementation of β carotene can be tried as a treatment modality in subjects who have already developed precancerous and cancerous conditions.

SUMMARY AND CONCLUSION

In the present study, totally 180 cases were included. They were divided into three groups - I, II and III. Group I consisted of 60 patients with clinically diagnosed oral sub mucous fibrosis of different stages. Group II consisted of 60 patients withclinically diagnosed and histopathologically proven oral squamous cell carcinoma. Group III is the control group which consisted of 60.The age of the patients ranged from 20 to 60 years. Both male and female were included under the study. None of the lesions were treated in any manner prior to sample collection.

Informed consent was obtained from all the patients prior to the investigation. Thorough clinical examination was done.Biopsy was performed for all the cases of oral squamous cell carcinoma diagnosis were established based on clinical and histopathological findings.All the findings were recorded on the structured proforma.

The Blood samples were collected and all samples were centrifuged and serum samples wereseparated. Then the serum samples were frozen at -20 degree Celsius until analysis. The concentration of Beta Carotene present in the serum samples was determined byBradley and Hornbeck method.[72]

The results of our study revealed a significant decrease in the concentration of serum β-carotene in oral squamous cell carcinoma patients than oral submucous fibrosis patients and healthy controls. Also, a significant decrease in concentration of serum β-carotene was noted in oral submucous fibrosis patients as compared with healthy controls but it was significantly higher when compared with oral squamous cell carcinoma patients. Oral submucous fibrosis was more commonly observed in younger age group of 20-30 years. It affected the males more predominantly. Majority

of the patients belonged to clinical stage II and functional stage A. In addition to this, a significant decrease in concentration of serum β-carotene was noted between TNM stage II and stage III, stage IV & different histological grading of oral squamous cell carcinoma.

From the present study, we conclude that Beta carotene plays an important role in pathogenesis of oral submucous fibrosis and oral squamous cell carcinoma, and that its level decreases with disease progression. These study findings suggest that a significant increase in the oxidative stress with concomitant decrease in the antioxidant enzymes in OSMF, OSCC patients. There is increased production of ROS by cancer cells and also suppression of antioxidant system.This oxidant–antioxidantimbalance is thought to be one of the factorswhich may be responsible for carcinogenesis and tumorgrowth and invasion. Thus maintenance of balancebetween the oxidant and antioxidants by appropriatetherapy may be of some help to limit the progression ofprecancerous condition towards malignancy. Further longitudinal studies with increased sample size are required to substantiate the role of Beta carotene level in precancerous condition and malignancy.

BIBLIOGRAPHY

1. Denisov ET, Afanas'ev IB. Oxidation and antioxidants in organic chemistry and biology. CRC: Taylor and Francis group;2005
2. Rodrigo R. Oxidative stress and antioxidants: their role in human disease. Nova Science Publishers:New York;2009
3. Irshad. M and Chaudhari. P. S. Oxidant-Antioxidant system: Role and significance in human body. Indian journal of experimental biology: 2002:40, 1233 – 1239.
4. Percival M. Antioxidants. Clin Nutr Insights 1998;31:1-4.
5. Thompson JL, Manore MM, Vaughan LA. The science of nutrition. 2nd ed. Pearson: Benjamin Cummings;2011.
6. Gupta S, Reddy MV, Harinath BC. Role of oxidative stress and antioxidants in aetiopathogenesis and management of oral submucous fibrosis. Ind J Clin Biochem 2004;19(1):138-141.
7. Warnakulasuriya.S. Global epidemiology of oral and oropharyngeal cancer. Oral Oncol. 2009 Apr-May;45(4-5):309-316.
8. Sankaranarayanan R. Oral cancer in India: an epidemiologic and clinical review. Oral Surg Oral Med Oral Pathol. 1990 Mar;69(3):325-30.
9. Holmes JD, Dierks EJ, Homer LD, Potter BE. Is detection of oral and oropharyngeal squamous cancer by a dental health care provider associated with a lower stage at diagnosis? J Oral Maxillofac Surg. 2003 Mar;61(3):285-91.
10. Gibanannda Ray and Syed Akhtar Husain, Oxidants, Antioxidants and Carcinogenesis. Indian journal of experimental biology: 2002: 40, 1213 – 1232.

11. Stahelin HB, et al. Plasma Antioxidant Vitamins and Subsequent Cancer Mortality in the 12-year Follow-up of the Prospective Basel Study. American Journal of Epidemiology, 133(8), April 15, 1991, p. 766-775.
12. George. E. Kaugars et al, Use of antioxidant supplements in the treatment of human oral leukoplakia. Oral surgery, Oral medicine, Oral pathology: 1996:81(1), 5 – 14.
13. Raina C, Raizada RM, Chaturvedi VN, Harinath BC, Puttewar MP, Kennedy AK. Clinical profile and serum beta carotene levels in oral submucous fibrosis. Ind J Otolaryngol Head Neck Surg 2005 Jul-Sep;57(3):191-195.
14. Barry Halliwell and Mathew Whiteman, Measuring reactive species and oxidative damage in vivo and in cell culture: How should you do it and what do the results mean? British journal of pharmacology: 2004:142, 231-255.
15. Helmutsies and Wilhelm Stahl, Vitamins E and C, β-carotene and other carotenoids as antioxidants. American journal of clinical nutrition: 1995: 62(suppl), 1315s – 1321s.
16. World Health Organization. Report of a meeting of investigators on the histological definition of precancerous lesions. Geneva: World Health Organization, 1973, Can/731.
17. Kramer IR, Lucas RB, Pindborg JJ, Sobin LH. Definition of leukoplakia and related lesions: an aid to studies on oral precancer. Oral Surg Oral Med Oral Pathol. 1978 Oct;46(4):518-39
18. S. Warnakulasuriya, Newell. W. Johnson, I. Van der Waal. Nomenclature and classification of potentially malignant disorders of the oral mucosa. J Oral Pathol Med 2007; 36: 575-580.

19. Pindborg JJ, Sirsat SM. Oral submucous fibrosis. Oral Surg Oral Med Oral Pathol 1966;22(6):764-779.
20. Shah N, Kumar R, Sing MK. J Ind Dent Assoc 1993;64(12):383-388.
21. Rajendran R. Oral submucous fibrosis: etiology, pathogenesis and future research. WHO Bulletin OMS 1994;72(6):985-996.
22. Joshi SG. Submucous fibrosis of the palate and pillars. Ind J Otolaryngol 1952;4:1-4.
23. Su IP. Idiopathic scleroderma of the mouth: report of three cases. Arch Otolaryngol 1954;59:330-332
24. Angadi PV, Rekha KP. Oral submucous fibrosis: a clinicopathologic review of 205 cases in Indians. Oral Maxillofac Surg 2011;15:15-19.
25. Lemmer J, Shear M. Oral submucous fibrosis: a possible case in a person of Caucasian descent. Br Dent J 1967;343-6.
26. Abrol BM. Clinicopathological, biochemical and immunological studies of idiopathic oral fibrosis. The Bombay hospital 1977; 19(1):50-60.
27. Ahmad MS, Ali SA, Ali AS, Chaubey KK. Epidemiological and etiological study of oral submucous fibrosis among gutkha chewers of Patna, Bihar, India. J IndSocPedodPrev Dent 2006 Jun:84-9.
28. Hayes ML, Richards AM. Oral submucous fibrosis in pediatric patients. Int J Pediatr Dentistry 2006;16(Suppl 1):3-4.
29. Kumar KK, Saraswathi TR, Devi UM, Elizabeth J. Oral submucous fibrosis: a clinic-pathological study in Chennai. Ind J Dent Res 2007;18(3):106-11.
30. Pandya S, Chaudhary AK, Singh M, Singh M, Mehrotra R. Correlation of histopathological diagnosis with habits and clinical findings in oral submucous fibrosis. Head & Neck Oncol 2009;1:10

31. Bathi RJ, Parveen S, Burde K. The role of gutka chewing in oral submucous fibrosis: A case-control study. Quintessence Int 2009;40:e19-e25.
32. Shirzaii M. Oral submucous fibrosis in a 15 year old boy: the first case report in Iran. Shiraz Univ Dent J 2011;11(Suppl):51-5.
33. Gupta PC, Ray CS. Epidemiology of betel quid usage. Ann Acad Med Singapore 2004;33(Suppl):31S-36S.
34. Pindborg JJ, Murti PR, Bhonsle RB, Gupta PC, Daftary DK, Mehta FS. Oral submucous fibrosis as a precancerous condition. Scand J Dent Res 1984 Jun;92(3):224-9.
35. Betel-quid and areca-nut chewing and some areca-nut-derived nitrosamines. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans (2003:Lyon, France) available from:
http://monographs.iarc.fr/ENG/Monographs/vol85/mono85.pdf
36. Seedat HA, Van Wyk CW. Betel nut chewing and submucous fibrosis in Durban. S Afr Med J 1988;74:568-571.
37. Haider SM, Merchant AT, Fikree FF, Rahbar MH. Clinical and functional staging of oral submucous fibrosis. Br J Oral Maxillofac Surg 2000;38:12-15.
38. Hazarey VK, Erlewad DM, Mundhe KA, Ughade SN. Oral submucous fibrosis: study of 1000 cases from central India. J Oral Pathol Med 2007;36:12-17
39. Javed F, Chotai M, Mehmood A, Almas K. Oral mucosal disorders associated with habitual gutka usage: a review. Oral Surg Oral Med Oral Pathol Oral RadiolEndod 2010;109:857-64.
40. Mehrotra R, Pandya S, Chaudhary AK, Kumar M, Singh M. Prevalence of oral pre-malignant and malignant lesions at a tertiary level hospital in Allahabad, India. Asian Pac J Cancer Prev. 2008;9(2):263-265.

41. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. CA Cancer J Clin. 2005;55(2):74-108
42. Kalyani R, Das S, Kumar ML. Pattern of cancer in adolescent and young adults--a ten year study in India. Asian Pac J Cancer Prev. 2010;11(3):655-9.
43. C. La Vecchia, A. Tavani, S. Franceschi, F. Levi, G. Corrao, E. Negri. Epidemiology and prevention of oral cancer. Oral Oncol 1997; 33: 302–312.
44. W.J. Blot, J.K. McLaughlin and D.M. Winn et al. Smoking and drinking in relation to oral and pharyngeal cancer. Cancer Res. 1988; 48: 3282–3287.
45. E. Petridou, A.I. Zavras and D. Lefatzis, The role of diet and specific micronutrients in the etiology of oral carcinoma. Cancer 2002; 94: 2981–2988.
46. R. Herrero, X. Castellsague and M. Pawlita et al. Human papillomavirus and oral cancer: the International Agency for Research on Cancer multicenter study. J Natl Cancer Inst 2003; 95: 1772–1783.
47. Pham-Huy LA, He H, Pham-Huy C. Free radicals, antioxidants in disease and health. Int J Biomed Sci 2008;4(2):89-96.
48. Levin G, Yeshurun M, Mokady S. In vivo antiperoxidative effect of 9-cis b-carotene compared with that of the all-trans isomer. Nutr Cancer 1997;27:293-7.
49. Human nutrition: Beta-Carotene. Products for the Dietary Supplement, Beverage and Food Industries – Technical Information. BASF- the chemical company, June 2007.
50. Milbury PE, Richer AC. Understanding the antioxidant controversy: scrutinizing the “fountain of youth”. Praeger:London;2008.
51. Rathore GS, Suthar M, Pareek A, Gupta RN. Nutritional antioxidants: a battle for better health. J Natural Pharmaceuticals 2011;2(1):2-14

52. Maserejian NN, Giovannucci E, Rosner B, Zavras A, Joshipura K. Prospective study of fruits and vegetables and risk of oral premalignant lesions in men. Am J Epidemiol 2006;164:556-66.
53. Collins AR. Antioxidant intervention as a route to cancer prevention. Eur J Cancer 2005;41:1923-30.
54. Babu S, Bhat RV, Kumar PU, Sesikaran B, Rao KV, Aruna P, Reddy PR. A comparative clinico-pathological study of oral submucous fibrosis in habitual chewers of pan masala and betelquid. J Toxicol Clin Toxicol 1996;34(3):317-22.
55. Uikey AK, Hazarey VK, Vaidhya SM. Estimation of serum antioxidant enzymes superoxide dismutase and glutathione peroxidase in oral submucous fibrosis: a biochemical study. J Oral Maxillofac Pathol 2003;7:44-5.
56. Subapriya R, Kumaraguruparan R, Nagini S, Thangavelu A. Oxidant-antioxidant status in oral precancer and oral cancer patients. ToxicolMech Methods 2003 Jan-Mar;13(1):77-81.
57. Metkari SB, Tupkari JV, Barpande SR. An estimation of serum malondialdehyde, superoxide dismutase and vitamin A in oral submucous fibrosis and its clinicopathologic correlation. J Oral Maxillofac Pathol 2007 Jan-Jun;11(1):23-27.
58. Jirge V, Shashikanth MC, Ali IM, Anshumalee N. Levamisole and antioxidants in the management of oral submucous fi brosis: a comparative study. J IndAcad Oral Med Radiol 2008;20(4):135-40.
59. Shariff AK, Patil SR, Shukla SP, Sontakke AV, Hendre AS, Gudur AK.Effect of oral antioxidant supplementation on lipid peroxidation duringradiotherapy in head and neck malignancies.Ind J Clin Biochem 2009;24(3):307-311.

60. Ching S, Ingram D, Hahnel R, Beilby J, Rossi E. Serum levels of micronutrients, antioxidants and total antioxidant status predict risk of breast cancer in a case control study. J Nutr 2002;132:302-6.
61. Bathi RJ, Rao R, Mutalik S. GST null genotype and antioxidants: Risk indicators for oral pre-cancer and cancer. Ind J Dent Res 2009;20(3):298-303.
62. Block, G. et al. Fruit, Vegetables, and Cancer Prevention: A Review of the Epidemiological Evidence. Nutr Cancer 1992;18(1):1-29
63. Tang G, Serfaty-Lacrosniere C, Camilo ME, Russell R. Gastric acidity influences the blood response to a beta-carotene dose in humans. Am J Clin Nutr 1996;64:622-626.
64. Berg G, Kohlmeier L, Brenner H. Use of oral contraceptives and serum beta-carotene. Eur J Clin Nutr1997;51:181-7.
65. Patrick L. Beta-carotene: the controversy continues. Altern Med Rev 2000 Dec;5(6):530-45.
66. Kitamura Y, Tanaka K, Kiyohara C, Hirohata T, Tomita Y, Ishibashi M et al. Relationship of alcohol use, physical activity and dietary habits with serum carotenoids, retinol and alpha-tocopherol among male Japanese smokers. Int J Epidemiol 1997 Apr;26(2):307-14.
67. Galan P, Viteri FE, Bertrais S, Czernichow S, Faure H, Arnaud J et al. Serum concentrations of β-carotene, vitamins C and E, zinc and selenium are influenced by sex, age, diet, smoking status, alcohol consumption and corpulence in a general French adult population. Eur J Clin Nutr 2005 Oct;59(10):1181-90.
68. Gabriel HE, Liu Z, Crott JW, Choi SW, Song BC, Mason JB. A comparison of carotenoids, retinoids, and tocopherols in the serum and buccal mucosa of chronic

cigarette smokers versus nonsmokers. Cancer Epidemiol Biomarkers Prev 2006;15(5):993-9.

69. Nierenberg DW, Stukel TA, Baron JA, Dain BJ, Greenberg RE. Determinants of plasma levels of beta-carotene and retinol. Am J Epidemiol 1989;130 (3):511-21.
70. Comstock GW, Menkes MS, Schober SE, Vuilleumier JP, Helsing KJ. Serum levels of retinol, beta-carotene, and alpha-tocopherol in older adults. Am J Epidemiol 1988; 127(1):114-23.
71. Chetan J Bhadage; Hemant R Umarji; Karan Shah; HannamariVälimaa. Vasodilator isoxsuprine alleviates symptoms of oral submucous fibrosis. Clinical oral investigations Aug 2012.
72. Daniel W .Bradley, Cecil L. Hornbeck .A clinical evaluation of an improved TFA micro method for plasma and serum vitamin A. Biochemical medicine 1973;7,78-86.
73. Murti PR, Bhonsle RB, Pindborg JJ, Daftary DK, Gupta PC, Mehta FS. Malignant transformation rates in oral submucous fibrosis over a 17 year period. Community Dent Oral Epidemiol 1985;13:340-1.
74. Chiu CJ, Chang ML, Chiang CP, Hahn LJ, Hsieh LL, Chen CJ. Interaction of collagen-related genes and susceptibility to betel quid-induced oral submucous fibrosis. Cancer Epidemiology, Biomarkers and Prevention 2002;11:646-53.
75. Tilakaratne WM, Klinikowski MF, SakuT,Peters TJ, Warnakulasuriya S. Oral submucous fibrosis: Review on aetiology and pathogenesis. Oral Oncol 2006;42:561-8.
76. S Gokul, VS Patil, R Jailkhani, K Hallikeri, KK Kattappagari.Oxidant–antioxidant status in blood and tumor tissue of oral squamouscell carcinoma patients. Oral Diseases (2010) 16, 29–33.

77. Nanci A. Tencate's oral histology: development, structure, and function. 7th ed. Mosby Publishers;2009.
78. Reddy V, Wanjari PV, Banda NR, Reddy P. Oral Submucous Fibrosis: Correlation of clinical grading to various habit factors. Int J Dental Clinics 2011:3(1):21-4.
79. Lai DR, Chen HR, Lin LM, Huang YL, Tsai CC. Clinical evaluation of different treatment methods for oral submucous fibrosis: a 10-year experience with 150 cases. J Oral Pathol Med 1995;24:402-6.
80. Ceena DE, Bastian TS, Ashok L, Annigeri RA. Comparative study of clinicofunctional staging of oral submucous fibrosis with qualitative analysis of collagen fibres under polarizing microscopy. Indian J Dent Res 2009;20(3):271-6.
81. Mehta FS and Hammer JE. Tobacco related oral mucosal lesions and conditions in India. Basic dental research unit - Tata Institute of Fundamental Research. Bombay:57-5.
82. Pindborg JJ and Singh B. Formation of vesicles in oral submucous fibrosis. Acta Pathol MicrobiolScand 1964;62:562-6.
83. Bhonsle RB, Murti PR, Daftary DK, Gupta PC, Mehta FS, Sinor PN et al. Regional variations in oral submucous fibrosis in India. Community Dent Oral Epidemiol 1987;15:225-9.
84. Johnson NW, Maher R, Trivedy C, Warnakulasuriya S. The clinical condition and pathology of oral submucous fibrosis. Oral Dis 1997;3:278-9.
85. Chiang CP, Hsieh RP, Chen TH, Chang YF, Liu BY, Wang JT. High incidence of autoantibodies in Taiwanese patients with oral submucous fibrosis. J Oral Pathol Med 2002;31:402-9.

86. AshishAggarwal,ArvindShetti,VaishaliKeluskar,AnjanaBagewadi.Estimation of serum beta carotene levels in patients with oral submucous fibrosis in india.Journal of Oral Science,Vol.53,No.4,427-431,2011.
87. Serge Hercber**g,** The history of β-carotene and cancers; From observational to intervention studies. What lessons can be drawn for future resea4ch on polyphenols. American journal of clinical nutrition: 2005:81(suppl), 218s – 222s.
88. Hans. F. Stich et al, Remission of precancerous lesions in the oral cavity of tobacco chewers and maintenance of the protective effect of β-carotene or Vitamin A. American journal of clinical nutrition: 1991: 53, 298s – 304s.
89. Anthony. T. Diplock, Safety antioxidant vitamins and β-carotene, American journal of clinical nutrition; 1998: 62(suppl), 1510s – 1516s.
90. Harinder. S. Garewal, Potential role of β-carotene in prevention of Oral cancer. American journal of clinical nutrition: 1991: 53, 294s – 297s
91. Helmutsies and Wilhelm Stahl, Vitamins E and C, β-carotene and other carotenoids as antioxidants. American journal of clinical nutrition: 1995: 62(suppl), 1315s – 1321s.
92. Geert Van Poppel et al**,**Epidemologic evidence for β-carotene and cancer prevention. American journal of clinical nutrition: 1995:62(suppl) 1393s – 1402s.
93. Christian. Y,"Oxidative stress and Alzhelmiers disease". Am J Clin Nutr. 71(2): 621s-629s. Feb, 2000.
94. Wood-Kaczmar. A, Gandhi. S, Wood. N."Understanding the molecular causes of Parkinsons disease". Trends Mol Med, 12(11): 521-8; 2006.
95. Davi. G, Falco. A, Patrono. C,"Lipid peroxidation in diabetes mellitus", Antioxid Redox Signal, 7 (1-2); 256-68; 2005.

96. Cooksman. M, Shaw. P, “Oxidative stress and motor neurone disease”. Brain Pathol 9(1): 165-86, 2004.
97. Aviram. M, “Review of human studies on oxidative damage and antioxidant protection related to cardiovascular diseases”. Free Radic Res 33 suppl: S85-97; 119-1279, 2000.
98. Rhee. S. G. “Cell signaling. H2O2, a necessary evil for cell signaling”. Science (journal) 312 (5782): 1882-3; 2006.
99. Serge Hercberg, The history of β-carotene and cancers; From observational to intervention studies. What lessons can be drawn for future resea4ch on polyphenols. American journal of clinical nutrition: 2005:81(suppl), 218s – 222s.

INSTITUTIONAL ETHICAL COMMITTEE
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Telephone No: 044 2534 0343
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R.C.No. 0430/DE/2010

Date: 07-03-2012

Title of the Work: **"ESTIMATION OF SERUM BETA CAROTENE IN PATIENTS WITH ORAL SUB MUCOUS FIBROSIS, ORAL SQUAMOUS CELL CARCINOMA AND HEALTHY CONTROLS"**

Principal Investigator: Dr. P. Vijayachandar, P.G. II year student

Department: Department of Oral Medicine and Radiology

The request for an approval from the Institutional Ethical Committee (IEC) was considered for the following on the IEC meeting held on **25-01-2012** at the Principal's Chambers, Tamil Nadu Government Dental College & Hospital, Chennai-3.

"Advise to proceed with the study"

The Members of the Committee, the Secretary and the Chairman are pleased to approve the proposed work mentioned above, submitted by the Principal Investigator.

The Principal Investigator and their team are directed to adhere the guidelines given below:

1. You should get detailed informed consent from the patients/participants and maintain confidentiality.
2. You should carry out the work without detrimental to regular activities as well as without extra expenditure to the Institution or Government.
3. You should inform the IEC in case of any change of study procedure, site and investigation or guide.
4. You should not deviate from the area of work for which you have applied for ethical clearance.
5. You should inform the IEC immediately in case of any adverse events or serious adverse reactions. You should abide to the rules and regulations of the Institution.
6. You should complete the work within the specific period and if any extension of time is required. You should apply for permission again and do the work.
7. You should submit the summary of the work to the ethical committee on completion of the work.
8. You should not claim funds from the Institution while doing the work or on completion.
9. You should understand that the members of IEC have the right to monitor the work with prior intimation.
10. Your work should be carried out under the direct supervision of your Guide/Professor.

Sd/ Vijayachandar 07/03/12
SECRETARY

07/3/12
CHAIRMAN

STUDY PROFOMA

ESTIMATION OF SERUM BETA CAROTENE IN PATIENTS WITH ORAL SUB MUCOUS FIBROSIS, ORAL SQUAMOUS CELL CARCINOMA

Date: Serial no:

Name: O.P No:

Age/Sex:

Address:

Phone no:

Occupation: Income:

Religion:

Presenting complaint with duration:

Past medical history:

Past Surgical history:

Past dental history:

Personal history:

A) Diet:

B) Teeth cleaning habits:

- Cleaning aids used:
- Frequency :

C) Smoking habit:
- Material used:
- Frequency :
- Duration of the habit:

D) Chewing habit:
- Material used:
- Frequency :
- Duration of the habit:

E) Other habits (alcohol, snuff):

Marital status:

Family history:

CLINICAL EXAMINATION

General examination:
- Appearance:
- Build and stature:
- Nutritional status:
- Any deformity:
- Temperature:
- Pulse:
- Blood pressure:

- Height & weight:
- Body Mass Index:
- Icterus: Present / Absent
- Clubbing of fingers: Present / Absent
- Cyanosis: Present / absent
- Lymph Node Examination:

Local examination:

Extraoral examination:

On Inspection:

On Palpation:

Intraoral examination:

- Mouth opening (Inter incisal distance):
- Teeth:

- Gingiva and alveolar mucosa:

- Labial and buccal mucosa:

- Hard and soft palate, Uvula:

- Tongue:

- Floor of the mouth:

- Retromolar trigone:

Clinical diagnosis:

Investigations:

1) Laboratory investigations:

A) Blood:
Total WBC count: Erythrocyte sedimentation rate:
Differential count: Bleeding time:
Haemoglobin %: Clotting time:
Peripheral smear:

B) Urine:
Glucose:
Albumin:

2) Radiological Examination:

3) Histological analysis:

Final diagnosis:

INFORMED CONSENT FORM

STUDY TITLE:

ESTIMATION OF SERUM BETA CAROTENE IN PATIENTS WITH ORAL SUB MUCOUS FIBROSIS, ORAL SQUAMOUS CELL CARCINOMA AND HEALTHY CONTROLS

Name: O.P.No:

Address: Serial No:

Age / Sex:

Tel. no:

I, _____age____years
Exercising my free power of choice, hereby give my consent to be included as a participant in the study "ESTIMATION OF SERUM BETA CAROTENE IN PATIENTS WITH ORAL SUB MUCOUS FIBROSIS, ORAL SQUAMOUS CELL CARCINOMA AND HEALTHY CONTROLS".

I agree to the following:

- I have been informed to my satisfaction about the purpose of the study and study procedures including investigations to monitor and safeguard my body function.
- I agree to use my serum sample for the study.
- I agree to cooperate fully and to inform my doctor immediately if I suffer any unusual symptom.
- I have informed the doctor about all medications I have taken in the recent past and those I am currently taking and other systemic illness that I have.
- I agree to report to the doctor for a regular follow-up as and when required for the research.
- I hereby give permission to use my medical records for research purpose. I am told that the investigating doctor and institution will keep my identity confidential.

Name of the patient Signature / Thumb impression

Name of the investigator Signature Date

ஒப்புதல் படிவம்

ஆராய்ச்சியின் தலைப்பு

வாயில் வரும் வாய் இறுக்குநோய், வாய்புற்றுநோய் மற்றும் நோயற்ற நபர்களின் குருதி ஊநீரில் உள்ள பீட்டா – கரோட்டினின் அளவை அறியும் ஆராய்ச்சி

பெயர் : புற நோயாளி எண்:
முகவரி : எண் :
வயது/ பால்:

தொலைபேசி எண் :

நான் ..... வயது ..... வருடம்

எனது முழுமனதுடன் வாயில் வரும் ''வாய் இறுக்குநோய், வாய்புற்றுநோய் மற்றும் நோயற்ற நபர்களின் குருதி ஊநீரில் உள்ள பீட்டா – கரோட்டினின் அளவை அறியும் ஆராய்ச்சி''யில் பங்கு பெற சம்மதிக்கிறேன்.

நான் கீழ்கண்டவற்றுக்கு சம்மதிக்கிறேன்

- நான் இந்த ஆராய்ச்சியின் நோக்கம் மற்றும் ஆராய்ச்சியின் முறைகள் பற்றி முழுமையாக தெரிவிக்கப்பட்டுள்ளேன்.
- நான் என் குருதி ஊநீரை ஆராய்ச்சிக்குப் பயன்படுத்த ஒப்புதல் அளிக்கிறேன்.
- நான் என்னுடைய மருத்துவருக்கு முழு ஒத்துழைப்பு தர சம்மதிக்கிறேன். நான் ஏதேனும் உடல் அசௌகரியம் ஏற்பட்டால் மருத்துவரிடம் உடனடியாகத் தெரிவிப்பேன்.
- நான் மருத்துவரிடம் இதற்கு முன்னர் மற்றும் தற்போது உட்கொள்ளும் மருந்துகள் பற்றியும், எனக்கு உள்ள மற்ற வியாதிகள் பற்றியும் முழுமையாகத் தெரிவிப்பேன்
- நான் ஆராய்ச்சியின் தன்மைக்கேற்ப தேவைப்படும் நேரத்தில் மருத்துவரைத் தொடர்புக் கொள்வேன்.
- நான் என் மருத்துவப் படிவங்களை ஆராய்ச்சிக்காக உபயோகப் படுத்த அனுமதிக்கிறேன். நான் மருத்துவர் மற்றும் மருத்துவமனை என்னுடைய அடையாளத்தை ரகசியமாக வைப்பார்கள் என்று கூறப்பட்டுள்ளேன்.

.....	.....	.....
நோயாளியின் பெயர்	கையொப்பம்/கைரேகை	தேதி
.....	.....	.....
ஆராய்ச்சியாளரின் பெயர்	கையொப்பம்	தேதி

INFORMATION SHEET

- We are conducting a study on estimation of serum beta carotene level in patients with oral sub mucous fibrosis, Oral squamous cell carcinoma and healthy controls. For that study, we are selecting patients.
- The purpose of this study is to estimate the level of serum beta carotene level in patients with oral sub mucous fibrosis, Oral squamous cell carcinoma and healthy controls.
- The identity of the patients participating in the research will be kept confidential throughout the study. In the event of any publication or presentation resulting from the research, no personally identifiable information will be shared.
- Taking part in the study is voluntary. You are free to decide whether to participate in the study or to withdraw at any time; your decision will not result in any loss of benefits to which you are otherwise entitled.
- The results of the special study may be intimated to you at the end of the study period or during the study if anything is found abnormal which may aid in the management or treatment.

Name of the patient Signature / Thumb impression

Name of the investigator Signature

Date:

ஆராய்ச்சி பற்றிய தகவல் படிவம்

1. வாயில் வரும் ‘‘வாய் இறுக்குநோய், வாய்புற்றுநோய் மற்றும் நோயற்ற நபர்களின் குருதி ஊநீரில் உள்ள பீட்டா – கரோட்டினின்’’ அளவை அறிதல் குறித்து ஆராய்ச்சி செய்யும் பொருட்டு தமிழ்நாடு அரசு பல் மருத்துவமனை மற்றும் கல்லூரிக்கு வரும் நோயாளிகள் தேர்வு செய்யப்படுகிறார்கள்.

2. இந்த ஆராய்ச்சியின் நோக்கம் வாயில் வரும் ‘‘வாய் இறுக்குநோய், வாய்புற்றுநோய் மற்றும் நோயற்ற நபர்களின் குருதி ஊநீரில் உள்ள பீட்டா – கரோட்டினின்’’ அளவை அறிதல் ஆகும்.

3. நோயாளி பற்றிய குறிப்புகள் பிறர் அறியாவண்ணம் ஆராய்ச்சி முடியும்வரை இரகசியமாக பாதுகாக்கப்படும். அதை வெளியிடும் நேரத்தில் எந்த நோயாளியின் தனி அடையாளங்களும் வெளியிட வாய்ப்பு கிடையாது.

4. இந்த ஆராய்ச்சியில் இங்கு பெறுவது நோயாளியின் தனிப்பட்ட முடிவு மற்றும் நோயாளிகள் இந்த ஆராய்ச்சியில் இருந்து எப்பொழுது வேண்டுமானாலும் விலகிக் கொள்ளலாம். நோயாளியின் இந்த தீடீர் முடிவு, அவருக்கோ அல்லது ஆராய்ச்சியாளருக்கோ எந்தவித பாதிப்பும் ஏற்படுத்தாது என்பதை தெரியப் படுத்துகிறோம்.

5. இந்த ஆராய்ச்சியின் முடிவுகள் நோயாளிகளுக்கு ஆராய்ச்சி முடியும் தறுவாயிலோ அல்லது இடையிலோ தெரிவிக்கப்படும். ஆராய்ச்சியின்பொழுது ஏதும் பின் விளைவுகள் ஏற்பட்டால் அதை சரி செய்ய தகுந்த உதவிகள் அல்லது தேவையான சிகிச்சைகள் உடனடியாக மேற்கொள்ளப்படும்.

நோயாளியின் பெயர்: **கையொப்பம்/கைரேகை**

DECLARATION

TITLE OF DISSERTATION	**"ESTIMATION OF SERUM BETA CAROTENE IN PATIENTS WITH ORAL SUB MUCOUS FIBROSIS, ORAL SQUAMOUS CELL CARCINOMA"**
PLACE OF STUDY	**Department of Oral Medicine and Radiology, Tamil Nadu Government Dental College and Hospital, Chennai-600003 and Department of Biochemistry ,Madras Medical College,Chennai-600003**
DURATION OF THE COURSE	**3 Years**
NAME OF THE GUIDE	**DR.S.Jayachandran M.D.S, Ph.D**
HEAD OF THE DEPARTMENT	**DR.S.Jayachandran M.D.S, Ph.D**

I hereby declare that no part of the dissertation will be utilized for gaining financial assistance/any promotion without obtaining prior permission of the Principal, Tamil Nadu Government Dental College and Hospital, Chennai-600003. In addition, I declare that no part of this work will be published either in print or in electronic media without the guide who has been actively involved in dissertation. The author has the right to reserve for publish of work solely with the prior permission of the Principal and Guide, Tamil Nadu Government Dental College & Hospital, Chennai-600003.

Guide and Head of the Department **Signature of the candidate**

TRIPARTITE AGREEMENT

This agreement herein after the "Agreement" is entered into on this day …….between the Tamil Nadu Government Dental College and Hospital represented by its **Principal** having address at Tamil Nadu Government Dental College and Hospital, Chennai - 600 003, (hereafter referred to as, 'the college')

And

Dr. S. Jayachandran, aged.49 years working as **Professor & HOD** in Department of Oral Medicine and Radiology at the college, having residence address at A.M 16, TNHB Quarters, Todhunter Nagar, Saidapet, Chennai – 600 015 (herein after referred to as the 'Principal Investigator')

And

Dr. P.Vijayachandar aged 26 years currently studying as **Post Graduate student** in Department of Oral Medicine and Radiology, Tamilnadu Government Dental College and Hospital, Chennai - 3 (herein after referred to as the 'PG student and co-investigator').

Whereas the PG student as part of his curriculum undertakes to research on "**ESTIMATION OF SERUM BETA CAROTENE IN PATIENTS WITH ORAL SUB MUCOUS FIBROSIS, ORAL SQUAMOUS CELL CARCINOMA"** for which purpose the Principal Investigator shall act as principal investigator and the college shall provide the requisite infrastructure based on availability and also provide facility to the PG student as to the extent possible as a Co-investigator.

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

Now this agreement witnessed as follows

1. The parties agree that all the Research material and ownership therein shall become the vested right of the college, including in particular all the copyright in the literature including the study, research and all other related papers.
2. To the extent that the college has legal right to do go, shall grant to licence or assign the copyright so vested with it for medical and/or commercial usage of interested persons/entities subject to a reasonable terms/conditions including royalty as deemed by the college.
3. The royalty so received by the college shall be shared equally by all the three parties.
4. The PG student and Principal Investigator shall under no circumstances deal with the copyright, Confidential information and know – how - generated during the course of research/study in any manner whatsoever, while shall sole west with the college.

The PG student and Principal Investigator undertake not to divulge (or) cause to be divulged any of the confidential information or, know-how to anyone in any manner whatsoever and for any purpose without the express written consent of the college.

5. All expenses pertaining to the research shall be decided upon by the Principal Investigator/Co-investigator or borne sole by the PG student.(co-investigator)
6. The college shall provide all infrastructure and access facilities within and in other institutes to the extent possible. This includes patient interactions, introductory letters, recommendation letters and such other acts required in this regard.
7. The Principal Investigator shall suitably guide the Student Research right from selection of the Research Topic and Area till its completion. However the selection and conduct of research, topic and area of research by the student researcher under guidance from the Principal Investigator shall be subject to the prior approval, recommendations and comments of the Ethical Committee of the College constituted for this purpose.
8. It is agreed that as regards other aspects not covered under this agreement, but which pertain to the research undertaken by the PG student, under guidance from the Principal Investigator, the decision of the college shall be binding and final.
9. If any dispute arises as to the matters related or connected to this agreement herein, it shall be referred to arbitration in accordance with the provisions of the Arbitration and Conciliation Act, 1996.

In witness whereof the parties hereinabove mentioned have on this the day month and year herein above mentioned set their hands to this agreement in the presence of the following two witnesses.

College represented by its **Principal** **PG Student**

Witnesses **Student Guide**

1.

2.