ESTIMATION OF SERUM L-FUCOSE (TUMOR MARKER) LEVEL AMONG PATIENTS WITH ORAL CANCER OF VARIOUS TNM (TUMOR NODE METASTASIS) STAGES

DISSERTATION

Submitted to The Tamil Nadu Dr. M.G.R. Medical University in partial fulfillment of the requirement for the degree of

MASTER OF DENTAL SURGERY

BRANCH IX

ORAL MEDICINE AND RADIOLOGY

2012 - 2015

CERTIFICATE

This is to certify that this dissertation titled "**Estimation of serum L-Fucose (Tumor Marker) level among Patients with Oral Cancer of various TNM (Tumor Node Metastasis) stages**" is a bonafide research work done by **Dr. P. Redwin Dhas Manchil** under our guidance during his Post Graduate study during the period of 2012-2015 under THE TAMIL NADU Dr. M.G.R. MEDICAL UNIVERSITY, CHENNAI, in partial fulfillment for the degree of MASTER OF DENTAL SURGERY IN ORAL MEDICINE AND RADIOLOGY, BRANCH IX. It has not been submitted (partial or full) for the award of any other degree or diploma.

Dr. J. EUGENIA SHERUBIN M.D.S

Guide

Reader, Department of Oral Medicine and Radiology

Sree Mookambika Institute of Dental Sciences,

Kulasekharam

Dr. TATU JOY. E M.D.S

Co-Guide

Professor and HOD

Department of Oral Medicine and Radiology

Sree Mookambika Institute of Dental Sciences,

Kulasekharam

SREE MOOKAMBIKA INSTITUTE OF DENTAL SCIENCES, KULASEKHARAM

ENDORSEMENT BY THE PRINCIPAL / HEAD OF THE INSTITUTION

This is to certify that this dissertation titled "**Estimation of Serum L - Fucose (Tumor Marker) Level among Patients with Oral Cancer of Various TNM (Tumor Node Metastasis) Stages**" is a bonafide research work done by **Dr. P. Redwin Dhas Manchil** under the guidance of Dr. J. Eugenia Sherubin MDS, Reader, Department of Oral Medicine and Radiology, Sree Mookambika Institute of Dental Sciences, Kulasekharam.

Dr. Elizabeth Koshi MDS,

PRINCIPAL,

Sree Mookambika Institute of Dental Sciences.

V.P.M Hospital Complex,

Padanilam. Kulasekharam,

KanyaKumari District,

Tamil Nadu - 629 161

ACKNOWLEDGEMENT

I extend my profound sense of gratitude to my Guide **Dr. J. Eugenia Sherubin** MDS, Reader whose constant strive for excellence was the beacon for my study. I thank her for the invaluable guidance, constant encouragement and immense patience with me at every step of this endeavour. I am most grateful for the wonderful teacher and my Co-guide **Dr. Tatu Joy .E** MDS, Professor & Head, Department of Oral Medicine and Radiology, Sree Mookambika Institute of Dental Sciences who has been very helpful and kind to me. In times of despair, his willful action and definite leadership helped me move ahead with confidence.

I would like to acknowledge the help and support given by **Dr.Velayuthan Nair** MBBS, MS Chairman and **Dr. Rema V Nair** MBBS, MD, DGO Director Sree Mookambika Institute of Dental Sciences without which my study would not have been possible.

I am extremely thankful to **Dr. S. Karthiga Kannan** Professor of Oral Medicine and Radiology for his unimpeded support. **Dr. Shashi Kiran M**. and **Dr. Hema. G**. Readers, Dept. of Oral Medicine and Radiology for their constant enthusiasm and support they showed me in completing this work.

I take this opportunity to express my sincere gratitude to my teacher **Dr. Raghupathy L. P**, Senior Lecturer, whose constant encouragement and support helped me through the course of the work.

It's my utmost privilege to acknowledge the debt I owe to Dr. S. Rajesh Sathya M.D., D.A.A., D.N.B Cardiologist. Director in charge of Kanyakumari Medical Mission C.S.I. International Cancer Centre. Neyyoor. Dr. V.C Sudhakaran, M.D, DMRT. Head, Radiation Oncology. International Cancer Centre. Neyyoor. Dr. Aseer M.S Plastic Surgeon. Staff nurses Mrs. Sunitha, Mrs. Gracelin Sujila, Mrs. Pamila, Mrs. Chitra, Mrs. Jayakumari, Mr. Vincent Christopher Pharmacist and Mr. J.Blanchian Devakirubai M.Sc (MLT)., PGDHT., Chief technician of Central Lab in Sree Mookambika Institute of Medical Sciences, for their technical help, constant encouragement and liberty they provided me without which my study would not have been possible.

I am thankful to Mr. K. Boopathi for providing me with his timely statistical analysis involved in this study.

I thank my Department fellow Postgraduates, Dr. Meera Mathai, Dr. Vineetha Vijayakumar, Dr. M. Farakath Khan, Dr. Indu Krishna, Dr. S. Melbia Shiny, Dr. Aravind B. S, Dr. Hema Mareeswari .G,

Postgraduate Friends from other Department and Undergraduate Students of this college for their help and suggestions which helped me a lot in the completion of the work.

I am thankful to my **Dad** Mr. Y. Praisen Jee Pius Singer and **Mom** Mrs. D. Grace Gettis, wife **Dr. Jenita Sereen**, son **Ryan Redwin,** and my in-laws **Mr. Beschi Joseph**, **Mrs. Sheba B. Joseph** for their Love, constant encouragement, appreciation and support all through my study works. With deep sense of gratitude, I remember the care, support and encouragement I received from my Uncle **Dr. Wilson,** Aunt **Mrs. Lakshmi** and, friends & family. I thank them with all my heart for being with me through my ups and downs.

Above all this was possible by the blessings and grace of **Almighty God.**

CONTENTS

LIST OF ABBREVIATIONS

L-Fucose – Levo Fucose

D-Form – Dextro Form

TNM – Tumor Node Metastasis

ANOVA – Analysis of variance

Tukey HSD – Honest Significant Difference

SCC – Squamous Cell Carcinoma

OSCC – Oral Squamous Cell Carcinoma

HNSCC – Head and Neck Squamous Cell Carcinoma

HPV – Human Papilloma Virus

Tp53 – Tumour Protein 53

DNA – Deoxy Ribonucleic acid

RNA – Ribo Nucleic Acid

cGVHD – Chronic Graft Versus Host Disease

OPMDs – Oral Potentially Malignant Disorders

Rb – Retinoblastoma protein

TSG – Tumor Suppressor Gene

IARC – International Agency for Research on Cancer

WHO – World Health Organisation

UICC – Union for International Cancer Control

TB – Toluidine Blue

TAS – Total Analysis Systems

FAD – Flavin Adenine Dinucleotide

NADH – Nicotinamide Adenine Dinucleotide plus Hydrogen

LCM – Lasers Capture Microdissection

CT – Computed Tomography

MRI – Magnetic Resonance Imaging

PET – Positron Emission Tomography

SPECT – Single Photon Emission Tomography

CA-125 – Cancer Antigen 125

GDP – Guanosine 5'-diphosphate

FucT – Fucosyltransferases

SLex – Sialyl Lewis x

TF – Total Fucose

GSH – Glutathione

OSMF – Oral SubMucous Fibrosis

LIST OF FIGURES

LIST OF TABLES

LIST OF GRAPHS

ABSTRACT

BACKGROUND:

Oral Cancer is a result of disordered cellular behavior initiated by various stimuli which is characterized by the alteration of serum glycoproteins consisting of different monosaccharides. One of the monosaccharides is L-Fucose, a methyl pentose. It is the terminal sugar in most of the plasma glycoproteins. Elevated levels of protein-bound Fucose have been reported in various malignancies as well as in a few chronic systemic diseases.

AIM:

To estimate the serum level of L-Fucose among various TNM stages on Oral Cancer patients.

MATERIALS AND METHODS:

The study was carried out on 90 subjects, including 30 healthy individuals and 60 Oral Squamous Cell Carcinoma cases. The serum Fucose level estimation was done based on the method as adopted by Winzler. Statistical analysis included Independent Sample's t test, one way ANOVA Test, Karl Pearson correlation test, Tukey HSD Post Hoc test to evaluate the significance and variability of values between groups.

RESULTS:

There was a significant increase in mean serum Fucose level in Oral Squamous Cell Carcinoma patients compared to healthy controls. And there was a

progressive rise in L-Fucose levels as the stage of severity increases. Serum Fucose levels were independent of age, sex and Histopathological Grading. The results correlated well with other studies.

CONCLUSION:

Serum L-Fucose levels were increased in Oral Cancer patients compared to healthy individuals and a positive correlation was observed between serum L-Fucose levels and the stages of Oral Cancer. Therefore it was concluded that serum L-Fucose can be used as an effective diagnostic biomarker in Oral Squamous Cell Carcinoma patients.

KEY WORDS:

Oral Cancer, Squamous Cell Carcinoma, Tumor Marker, L-Fucose

INTRODUCTION

Cancer is the major causes of fear, morbidity and mortality all the world over. The world sees immense mortality due to cancer with Oral Cancer ranking as the sixth most common cause of Cancer-related death. [1] Approximately, around 95 % of Oral Cancer is found to be Squamous Cell Carcinoma (OSCC). [2] Inspite of recent advances in tumor surgery and multimodal treatment regimes, the prognosis of Oral Squamous Cell Carcinoma remains relatively poor. [1, 3] This indicates that the presence of the Carcinoma is often detected when the tumor is in an advanced stage. To overcome these problems, it would be very useful to find biochemical markers that allow suspecting the presence of Carcinoma at early stages. During the course of tumor development, quantitative changes will occur in a variety of substances in serum. These substances are collectively referred to as biochemical markers or tumor markers. [1]

The systematic study of glycans and glycan-binding proteins in various biological systems is known as Glycomics. It is an emerging field in the post-genomics and postproteomics era. Glycoproteins can be defined as proteins that have carbohydrate as a functional group covalently attached to their peptide portion. These glycoproteins are found as enzymes, hormones, blood group substances and as constituents of extracellular membranes. These are organic compounds, composed of both a protein and carbohydrate monosaccharides, usually hexose, hexosamine, Fucose and Sialic acid, joined together covalently linked to polypeptide chain.[4] Oligosaccharides are one of the most important factors in the post translational modification of proteins and lipids. [5] These structures undergo changes during malignant transformation, which lead to remodeling of cell

surface glycoproteins and glycolipids. They are associated with the biological behavior of tumor cells. Fucose is a constituent of oligosaccharides, and is notably associated with cancer. In the 1980s, the development of monoclonal antibodies against carbohydrate antigens triggered research to detect Cancer-associated aberrant glycosylation. [6]

Glycoproteins coat all eukaryotic cells and play an important role in many aspects of tumour progression. Cancer transformation causes alterations in the synthesis and expression of specific sugar structures. This is connected with the incorrect glycosylation of proteins.[7] Fucosylation pattern of these molecules in the tissues of Cancer patients show changes due to fucosyl transferase activity, which is especially high in the serum of patients suffering from Malignant or Metastatic Tumors.

It has been observed that serum fucose levels are raised in different groups of malignancies such as Breast Cancer, Ovarian Cancer, Colorectal Adenocarcinoma, Leukemia, Brain Tumors and recently in Oral Cancer. But rise in serum Fucose level is not specific for Cancers alone, as elevated serum Fucose levels have also been reported in various pathological states. However, in association with clinical diagnostic procedures, serum L-fucose levels can be used as an effective biochemical indicator in Oral Cancer and may be useful in monitoring recurrences, and effectiveness or response to treatment. There is minimal published data on serum L-Fucose levels in Oral Cancer in the Indian population. This study was done to determine the level of serum L-Fucose in Oral Cancer patients with increasing severity.[8]

AIM AND OBJECTIVES

AIM:

To estimate the serum levels of L-Fucose in the various TNM stages of Oral Cancer patients.

OBJECTIVES:

- To estimate the serum levels of L-Fucose in healthy individuals.
- To compare the serum levels of L-Fucose among Oral Cancer patients and healthy individuals.

REVIEW OF LITERATURE

ORAL CANCER:

A neoplasm can be defined as an abnormal mass of tissue, the growth of which exceeds and is uncoordinated with that of normal tissue and persists in the same excessive manner after cessation of the stimuli which evoked the change (Willis 1952).[9] Oral Squamous Cell Carcinoma is a heterogeneous group of malignant neoplasms seen in the oral cavity usually found arising from the mucosal surface of oropharynx, hypopharynx, larynx, sinuses and other sites restricted within the upper aerodigestive tract. [10] It is the most common neoplasm of the oral cavity. Clinically, it is often misdiagnosed as it gives varying appearances. [11] Early stages of the lesion are asymptomatic and may sometimes present with mild symptoms. But in the case of advanced lesions it may present with pain, halitosis, difficulty in mouth opening, speaking, swallowing, and chewing.[12]

EPIDEMIOLOGY:

- INCIDENCE:

According to GLOBOCAN 2008, Global incidence for head and neck Squamous Cell Carcinoma is 5, 40,000 per annum and in India it's around 70,000. [13] In Western countries over the past 30 years, frequency of Squamous Cell Carcinoma in the oropharynx has been found to increase rapidly while the incidence of Squamous Cell Carcinoma in the oral cavity has been gradually decreasing. [10] The main causative factor for the varying differences in the incidence rates are due to increased exposure to tobacco and alcohol, socioeconomic status, diet, age, gender and site. [14]

- GENDER

Oral Squamous Cell Carcinoma has a slight predilection for men accounting for 20% more than woman. Among females, the plantation workers who are addicted to pan chewing and other forms which include catechu, lime, piper betel leaf filled with sliced areca nut and spices chewed with or without tobacco and poor oral hygiene are more prone to it. [13]

- HABITS

The main etiological factor for Oral Cancer is mainly due to tobacco, alcohol abuse and areca nut consumption, severity of which is enhanced in the presence of poor dental health and diet. The usage of beedi which is the age old form of smoking is still being used by over 100 million Indians in South India. The risk of Oral Cancer is increased nearly five times by tobacco chewing, according to a cohort study in Kerala. [13]

- SITE:

The floor of the mouth and tongue are the most common sites for developing Oral Cancer, the tongue being the most common site(40—50%) among the European and American population. Due to habitual usage of betel quid and tobacco chewing habits, the most common site for Oral Cancer among the Asian population is in the buccal mucosa. Some individuals are at risk of developing palatal Cancer which is usually rare, for those who have the habit of reverse smoking with cheroot or cigarette held inside the mouth, this is practiced in a few places in Andhra Pradesh. [13]

The involvement of anterior sites has a better prognosis than the posterior sites (i.e. oropharyngeal). Incidence of survival is reduced by nearly 5 years for tumour located far more posteriorly. This is based on the influence of tumor site on nodal metastasis. The lymphatic drainage of the posterior part of the tongue is bilateral and it drains far more inferiorly while the lymphatic drainage of the anterior part of the tongue is unilateral and it drains to the upper part of the cervical lymph nodes. [15]

- AGE:

Squamous Cell Carcinoma in the oropharynx and oral cavity is usually regarded as a disease of the elderly. Since 1960's the International incidence of HNSCC particularly in oropharynx and tongue has increased in young adults. In the West, among the younger age groups, the increasing incidence of Oral Cancer appearing in the base of tongue and oropharynx is related to Human Papilloma Virus (HPV) infection. [13] It is noted that in developing countries like South and Southeast Asia, reason for increased rates of oral cancer is due to the rising incidence of young adults chewing betel quid.[10]

- MORTALITY

According to GLOBOCAN 2008, Global mortality rate for head and neck SCC are 2, 71,000 per annum and in India it is more than 48,000. It is reported that over five people die from Oral Cancer every hour, every day in India and similar reports were given for the cancer of oropharynx and hypopharynx.[13]

ETIOLOGY AND RISK FACTORS:

Squamous Cell Carcinoma (SCC) develops due to genetic and epigenetic changes in a variety of cellular pathways. This may lead to clonal expansion of those cells that has the most favorable genetic aberrations, resulting in the development of tumor and it finally leads to the progression of invasive carcinoma. There is mutation of Tp53, a tumor suppressor gene on chromosome 17p12 in almost half of all SCC. Malignant transformation is due to the functional loss of Tp53 which is seen in most of the cancers. Premalignant lesion, with such mutation shows dysplastic changes. The incidence of these mutations increases, as the tumour progresses from dysplasia to invasive carcinoma. [11, 16] Conversely, it doesn't show any remarkable difference between patients aged less than 35 years and greater than 75 years. [10]

NOTCH1 is the second most commonly mutated gene which plays a significant role in regulating normal cell differentiation, lineage commitment, and embryonic development. Based on the position and characteristics of the mutations and the inactivation of both alleles NOTCH1 is also a tumor suppressor gene in SCC. [16]

- TOBACCO:

The main risk factor of OSCC is tobacco associated intra-oral carcinogens, which has a synergistic role in oral tumor genesis. [2] All types of tobacco are not similar; it varies widely by the mode of use, processing and botanical type. And accordingly the toxicity and carcinogenicity varies. All forms of tobacco widely used in India are highly toxic to multiple body systems. [13]

- SMOKING (Beedi / Cigarette):

Beedi contain about 0.2–0.5 g of raw, dried and ground tobacco flakes, which are naturally cured, and wrapped in a temburni leaf; it produces 45–50 mg of tar, as compared to 18–28 mg delivered in cigarette. A three-fold increased risk for Oral Cancer in beedi smokers was found on a meta-analysis studied under 10 case–controls from India by Rahman et al. This risk is comparable to that of cigarette smokers.[13]

And this risk is related with the intensity and duration of smoking habit.[17] Individuals who smoke more than 20 cigarettes a day and consume more than 100 g of alcohol a day are at high risk of developing oral epithelial dysplasia.[10] In tobacco smoke, pro-carcinogens such as benzo - [α] - pyrene are metabolized by oxidizing enzymes, particularly cytochrome p450 and it may result in the production of reactive carcinogenic intermediates.[2, 18]

- SMOKELESS TOBACCO:

Smokeless tobacco is consumed by chewing it as an ingredient in pan/betel quid, packaged pan masala or gutkha (a chewable tobacco containing areca nut), and mishri (a powdered tobacco rubbed on the gums as toothpaste). The use of smokeless tobacco is socially accepted by the people in Eastern, Northern and North Eastern parts of India. The use of commercially available blends of pan masala and gutkha is increasing, not only among men, but also seen among children, teenagers and women.[13]

- ALCOHOL:

It has been found that the combined effects of smoking and alcohol consumption on the risk for Oral Cancer are highly synergistic. In a South Indian based study, a multiple interaction between the consumption of alcohol and tobacco products, respectively, was observed to produce a 24-fold increase in risk for Oral Cancer. [19, 20]

- BETEL QUID:

It is estimated that almost 10% of the world population has the habit of consuming betel nut in different forms. It includes crude fiber, carbohydrates, fats, polyphenols, alkaloids, proteins, tannins and water. [21] The use of betel quid, which has both areca nut and tobacco, is associated with a high risk of Oral Cancer. [22]

The fourth most commonly used psychoactive substance in the world after caffeine, nicotine and alcohol is the areca nut. Composition includes arecoline and 3-(Methylnitrosamino)propionitrile, and lime which provides nascent oxygen radicals, each of which has a role in oral cancer induction. Supari consists of small roasted and flavoured bits of arecanut, which is prepared commercially, and as a cultural practice is served after meals in North India. In Northeastern parts of India, fermented areca nut called ‘tamul’ is used habitually. In Gujarat, ‘mawa’, a mixture of slender shavings of areca nut combined with tobacco and slaked lime is used by the youth population. Areca nut, in combination with tobacco in the form of gutkha, and without tobacco in the form

of pan masala, is widely available in prepackaged forms and is advertised as a safe product and even as a mouth freshener.

However, gutka is carcinogenic and areca nut in all its forms is the major cause of the potentially malignant disorder. In several parts of India like Maharashtra, Gujarat and Bihar areca nut chewing is a well-accepted practice socially and is indulged in by even young children.[13]

- MARIJUANA:

The tar component of marijuana contains chemicals and carcinogens similar to tobacco, but each marijuana cigarette may be more harmful than a tobacco cigarette because of the greater inhalation of tar and longer retention of marijuana smoke.[10]

- VIRUS:

Human papillomaviruses (HPVs) which constitute the Papilloma viridae family are a heterogeneous group of small non-enveloped epitheliotropic DNA viruses. They target the basal cells of stratified epithelia at either mucosal or cutaneous sites.[23] They are closely associated with several benign and malignant oral and cutaneous lesions.[2] Potential causes of viral colonization in the oral mucosa includes vaginal or/and oral sexual partners and young age of onset of sexual activity. [10, 24]

- FUNGUS;

Candida albicans, with the ability to convert procarcinogens to carcinogens is the most common candida species which occurs and can induce epithelial proliferation.[25] Chronic hyperplastic candidiasis may present as nodular or speckled-white plaques on the mucosa and are potentially malignant oral epithelial lesions.[2]

- CHRONIC GRAFT VERSUS HOST DISEASE (cGVHD):

Oral cGVHD is a significant risk factor for the development of OSCC. This is mainly due to cGVHD-related inflammation, prolonged immunosuppression which frequently follows cGVHD therapy, immunologic dysfunction related to the therapy, and carcinogenic and cytotoxic medication effects.[25]

- DIET:

According to Dietary studies and Laboratory data, diet plays a role in Cancer etiology by an indirect relationship between the consumption of selected food constituents and its occurance.[26] Fruits and vegetables, high in vitamins A and C and vitamin E with antioxidant properties give protection from oral neoplasia, whereas meat and chilies are risk factors.[27, 28] Dietary Iron may play a protective role in maintaining the integrity of the epithelium. [2] Its deficiency results in oral epithelial atrophy and the Plummer-Vinson (Patterson Brown Kelly) syndrome, which is associated with Cancer of the aerodigestive tract. [2, 29]

- HEREDITARY:

Epidemiological evidence from case-control studies of Squamous Cell Carcinoma indicated that a family history of head and neck Cancer is also a risk factor. [2] Retrospective statistical studies have revealed a two to four fold risk of Cancer occurrence at the same site, as that of close relatives affected by the same.[30]Relative risk of Squamous Cell Carcinoma was high when first degree family members suffered from HNSCC, especially if the onset occurred before 50 years of age. [10]

In our country the majority of Oral Cancers arise from pre-existing longstanding lesions, which is termed as 'Oral Potentially Malignant Disorders' (OPMDs). [13]

- PRECANCEROUS LESION:

A precancerous lesion is a morphologically altered tissue in which Oral Cancer is more likely to occur than in its apparently normal counterpart. [31]

The precancerous lesions are: [32]

1. Leukoplakia:
2. Erythroplakia
3. Palatal changes among smokers (smoker's palate) [31]

PRECANCEROUS CONDITION:

A precancerous condition is a generalized state associated with a significantly increased risk of Cancer. [31]

The precancerous conditions are: [9, 32]

1. Oral SubMucous Fibrosis
2. Oral lichen planus[19]
3. Sideropenic dysphagia
4. Discoid Lupus Erythromatosis[31]
5. Syphilis
6. Xeroderma Pigmentosum
7. Epidermolysis Bullosa

PATHOGENESIS:

OSCC is defined as "A malignant epithelial neoplasm exhibiting squamous differentiation as characterized by the formation of keratin and/or the presence of intercellular bridges - 1997, pindborgJJ et al." [22]

The oncogenes and anti-oncogenes are the two classes of regulatory genes directly involved in carcinogenesis. ***Oncogenes*** are positive regulators of carcinogenesis. In non-transformed cells, they are inactive and called as proto-oncogenes. Several proto-oncogenes were first identified through viral transformation of cellular genome, eg) c-erbB, cmos, c-myc, c-myb, C-H-ras. Many of the mutations in specific oncogenes eg) ras, myc, etc - are intimately associated with different types of malignant neoplasms.

Anti-oncogenes or tumor suppressor genes: They are negative growth regulators that are they limit or restrict the formation of tumors. The anti-oncogenes

function as tumor suppressor genes. In healthy cells they control cell proliferation by regulating cell cycle progression.

The two most widely studied tumor suppressor genes are the Rb gene and Tp53 gene. Tp53 has a significant role in maintaining the genomic stability and cellular equilibrium. In normal cells, this gene promotes apoptosis, regulates cell cycle through G1 - S checkpoint control and causes induction of cell differentiation. [13]

Levine, Crawford, and Lane in 1979 first described the Tp53 protein. TP53 (tumor protein 53) is a tumor suppressor gene (TSG) which is located on the short arm (p) of chromosome 17.[22, 33] It helps in maintaining the integrity of the genome and therefore Lane (1992) described p53 as "Guardian of the Genome". [27, 33, 34] Alterations in Tp53 can occur through loss of heterozygosity, point mutations, deletions, insertions, or interaction with viral proteins. These alterations are common and they mark early events in head and neck carcinogenesis. [34]

Mutations in the Tp53 gene are the most common genetic change observed in a large number of human malignancies; at least 50% of all human cancers have been found to contain Tp53 abnormality.[13]

PROCESS OF CARCINOGENESIS:

Cancer development is a multistep process. The concept of multi-stage carcinogenesis was first proposed by Berenblum and Schubik in 1948

- INITIATION:

Initiation involves one or more stable cellular changes arising spontaneously or induced by exposure to a carcinogen. This is considered to be the first step or the initiating step in carcinogenesis. Oncogenes are the human DNA sequences, which are responsible for transformation. More than one oncogene has to be activated for neoplastic transformation; however the initiation may be induced even by a single point mutation. This can lead to deregulation of genes responsible for cellular communication, development and differentiation. The transformed cell undergoes continuous division with further mutations, leading to a malignancy being manifested.[35, 36]

- CONVERSION OF PROTO ONCOGENE TO ACTIVE ONCOGENES [35]

The following mechanisms are considered for the conversion of proto-oncogenes to active oncogenes (Land et al. 1983)

(1) Overexpression of proto-oncogene following acquisition of a novel transcriptional promoter.

(2) Over-expression due to amplification of the proto-oncogene or oncogene.

(3) Influences on the levels of transcription and, in turn, the amount of gene product.

(4) Juxtaposition of the oncogene and immunoglobulin domains, following chromosomal translocations.

(5) Structural alteration of the oncogene protein.

- PROMOTION:

The transformed (initiated) cell can remain passive, unless and until it is aggravated to undergo further proliferation, creating the cellular imbalance. The transformation from an initiated cell to neoplasia is a multistep process and requires repeated and prolonged exposures to the offending stimuli.

Neoplastic development is influenced by the intra and extracellular environment. Initial mutation will depend not only on interaction with other oncogenic mutations but also on factors that may temporarily change the patterns of specific gene expression. This may result in an amplification of cellular growth potential and/or an uncoupling of the intercellular communication that restricts cellular autonomy and which coordinate tissue maintenance and development. [35, 36]

- PROGRESSION:

The successive changes in the neoplasm give rise to increasingly malignant sub-populations. The process may be promulgated by repeated exposures to carcinogenic stimuli or by selection pressures favoring the autonomous clonal derivatives. The initiated cells continue proliferating, causing a rapid increase in the

tumor size. As the tumor grows in size, the cells may undergo further mutations and alterations, leading to increasing heterogeneity of the cell population.

In the first phase of progression (neoplastic conversion) the pre-neoplastic cells are transformed to a state in which they are more committed to malignancy. This may involve further gene mutations accumulating within the expanding pre-neoplastic cell clone. The dynamic cellular heterogeneity which is a feature of malignancy, may, in many instances, be a result of the early acquisition of gene specific mutations that destabilize the genome. [35, 36]

- TUMOR METASTASIS: [35]

With tumor progression, the cells absolve their property of adherence, dissociate from the tumor mass and invade the surrounding tissues. In addition to this local invasion, the detached cells also enter the circulating blood and lymph and are transported to other organs/tissues away from the site of the primary growth and develop into secondary tumors at new sites. These form the distant metastases, resulting in wide spread Cancer. Cancer metastasis consists of a number of steps; the main steps are common for all tumors. The progress of the neoplastic disease depends on changes that facilitate:

(a) Invasion of Local Normal Tissues,

(B) Entry and transit of neoplastic cells in the blood and lymphatic systems, and

(C) The subsequent establishment of secondary tumor growth at distant sites.

Many of the steps in tumor metastasis involve cell-cell and cell-matrix interactions, involving specific cell surface molecules. Malignant cells are thought to have reduced ability to adhere to each other, so that they detach from the primary tumor and invade the surrounding tissues. The behavior of tumor is influenced by the cell adhesion molecules, one of the most important of which is cadherins. It is the metastatic process and tumors local invasion that are mainly responsible for the lethal effects of many common tumors. In many cases gene mutations are believed to be the driving force for tumor metastasis, with the development of tumor vasculature playing an important role in disease progression. [35]

- TUMOR ANGIOGENESIS:

Tumor growth depends on the supply of growth factors and efficient removal of toxic molecules, which is ensured through adequate blood supply. In solid tumors, efficient oxygen diffusion from capillaries occurs to a radius of 150-200μm, beyond which the cells become anoxic and die. Therefore, increase in tumor mass to more than 1-2 mm will depend on adequate blood supply through development of blood capillaries (angiogenesis). [35]

CLINICAL PRESENTATIONS:

It may take various clinical forms which may eventually develop into an ulcer with irregular, raised indurated borders, or into a broad based exophytic mass with a verrucous, pebbled or relatively smooth surface or it may develop into an Endophytic, or fungating lesion. When traumatized, OSCC tends to bleed readily and often becomes secondarily infected. OSCC is usually painless, unless it is secondarily infected. Large lesions may interfere with normal speech, mastication or swallowing. [37]Vascular and lymphatic networks, which vary between different anatomic sites, may influence tumor evolution and the outcome of the disease. [38]The site distribution of the lesions in the oral cavity can vary based on the type of habit, and the type, form and frequency of tobacco used.[39, 40]

HISTOLOGICAL GRADING OF ORAL SQUAMOUS CELL CARCINOMA:

BORDER'S SYSTEM (DESCRIPTIVE SYSTEM): [41, 42]

Tumors were graded as follows

Well differentiated (Grade I)	= <25 % undifferentiated cells
Moderately Differentiated (Grade II)	= <50% undifferentiated cells
Poorly Differentiated (Grade III)	= <75% undifferentiated cells
Anaplastic / pleomorphic (Grade IV)	= >75% undifferentiated cells

There are several subtypes of oral SCC which includes Spindle-cell carcinoma, Papillary SCC, AdenoSquamous carcinoma, Acantholytic SCC and carcinoma Cuniculatum. All these were considered in the classification adopted by the IARC–WHO. [43]

TNM STAGING

The Tumor-Node-Metastasis (TNM) staging system was first reported in the 1940s by Pierre Denoix. The Union for International Cancer Control (UICC) adapted the system and compiled the first edition of the TNM staging system in 1968 for 23 body sites. TNM staging is simply an anatomic staging system that describes the anatomic extent of the primary tumor as well as the involvement of regional lymph nodes and distant metastasis. [44]

T staging for tumors of the lip and oral cavity

TX	Primary tumor cannot be assessed
T0	No evidence of primary tumor
Tis	Carcinoma in situ
T1	Tumor 2 cm or less in greatest dimension
T2	Tumor more than 2 cm but not more than 4 cm in greatest dimension
T3	Tumor more than 4 cm in greatest dimension
T4a	**Lip:** Tumor invades through cortical bone, inferior alveolar nerve, floor of mouth, or skin of face (i.e., chin or nose) **Oral Cavity:** Tumor invades through cortical bone, into deep extrinsic muscle of tongue (genioglossus, hyoglossus, palatoglossus, and styloglossus), maxillary sinus, or skin of face
T4b	Tumor involves masticator space, pterygoid plates, or skull base and/or encases internal carotid artery

N staging for all Head and Neck sites except the nasopharynx and thyroid

Nx	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	Metastasis in a single ipsilateral lymph node, 3 cm or less in greatest dimension
N2	Metastasis in a single ipsilateral lymph node, more than 3 cm but not more than 6 cm in greatest dimension; or in multiple ipsilateral lymph nodes, none more than 6 cm in greatest dimension; or in bilateral or contralateral lymph nodes, none more than 6 cm in greatest dimension
N2a	Metastasis in a single ipsilateral lymph node more than 3 cm but not more than 6 cm in greatest dimension
N2b	Metastasis in multiple ipsilateral lymph nodes, none more than 6 cm in greatest dimension
N2c	Metastasis in bilateral or contralateral lymph nodes, none more than 6 cm in greatest dimension

M staging for Head and Neck tumors

Mx	Distant metastasis cannot be assessed
M0	No distant metastasis
M1	Distant metastasis

Stage Grouping for All Head and Neck sites except the nasopharynx and thyroid

Stage Group	T Stage	N Stage	M Stage
0	Tis	N0	M0
I	T1	N0	M0
II	T2	N0	M0
III	T3	N0	M0
	T1	N1	M0
	T2	N1	M0
	T3	N1	M0
IVA	T4a	N0	M0
	T4a	N1	M0
	T1	N2	M0
	T2	N2	M0
	T3	N2	M0
	T4a	N2	M0
IVB	T4b	Any N	M0
	Any T	N3	M0
IVC	Any T	Any N	M1

APPROACHES TO EARLY DETECTION OF DYSPLASIA AND ORAL CANCER: [15, 16, 20, 45]

There are numerous non-invasive and invasive diagnostic aids which help to detect cancer in the early stage.

NONINVASIVE DIAGNOSTIC AIDS:

i. Toluidine Blue (Vital Staining)

Toluidine Blue (TB) staining is an acidophilic dye that selectively stains acidic tissue components such as DNA and RNA. It is simple, cost effective and sensitive adjunct tool for identifying early OSCC and high-grade dysplasia.

Patients have to rinse the oral cavity with water for 20 sec. to remove debris prior to rinsing with 1% acetic acid for 20 sec. Toluidine blue (1% w/v) was applied as an oral rinse for 20 sec. and then 1% acetic acid was used for 20 sec to eliminate mechanically retained stain. [16] Lesions that showed dark blue staining were considered to be positive for premalignant or malignant tissue, while those with light staining, or totally not coloured, were considered negative. [46]

ii. Methylene Blue

Methylene blue dye system had 2 solution bottles.

Bottle A (The dye rinse solution) - Contain 1% methylene blue, 1% malachite, 0.5% eosin, glycerol, and dimethyl sulfoxide.

Bottle B (Pre- and post-rinse solution) –Contains 1% lactic acid, and purified water.

The patient should rinse their mouth with 1% lactic acid & distilled water for 30 seconds to remove food debris and excess saliva. The suspected mucosal area should be dried with gauze and power air spray to ensure that the lesion was not being contaminated with saliva. First the dye should be directly applied on the lesion with help of a cotton bud and then Methylene blue should be used as a mouth rinse for 30 seconds. Followed this, 1% lactic acid for 30 seconds should be used to wash out the excess dye. The pattern of dye retention was assessed by the intensity of stain on the lesion. Local, and deep blue stains were marked as positive (+) reaction. Wide, shallow or faint blue stains were marked as negative (–) reaction. [47]

iii. Lugol's Solution :

It contains Iodine 2g and Potassium Iodide 4 g. It should be applied with a cotton bud for 10-20 sec. Normal mucosa will be stained brown while the area without any retention of stain were considered as positive. [48]

iv. Oral Brush Biopsy

In this procedure cells will be collected from the full thickness of oral epithelium. It is a chair-side, easy to perform, painless test that can be used to evaluate any suspected lesion including common red and white lesions to rule out dysplasia. Sensitivity and specificity is over 90%.[49]

v. Lab-on-a-Chip:

Broadly, microfluidics technologies are referred to as lab-on-a-chip or micro-Total-Analysis Systems (TAS). It is the adaptation, miniaturization, integration, and automation of analytical laboratory procedures into a single device or "chip."

vi. Light-Based Detection Systems: (Optical Biopsy).

a) Chemiluminescence (ViziLite Plus; Microlux/ DL, Orascoptic-DK).

Oral cavity should be rinsed with acetic acid to dehydrate the lesion. This highlights the nuclear density and imparts an acetowhite appearance to tissue. Then it should be examined with an illuminated chemiluminescent light stick which further amplifies it. [50]

b) Tissue Fluorescence Imaging (VELscope)

It is a handheld device that uses visible light of 430 nm wavelength in order to cause fluorescent excitation of certain components of cell metabolism in the tissues like FAD and NADH.[16]

c) Tissue Fluorescence Spectroscopy.

Alterations in the structure and biochemistry of tissues, indicates the pathology. This was found by a significant increase of NADH fluorescence in neoplastic epithelium and a significant reduction of collagen fluorescence from the structure under the diseased layer. [16]

vii. Lasers Capture Microdissection. (LCM)

LCM provides an ideal method for the extraction of cells from specimens in which the exact morphology of both the captured cells and the surrounding tissue are preserved. [16]

viii. Saliva-Based Oral Cancer Diagnosis

ix. Cytological Techniques

x. Biomarkers[51]

INVASIVE DIAGNOSTIC AID[15, 16, 45]

(i) Surgical Biopsy.

RADIOGRAPHIC DIAGNOSTIC AID

Oncology highly depends upon radiologic imaging for diagnosis, response assessment and follow-up. Anatomic imaging modalities such as Computed Tomography (CT) and Magnetic Resonance Imaging (MRI) provides fine details regarding lesion location, size, morphology, and structural changes to adjacent tissues; but little details regarding the tumor physiology. Positron Emission Tomography (PET) and Single Photon Emission Tomography (SPECT) are used to provide tumor's biologic functions and its surrounding microenvironment. But it lacks ability to provide anatomic detail. The availability of hybrid imaging with PET/ CT, SPECT/CT and PET/MR improves our

ability to characterize lesions and to give proper treatment decisions and patient management. [52]

TUMOR MARKERS

These are substances that are produced by the tumor or by the body in response to the presence of Cancer or certain benign conditions, which can aid in the diagnosis of Cancer and in the assessment of tumor burden. [53]

USES OF TUMOR MARKER:

Tumor markers can be used for screening and early detection, to differentiate Cancer from other lesions (for example CA-125 helps to differentiate Ovarian Cancer from other conditions), to find the clinical stage of Cancer, as a supportive measure for diagnosis, to determine prognosis and to find the response to treatment. [54]

LIMITATIONS OF TUMOR MARKER:

Its limitation includes, Difficulty in identifying minute quantities in serum, It is proliferation related rather than tumor associated antigen, Cross reactive antigens for instances a common domains in different proteins, Cross reaction with degradation products of normal proteins taken up by tumor cells, The financial and psychological cost for the society on routine screening for early cancers using currently available tumor marker would be prohibitive. [53]

CLASSIFICATION

I. According to Spieght and Morgan (1993)[55]

- Proliferative markers: PCNA, Ki67, BrU, Histones & AgNORs
- Genetic markers: Ploidy
- Oncogene: C-myc
- Tumor suppressor markers: P53 mutations
- Cytokines
- Blood group antigens
- Integrins ECM ligands

II. According to Schliephake H (2003)[56]

A. Tumor Growth Markers

- Epithelial growth (EGF)
- Cyclin
- Nuclear cell proliferation antigens
- AgNORs (Agryophilic Nucleolar Organizer Region)
- Skp2(S-phase kinase-interacting protein 2)
- HSP 27 and 70 (Heat Shock Protein)
- Telomerase

B. Markers of tumor suppression and anti-tumor response

- Retinoblastoma protein (pRb)
- Cyclin dependent kinase inhibitors
- Tp53
- bax
- Fas/FasL

C. Angiogenesis markers

- VEGF/VEGF-R (Vascular endothelial growth factor/receptor)
- PD-ECGF (Platelet-derived endothelial cell growth factor)
- FGFs (Fibroblast growth factor)

D. Markers of tumor invasion and metastatic potential

- MMPs (matrix-metallo proteases)
- Cathepsins
- Cadherins and catenins
- Desmoplakin

E. Cell surface markers

- Carbohydrates
- Histocompatibility antigen
- CD57 antigen

F. Intracellular markers

- Cytokeratins

G. Markers of anomalous keratinization

- Filagrins
- Involucrin
- Desmosomal proteins
- Intercellular substances antigen
- Nuclear analysis

H. Arachidonic acid products

- Prostagladin E2
- Hydroxyeicosatetraenoic acid
- Leucotriene B4

I. Enzymes

- Glutathione S-transferase

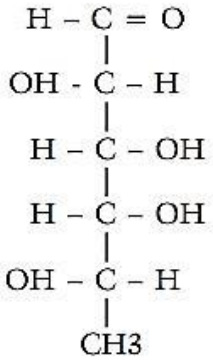
FUCOSE:

Fucose is one of the cell surface tumor markers which gained significant interest in the medical field. It exists in both D forms as well as L form. [57] And it is the only sugar which is present in L form. All other sugars, including D-glucose, D-galactose, D-manonose, D-glucosamine, and so on, exists in D-Form.[58]

D-Fucose (D-6-deoxygalactoses monosaccharide) is found in simple glycosides comprising only a few sugar units, limited to plant products, microbial and antibiotic substances.[57]

L-Fucose (6-deoxy-L-galactose) is a monosaccharide, which is a common component of many N- and O-linked glycans and glycolipids produced by mammalian cells. Lack of a hydroxyl group on the carbon at the 6-position (C-6) and the L-configuration are the two structural features which help to distinguish fucose from other six-carbon sugars present in mammals.

STRUCTURE OF L – FUCOSE[58]



Fucose frequently exists as a terminal modification of glycan structures. In mammals, Fucose-containing glycans have important roles in blood transfusion reactions, selectin mediated leukocyte-endothelial adhesion, host-microbe interactions, and numerous ontogenic events, including signaling events by the Notch receptor family.[59, 60]

OCCURRENCE OF L - FUCOSE AND ITS DERIVATIVES:

L-Fucose occurs in several human milk oligosaccharides, in Plant polysaccharides, Both N- and O-glycosyl chains of human or animal Glycoproteins and the lipopolysaccharides of Gram-negative bacteria and animal glycosphingolipids. **[61]**

REGULATORY MECHANISM FOR FUCOSYLATION:

Fucosylation (process of adding Fucose to a molecule) is catalyzed by fucosyltransferases, Guanosine 5'-diphosphate (GDP)-fucose synthetic enzymes, and GDP-fucose transporter(s). [62] GDP-fucose, which is a common donor substrate to all fucosyltransferase, is synthesized in the cytosol via two pathways, namely the salvage pathway and the de novo pathway.

The salvage pathway synthesizes GDP-fucose from free L-fucose, derived from extracellular or lysosomal sources via two steps: catalyzation by L-fucokinase and then GDP-fucose pyrophosphorylase. [59]

The de novo pathway transforms GDP-mannose into GDP-fucose via three steps: catalyzation by GDP-mannose-4, 6-dehydratase (GMDS) and GDP-4-keto-6-

deoxymannose-3, 5-epimerase-4-reductase (FX). The salvage pathway is responsible for only about 10% of the cellular pool of GDP-fucose. Thus, cellular GDP-fucose is mainly produced by the de novo pathway. A defect of this pathway leads to a virtually complete deficiency of cellular global fucosylation, including α1-2, 1-3/4, 1-6, and O-fucose. [59]

After GDP-fucose has been synthesized in the cytosol, it is transported to the Golgi apparatus through GDP-fucose transporter to serve as a substrate for fucosyltransferases.[6]

L-FUCOSE IN MALIGNANCY

Glycosylation is the most universal form of posttranslational modification of proteins. It is important in many of the signaling pathways which turns a normal cell into a cancer cell. The protein diversity is achieved by different sequence and structure of sugar moieties or glycan attachment. [63] Cellular glycosylation changes are associated with diverse types of neoplastic transformation. Mammalian cells either express or mediate many of their properties through the cell surface.[1] Altered glycosylation of cell surface proteins is critically important in cancer progression, especially the terminal epitopes of glycoproteins, which have been proposed to play a significant role in cell-cell interactions, development of cell adhesion, malignant transformation, and metastasis.[61]

Fucosylation of glycoproteins (the addition of L-fucose at the terminal end of the oligosaccharide chain) is one of the most important features that mediate several specific biologic functions. Tumor cells modulate their surface by increasing

fucosylation levels to escape recognition, which contribute to several abnormal characteristics of tumor cells, such as decreased adhesion and uncontrolled tumor growth. Several studies have suggested that estimating the serum/tissue fucose levels could be a promising approach for the early detection, diagnosis, and prognosis of various cancer types. [61]

Group of enzymes catalyze incorporation of fucose from activated nucleotide donor Guanosine Diphosphate (GDP)-fucose to the reducing end of complex glycans in a linkage-specific manner. They are known as Fucosyltransferases (FucT). These enzymes are expressed in many tissues and are increased in serum and tumors of cancer patients. [61] It has been reported that increased fucosylation is associated with elevated FucT activity.

Cancer cells, which are shed or released into circulation from the primary tumor often over express fucosylated glycans on their surface. [1] The expression of fucosylated glycoproteins (ie, fucoproteins) has been detected by means of specific lectins. Several lectin-based studies have indicated that fucoproteins are increased in various cancers. Profound fucosylation of the serum microenvironment may be a factor that interrupts adhesion and influences the formation of metastases. (For example) several fucose-containing 'natural ligands' reportedly are involved in the migration of tumor cells. Increased expression of fucosylated cell surface antigens, such as Lewis x/y (Lex/y) or sialyl Lex/ α, and the up-regulation of α1, 3/4-FucT have been associated with malignant transformation and increased metastatic potential of tumors, which result in a

poor prognosis of patients with cancer. α-L-fucosidase is a lysosomal enzyme that catalyzes the hydrolytic cleavage of terminal fucose residue that is involved in maintaining the homeostasis of fucose metabolism.[64]

Extensive studies on the nature of metastasis have shown that only a small subpopulation of cells in tumors possess the characteristics necessary for their release from the primary tumour and transport to and establishment of tumour foci in distant organs. Carbohydrate moieties on cell surface glycoconjugates play an important role in this metastatic spread since it could be demonstrated that they are involved in adhesion processes.[61, 64]

L - FUCOSE IN INFLAMMATION:

Sialyl Lewis x (SLex) is a complex sugar molecule located at the surface of leucocytes. It is a derivative of a sugar which has the three carbohydrate rings galactose, glucosamine and **fucose** joined together. The fourth ring is sialic acid, a sugar with additional ethylene glycol and carboxylic acid component. When it gets a signal of tissue damage, it attaches to a protein E-selectin, produced on the surface of blood vessels near damaged tissue. This triggers the blood vessel to expand, allowing other white blood cells to enter the damaged tissue and destroy intruding bodies. However, if innumerous white blood cells get into the lungs or kidneys, or into transplanted organs, they can damage healthy tissue.[61]

An injection of SLex into the bloodstream will block the E-selectin on the surface of the blood vessels and so hide the chemical message that alerts the white blood cells. SLex is also found on the surface of lung cancer cells and Colon Cancer cells, which also attach to E-selectin as they spread around the body. Administration of a SLex-like material could prevent metastasis.[61]

OTHER DISEASES

Serum L-fucose levels may serve as an indicator of disease activity in the follow up of patients. The clinical significance of L-fucose in rheumatoid arthritis was investigated by Kamel and Serafi. In rheumatoid arthritis decreased levels of serum L-fucose correlated with the duration of rheumatoid disease, number of involved joints and bone erosions. Recently for Immunization, artificial antigens mainly containing L-Fucose have been prepared by coupling oligosaccharide determinants to protein. When injected into animals, antibodies are generated that react with the glycans and also with intact bacteria.

In this context, preparation of L-fucose or L-fucose-containing oligosaccharides for the synthesis of immunogens is needed. In contrast to D-galactose, L-fucose represents a rather rare and expensive sugar and presently the treatment of a large amount of patients with high doses is hardly feasible. It is therefore a prominent goal to achieve an improved access to L-fucose and L-fucose containing oligosaccharides.[61]

Considering the role of L-Fucose in cancer, several Studies have been conducted including few in oral cancer. The values varied in every study but the net result supported the use of L-Fucose as a biomarker in oral cancer, this can be understood from the following studies and their similar positive results.

A study was conducted to determine Serum protein-bound Fucose in the total serum and the seromucoid fraction of 89 histologically confirmed female patients with a breast lesion. As a result no significant difference exists between the serum levels in patients with malignant lesion from benign lesions. So they concluded that the serum protein-bound Fucose might be a useful screening procedure for patients with breast masses but not much helpful to differentiate between benign from malignant masses. [65]

A study was done to measure the serum level of glycoprotein Fucose and N acetyl neuraminic acid (total and free) in a group of 59 patients with benign breast lesions and 107 patients with breast cancers. They found that patients were proven to have carcinoma whose values were above 3.35×10^{-3} mg. Fucose/mg. protein and patients with benign lesions had levels below this arbitrary point. [66]

A study was conducted to analyze the Utility of Serum Protein-bound Neutral Hexoses and L-Fucose for Estimation of Malignant Tumor extension and Evaluation of Efficacy of Therapy. As a result patients with confirmed diagnosis of malignant neoplasia indicated excellent correlation for presurgical estimation of tumor activity and post-surgical therapeutic efficacy. [67]

A study was conducted to find out Clinical Significance of Fucose Level in Glycoprotein Fraction in Patients with Malignant Tumors. As a result increase in fucose level in glycoprotein fraction is seen in patient with malignant diseases in contrast to benign diseases. However, no significant difference was noted in the fucose levels in the mucoprotein fraction. The increased fucose level in glycoprotein in malignant diseases was parallel to the increment in total fucose content in serum. They concluded that increased levels in total fucose in malignant diseases are primarily due to the increase in fucose-containing glycoprotein. [68]

A study was conducted to measure the Serum concentrations of fucose, sialic acid, and eight acute phase proteins in single specimens from patients with cancer to determine whether the raised concentrations of protein bound sugars commonly found in Cancer correlate with increased concentrations of the acute phase proteins. Serum fucose was raised more often in patients with advanced disease than in those in whom the spread of the tumour was more restricted; increased sialic acid concentrations, however, were found with a similar frequency in both these groups. Combined use of fucose and sialic acid values gave a high degree of marker positivity which could be only slightly improved on by including measurement of acute phase proteins. Therefore they concluded that the sialic acid provides an index of the acute phase response and the fucose a measure of the tumour spread. [69]

A study was conducted on rapid, simple enzymatic assay of free L-fucose in serum and urine, and its use as a marker for Cancer, cirrhosis, and gastric ulcers. They

measured L-fucose in healthy subjects, Cancer patients, and patients with other diseases. As a result the concentrations of L-fucose were significantly higher in patients with gastric ulcers, cirrhosis of liver and Cancer. And since urinary analysis is rapid and inexpensive, it is suitable for mass screening.[70]

A study was conducted to investigate the biochemical basis of aberrant Fucose-containing antigen expression by comparing the activity of fucosyltransferases (FTase) and α-L-fucosidase in tissue biopsies, from 18 normal and 20 malignant endometrium patients. As a result they suggested that aberrant expression of fucose-containing antigens, such as the H and the Lewis blood-group antigens, in endometrial carcinoma is consequential to the change in FTase rather than in a-L-fucosidase activity. In addition, the investigation suggests that different glycosylation mechanisms are operative in different subtypes of endometrial cancer. [71]

A study was conducted to estimate the serum level of total sialic acid; lipid bound sialic acid and Fucose in patients with benign and malignant tumors of the breast. As a result, more marked increase in level of all the three parameters were noted in malignancy, when compared with benign and controls. After surgery, there was an elevation in the serum levels of the above parameters than the values prior to surgery. And 2 months after the surgery a decline was noted, although none of the values reached the normal range. They concluded that there is a close association of the glycoproteins with the tumour burden and further signified its role in early detection and staging of Breast Cancer. [72]

A study was conducted on 30 histopathologically confirmed Oral Cancer patients, before onset of any treatment. The levels of glycoprotein-associated carbohydrates such as Hexose, hexosamine, Fucose and Sialic acid were estimated and as a result significant rise in levels of glycoprotein associated carbohydrates is found when compared to control subjects. And most importantly progressive rise in these markers was found as the stages of Oral Cancer advances. [73]

A study was conducted to find the possibility of using α-L-Fucose and reduced glutathione (GSH) as a biomarker in the diagnosis of prostate cancer patients. 40 patients with histopathologically proven prostate cancer were involved in the study. As a result the serum GSH decreases in prostate cancer, while serum Total Fucose increase (TF) in the same patients shows an inverse relationship between the 2 parameters. Finally it is concluded that prostate cancer affects both TF and GSH levels in the patient's serum. [74]

A study was conducted to analyze the role of serum Fucose as a biomarker and to correlate with other studies for its effective clinical application. The study was carried out on 67 subjects, including 14 healthy individuals and 53 Oral Squamous Cell Carcinoma patients. As a result there was a significant increase in mean serum fucose level of Oral Squamous Cell Carcinoma patients compared with healthy controls and they were found to be independent of age and sex. [75]

A study was conducted to determine the significance of serum L-fucose levels in head and neck malignancies. The study was conducted on 50 healthy controls and 50 cancer patients. In which the most common site of primary lesion is in the oral cavity, followed by larynx, hypopharynx and oropharynx respectively. As a result Comparison of glycoprotein L-fucose in two groups showed marked increase in levels in cancer patients than controls. And there was no relationship between serum fucose levels and age, sex and tumour differentiation. It is concluded that Serum glycoprotein L-fucose levels can be used as an effective biomarker in conjunction with clinical diagnostic procedures in head and neck neoplasia and also for monitoring recurrences. [76]

A study was conducted on Quantitative evaluation and correlation of serum glycoconjugates such as Protein bound Hexoses, Sialic acid and Fucose in Leukoplakia, Oral Sub Mucous Fibrosis and Oral Cancer. In this study 27 newly diagnosed Oral leukoplakia, 27 OSMF and 26 Oral Cancer patients, 40 healthy controls who are non-tobacco users and 40 healthy controls who are tobacco users were selected. In all these groups serum glycoconjugates were estimated. As a result no difference in serum glycoconjugates levels between tobacco and non-tobacco controls were found, but very high levels in Oral Cancer, Leukoplakia and Oral Sub Mucous Fibrosis (OSMF) patients, when compared to control groups. Fucose levels were significant of all the glycoconjugates between OSMF and Leukoplakia. It is concluded that serum glycoconjugates whose levels were very high in OSMF, Leukoplakia and Oral Cancer, do have a significant diagnostic and prognostic value in these diseases. [77]

MATERIALS AND METHODS

SOURCE OF THE DATA:

This study was carried out in the Department of Oral Medicine and Radiology. Sree Mookambika Institute of Dental Sciences, Kulasekharam, KanyaKumari district and The International Cancer Centre, Neyyoor, KanyaKumari District.

METHODS OF SELECTION OF DATA:

I. SAMPLE SIZE

1. Total number of subjects: 90
2. Total number of oral cancer patients: 60
3. Total number of healthy volunteers: 30

II. SELECTION OF CASES

Inclusion criteria:

- Patients (both male & female) of age between 25-75 years with histologically proven oral cancer but before onset of any form of treatment.

Exclusion criteria:

Other Cancers

Liver disease

Tuberculosis

Diabetes

Cardiovascular diseases

Any other chronic systemic diseases

III. SELECTION OF CONTROLS

Inclusion criteria:

- Healthy volunteers (both male & female) of age between 25 – 75 years without any history of debilitating systemic illness.

Exclusion criteria:

- Healthy controls were further excluded on the basis of tobacco consumption and alcoholism

PROCEDURE:

1. Complete study is explained to the Patients and healthy individuals and written Consent is taken in a prefilled form.
2. Case history is recorded
3. TNM (Tumor Node Metastasis) staging is done in all oral cancer patients.
4. Biopsy is taken in all suspected cancer patients for histopathological confirmation and grading.

SAMPLE COLLECTION, STORAGE AND HANDLING:

1. The subject is seated comfortably with the arm supported. Aseptic measures are used and tourniquet is applied 2 inches above the elbow of the upper arm. The site of the puncture is cleaned using sterile gauze dipped in 100% alcohol. Using a 3ml syringe with the needle size of 0.55×25mm. 3ml of blood is drawn from the anticubital vein.
2. The blood is allowed to clot and the serum separated by centrifugation and stored at 4°C
3. Standard L-Fucose is procured from **Megazyme Chemical Company, Ireland.**
4. Serum L-Fucose level is estimated by using Clinical chemistry Auto-Analyzer based on Winzler method

MATERIALS REQUIRED:

1. L-Fucose Assay kit (**Megazyme Chemical Company, Ireland)**
2. Clinical chemistry Auto-Analyzer (**gesan Chem 200**)
3. Micro-pipettes.
4. Bench Top Centrifuge

PRINCIPLE:

L-Fucose is oxidized by the enzyme L-fucose dehydrogenase in the presence of nicotinamide-adenine dinucleotide phosphate (NADP+) to L-fucono-1,5 lactone with the formation of reduced nicotinamide-adenine dinucleotide phosphate (NADPH).

$$\text{L-Fucose} + \text{NADP}^+ \xrightarrow{\text{(L-fucose dehydrogenase)}} \text{L-fucono-1, 5 lactone} + \text{NADPH} + \text{H}^+$$

The amount of NADPH formed in this reaction is stoichiometric with the amount of L-fucose. It is the NADPH which is measured by the increase in absorbance at 340 nm.

PREPARATION OF REAGENT SOLUTIONS (SUPPLIED):

Bottle 1: Buffer (44 mL; pH 9.5) plus sodium azide (0.02% w/v) as a preservative.

Bottle 2: NADP+. Freeze dried powder.

Bottle 3: L-Fucose dehydrogenase suspension (2.2 mL).

Bottle 4: L-Fucose (5 mL, 0.5 mg/mL).

- Contents of bottle 1 are used as supplied.
- Contents of bottle 2 are dissolved in 11 mL of distilled water. To avoid repetitive freeze / thaw cycles, divided into appropriately sized aliquots and stored in polypropylene tubes.
- Contents of bottle 3 are used as supplied.
- Contents of bottle 4 are used as supplied.

AUTO-ANALYSER ASSAY PROCEDURE:

This kit is suitable for the preparation of 280.5 mL of reagent (equivalent to 1020 reactions of 0.275 mL). Reagent preparation is performed as follows:

Preparation of R1:

COMPONENT	VOLUME
Distilled water Solution 1 (buffer) Solution 2 (NADP+)	40 mL 8.8 mL 2.2 mL (after adding 11 mL of H_2O to bottle 2)
Total volume	51 mL

Preparation of R2:

COMPONENT	**VOLUME**
Distilled water Suspension 3 (l-FDH)	4.66 mL 0.44 mL
Total volume	5.1 mL

R1: 0.250ml

Sample: 0.01ml

R2: 0.025ml

Reaction time: 10 min at 37°C

Wavelength: 340 nm

Calculation: endpoint

Reaction direction: Increase

Linearity: 0.01-1 g/L of L-fucose using 0.01 mL sample volume

FIGURE 1: CLINICAL PICTURE OF ORAL SQUAMOUS CELL CARCINOMA

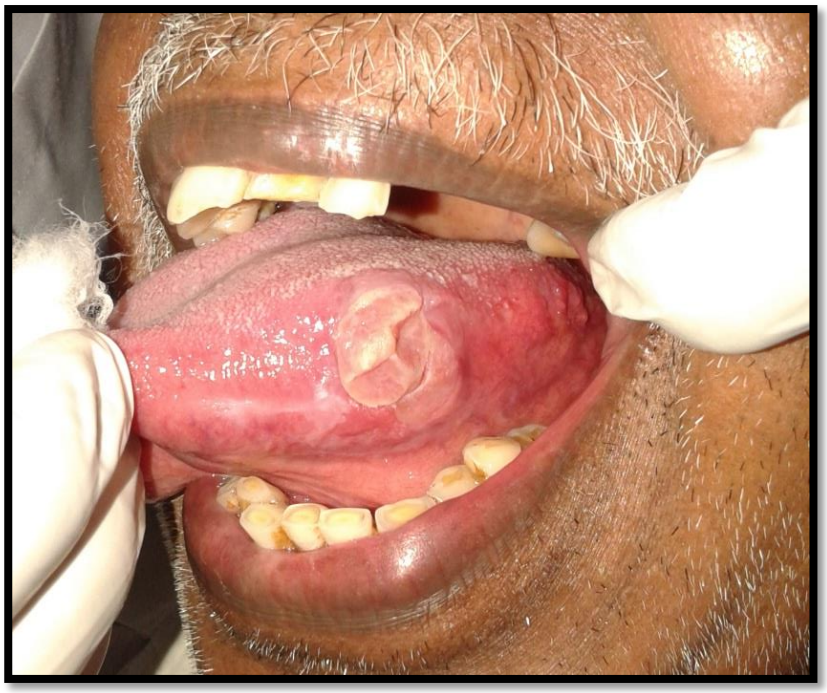


FIGURE : 2 PHOTOMICROGRAPH SHOWING MODERATELY DIFFERENTIATED ORAL SQUAMOUS CELL CARCINOMA

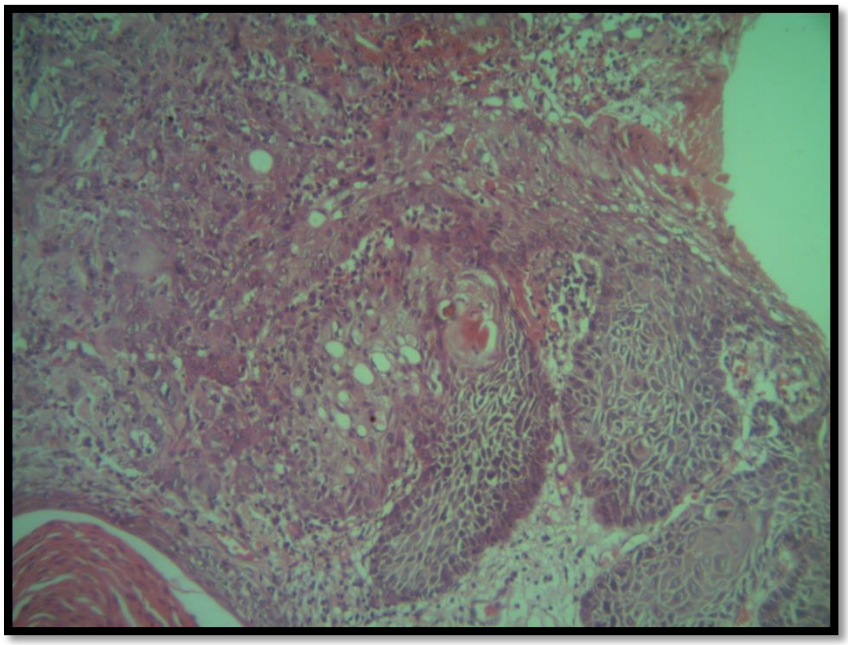


FIGURE 3: BENCH TOP CENTRIFUGE

FIGURE 4: SERUM SAMPLES

FIGURE 5: L-FUCOSE ASSAY KIT

FIGURE 6: MICROPIPETTES

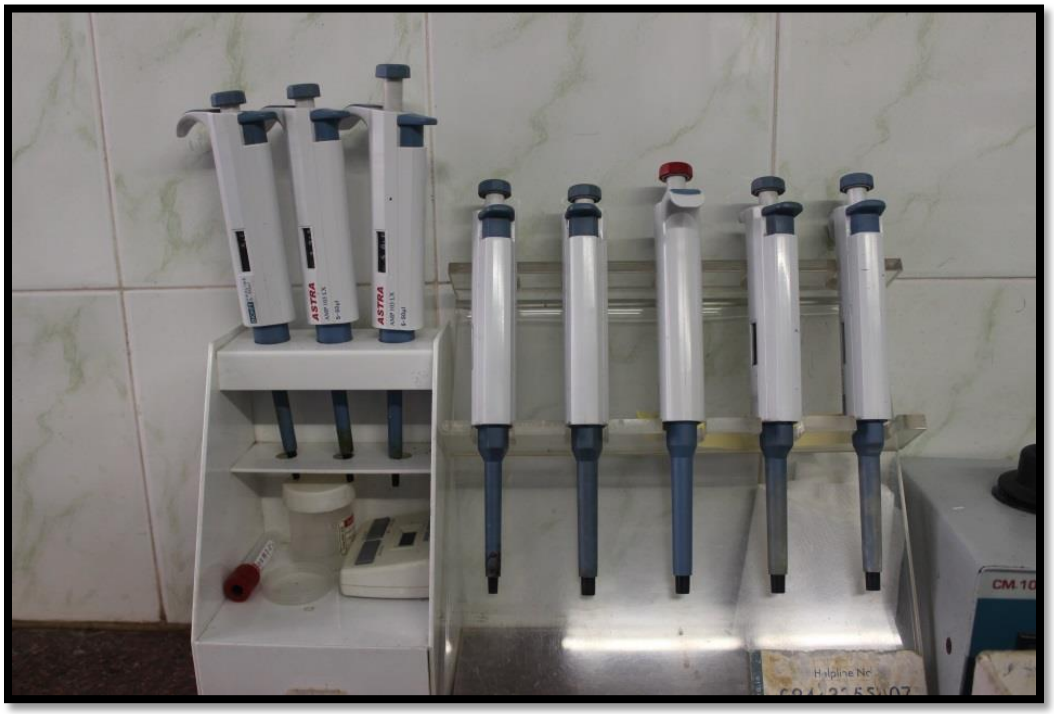


FIGURE 7 CLINICAL CHEMISTRY AUTO-ANALYZER

RESULTS AND OBSERVATION

The present study was undertaken to estimate the serum L-Fucose levels among various TNM stages of Oral Cancer. It was carried out on a study group comprising of 30 healthy individuals as controls in comparison with 60 OSCC patients in various stages. A comparison of the overall serum L- Fucose levels between the OSCC patients and healthy controls showed the mean value of serum L-Fucose levels of OSCC patients to be 10.85mgdl, whereas the healthy controls had mean serum L-Fucose level 3.47mg/dl, thereby showing a wide range of difference of 7.38mg/dl. Among the 60 OSCC, 2 belong to stage I with a mean serum L-Fucose level of 8.13mg/dl. The serum L-Fucose level progressively increased with its level being 9.18 mg/dl in stage II, 10.53mg/dl in stage III and 11.59 mg/dl in stage IV. Stage IV showed the highest level with a minimum value of 10.55mg/dl and maximum value of 12.54 mg/dl among the subjects. An attempt to correlate the serum L-Fucose levels with histopathological grading, between genders and ages yielded no significant results thereby inferring that serum L-Fucose levels are directly indicative of the degree of tissue destruction.

To compare the mean values between two groups (between genders) independent samples t-test was used. To compare the mean values between three or more groups (stages of cancer) one way ANOVA was used.

SPSS version 17.0 Software(s) was used for statistical analysis

(If P-Value < 0.05 then statistically significant)

TABLE 1

Serum level of L-Fucose in Oral Cancer

Fucose Level	Group		
	Cancer	Control	Total
N	60	30	90
Mean	10.85	3.47	8.39
Std. Dev	1.01	0.35	3.60
Minimum	8.01	2.89	2.89
Maximum	12.54	4.00	12.54

INFERENCE: Mean value of cancer group (10.85mg/dl) and control group (3.47mg/dl) shows a wide range of difference (7.38mg/dl)

GRAPH 1

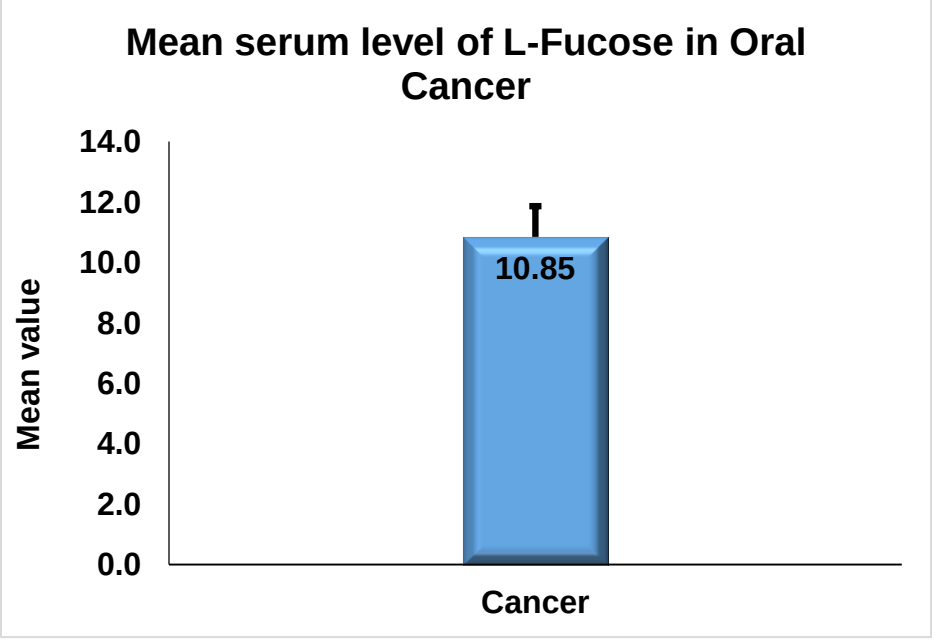


TABLE 2

TNM staging in Oral Cancer patients

TNM Stage	N	%
Stage-I	2	3.3
Stage-II	6	10.0
Stage-III	22	36.7
Stage-IV	30	50.0
Total	60	100.0

INFERENCE: The above table mentions the number of Cancer patients in each TNM Stage. Maximum number of patients belongs to TNM stage - IV

GRAPH 2

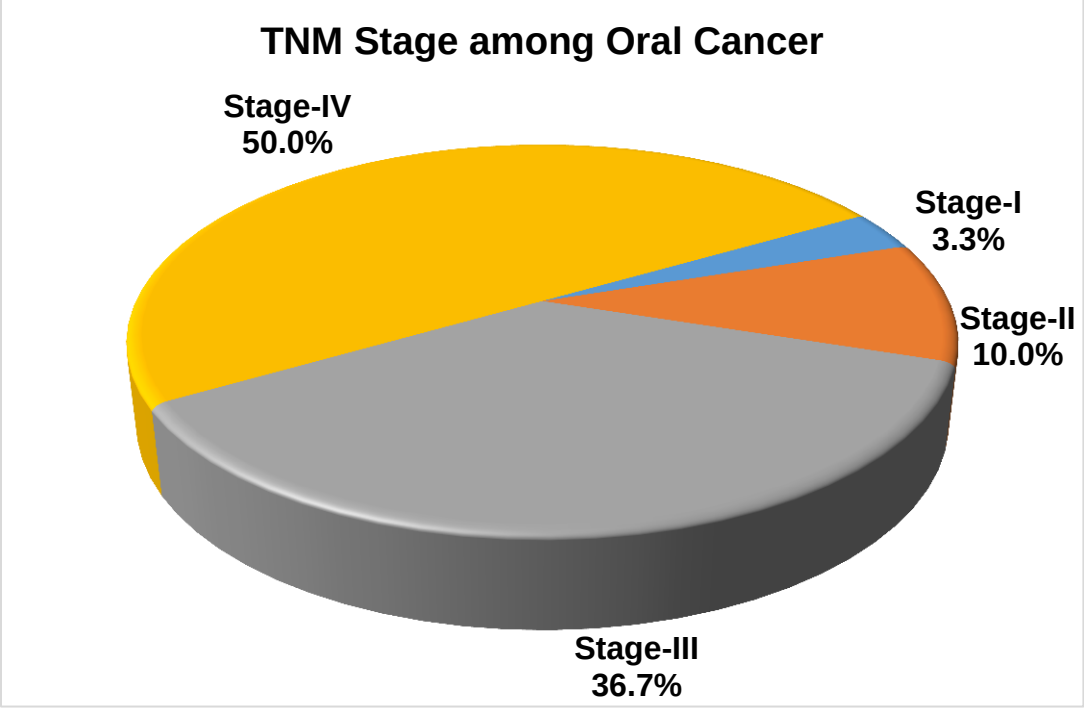


TABLE 3

Histopathological Grading In Oral Cancer Patients

Histopathological Grading	N	%
Poorly differentiated	2	3.3
Moderately differentiated	27	45.0
Well differentiated	31	51.7
Total	60	100.0

INFERENCE: The above table mentions the number of Cancer patients in each Histopathological grading. Maximum numbers of patient have well differentiated histological type of Oral Squamous Cell Carcinoma.

GRAPH 3

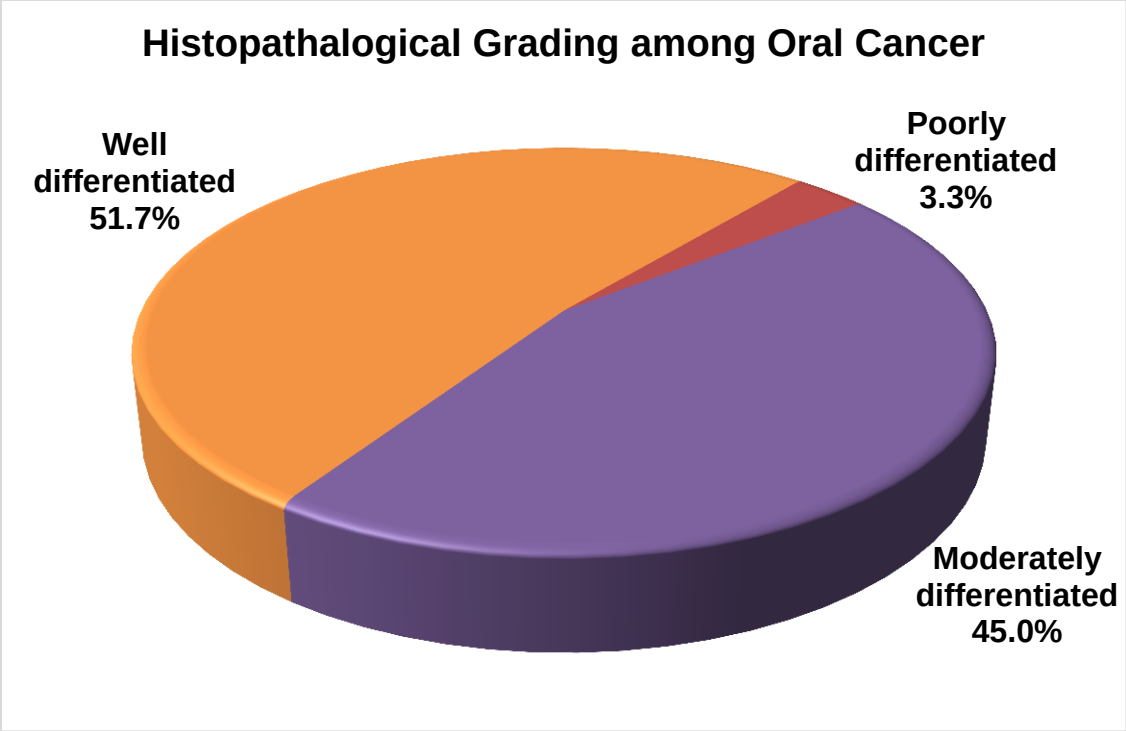


TABLE 4:

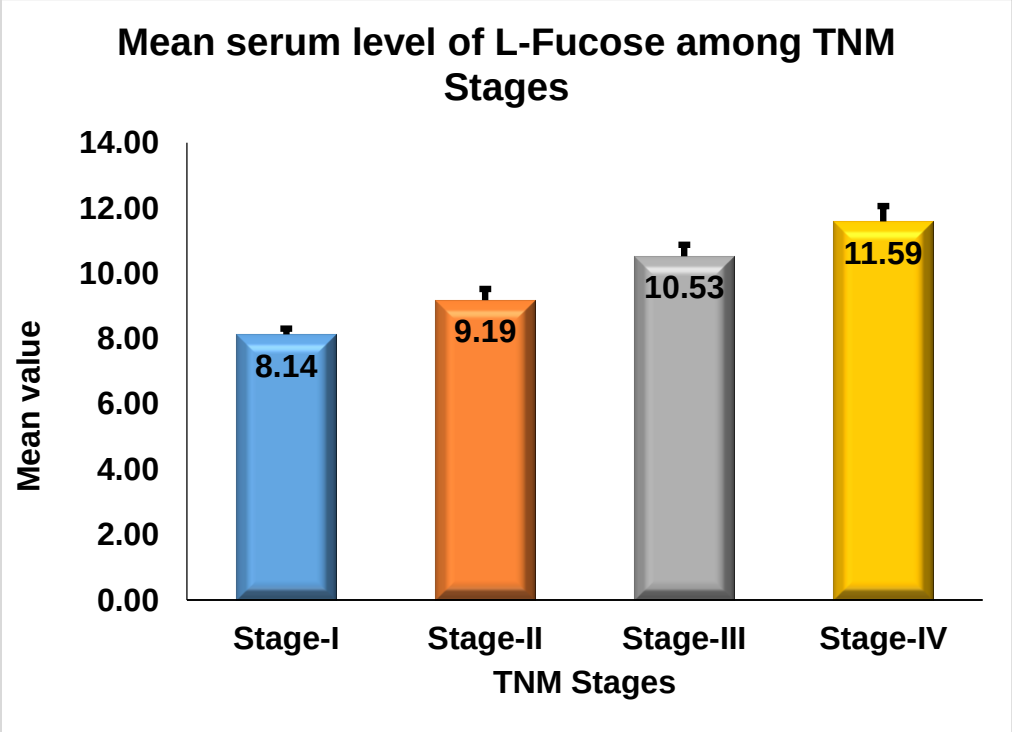
a) Serum level of L-Fucose in various Stages of Oral Cancer.

One way ANOVA was used to compare the mean Fucose level between stages of Cancer.

Stages	N	Mean	Std. Dev	Minimum	Maximum	F-Value	P-Value
Stage-I	2	8.1350	0.17678	8.01	8.26		
Stage-II	6	9.1850	0.34524	8.97	9.87		
Stage-III	22	10.5309	0.34355	10.02	11.43	98.774	<0.001
Stage-IV	30	11.5930	0.46893	10.55	12.54		
Total	60	10.8475	1.00557	8.01	12.54		

INFERENCE: Comparison of all the 4 TNM stages in Cancer group shows a significant P-value ($P < 0.001$)

GRAPH 4



b) ANOVA Table

Sum of Squares		df	Mean Square	F-Value	P-Value
Between Groups	50.177	3	16.726	98.774	<0.001
Within Groups	9.483	56	.169		
Total	59.660	59			

C) Tukey HSD Post Hoc Tests for multiple comparisons

TNM Stage		Mean Difference	P-Value
Stage-I	Stage-II	-1.050	0.015
	Stage-III	-2.400	<0.001
	Stage-IV	-3.458	<0.001
Stage-II	Stage-III	-1.346	<0.001
	Stage-IV	-2.408	<0.001
Stage-III	Stage-IV	-1.062	<0.001

INFERENCE: Comparison of one stage with another in Cancer group shows a significant P-value ($P < 0.05$).

TABLE 5

Serum level of L-Fucose between Oral Cancer and Healthy Individuals.

Independent samples T-Test to compare the Mean Fucose level between control and Cancer groups

	Group	N	Mean	Std. Deviation	t-Value	P-Value
Fucose Level	Cancer	60	10.8475	1.00557	38.885	<0.001
	Control	30	3.4750	.35267		

INFERENCE: Comparison of cancer group and the control group shows a significant P-value (P < 0.001)

GRAPH 5:

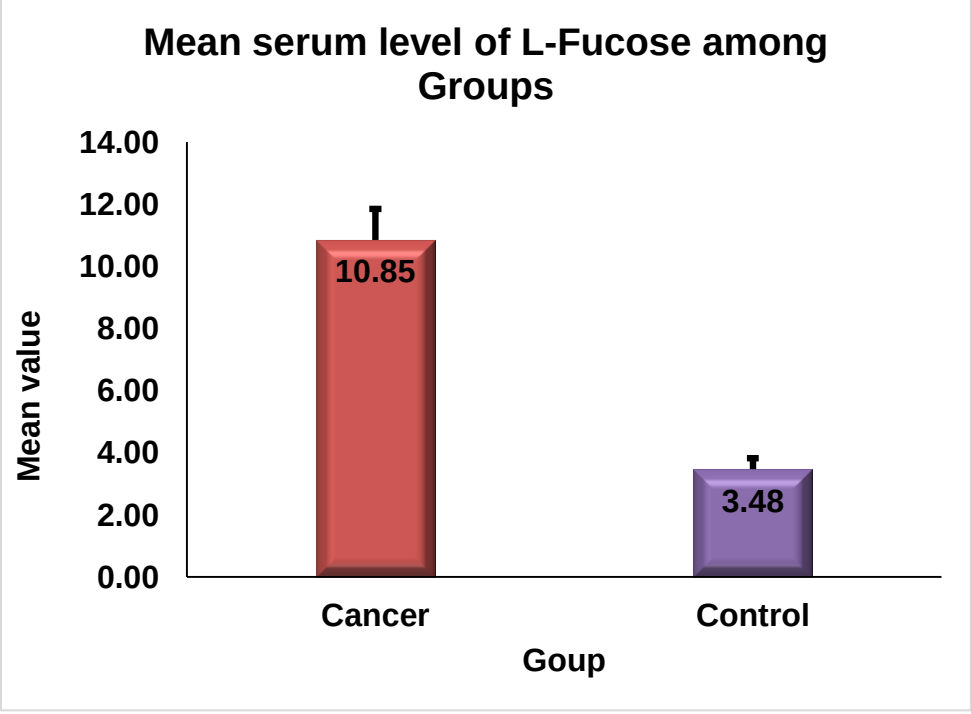


TABLE 6:

a) Serum Level of L-Fucose Between Histopathological Gradings

One Way ANOVA to compare the Mean Fucose Level between Histopathological Gradings.

Histo pathological Grading	N	Mean	Std. Dev	Minimum	Maximum
Poorly differentiated	2	12.0350	.10607	11.96	12.11
Moderately differentiated	27	10.9070	1.17426	8.01	12.54
Well differentiated	31	10.7190	.82401	8.26	11.93
Total	60	10.8475	1.00557	8.01	12.54

b) ANOVA Table

Sum of Squares		df	Mean Square	F-Value	P-Value
Between Groups	3.428	2	1.714	1.737	0.185
Within Groups	56.232	57	.987		
Total	59.660	59			

GRAPH 6:

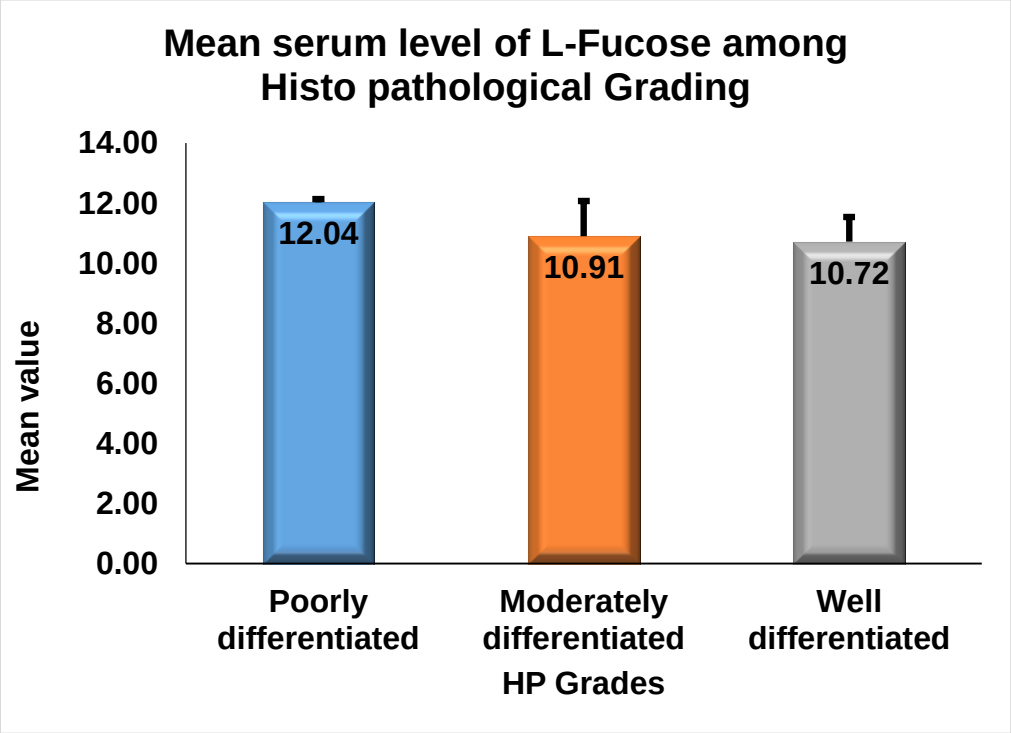

Mean serum level of L-Fucose among Histo pathological Grading
14.00
12.00
10.00
8.00
6.00
4.00
2.00
0.00
Mean value
12.04
10.91
10.72
Poorly differentiated
Moderately differentiated
Well differentiated
HP Grades

TABLE 7:

Serum level of L-Fucose between Male and Female Oral Cancer Patients

a) Independent samples T-Test to compare the Mean Fucose level between genders

	Gender	N	Mean	Std. Deviation	t-Value	P-Value
Fucose Level	Male	67	8.9842	3.32520	2.578	0.014
	Female	23	6.6591	3.86137		

INFERENCE: Comparison of the L-Fucose level between genders shows a significant P value (0.014)

b) Independent samples T-Test to compare the Mean Fucose level between genders among cancer and control group separately

Group	Variable	Gender	N	Mean	Std. Deviation	t-Value	P-Value
Cancer	Fucose Level	Male	50	10.8312	1.06331	0.279	0.782
		Female	10	10.9290	0.68139		
Control	Fucose Level	Male	17	3.5518	0.36174	1.385	0.177
		Female	13	3.3746	0.32687		

INFERENCE: Comparison of the L-Fucose level between genders among cancer and control group separately shows an insignificant P-value (>0.05)

GRAPH 7

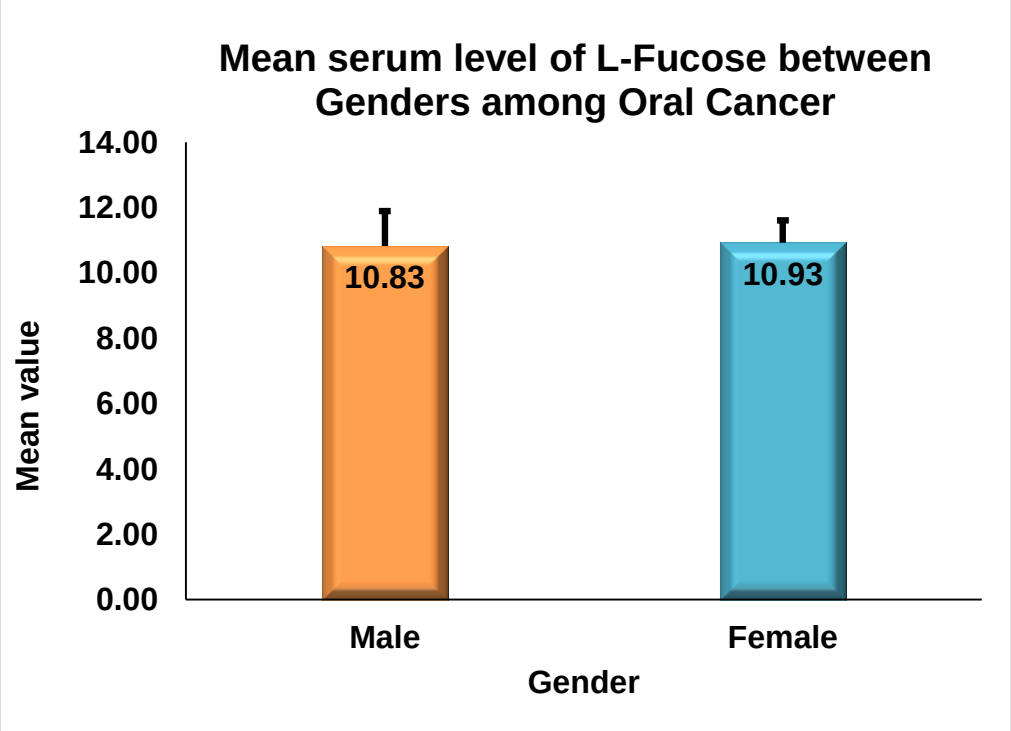


TABLE 8:

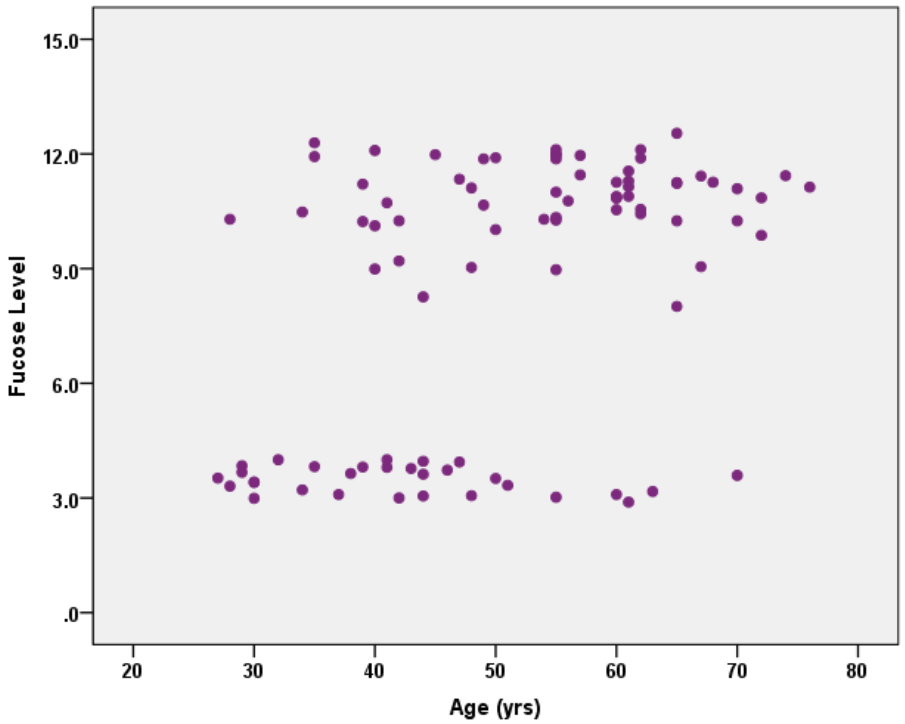
Serum level of L-Fucose in Oral Cancer Patients of varying ages

a) Correlations between age and Fucose levels

		Fucose Level
Age (yrs)	Pearson Correlation	0.465
	P-Value	<0.001
	N	90

INFFERENCE: Comparison of the L-Fucose level in oral cancer patients of varying ages shows significant P-Value (<0.001)

GRAPH 8



b) Correlations between age and Fucose levels among Cancer and control groups separately

Group			**Fucose Level**
Cancer	Age (yrs)	Pearson Correlation	0.069
		P-Value	0.598
		N	60
Control	Age (yrs)	Pearson Correlation	-0.266
		P-Value	0.155
		N	30

INFFERENCE: Comparison between age and Fucose levels among Cancer and control groups separately shows insignificant P-value (>0.05)

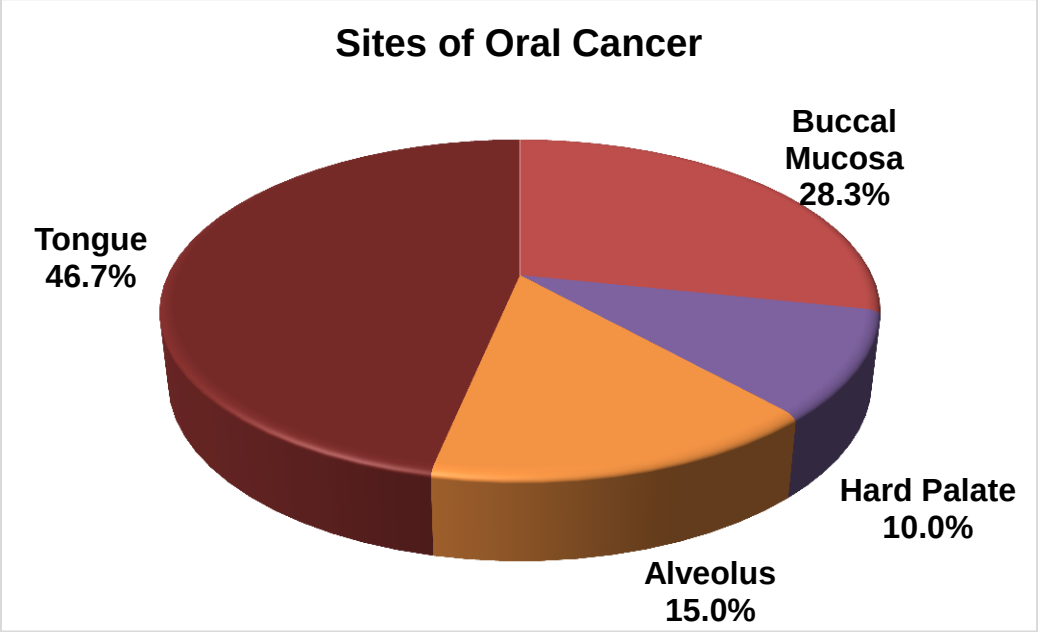
TABLE 9:

Site distribution of Oral Cancer

Site of Cancer	N	%
Buccal Mucosa	17	28.3
Hard Palate	6	10.0
Alveolus	9	15.0
Tongue	28	46.7
Total	60	100.0

INFERENCE: The above table mentions the site distribution of Oral Cancer, with the maximum of 28 patients in tongue and minimum of 6 in hard palate.

GRAPH 9



DISCUSSION

Group of neoplasms affecting any region of the oral cavity is termed as Oral cancer, among which oral squamous cell carcinoma represents more than 90% of all the neoplasm. Early diagnosis is important for the successful treatment of oral cancer. [78] Recently the knowledge about Cancer biomarkers has increased and numerous studies have been carried out regarding these. This has paved the way for improving the management of cancer patients by enhancing the accuracy of detection and efficacy of treatment. Understanding the relevance of biomarkers before using them is very important for diagnosis, treatment and proper follow-up. [79]

In our study serum L-Fucose was estimated in Oral Cancer patients (Oral Squamous Cell Carcinoma) of various TNM stages and compared with the control group. The aim of our study was to analyze the potential of L-fucose as a biomarker in detecting oral cancer.

This study was conducted in the Department of Oral Medicine and Radiology. Sree Mookambika Institute of Dental Sciences, Kulasekharam, KanyaKumari district and The International Cancer Centre, Neyyoor, KanyaKumari District. The total sample size was 90. This included 2 groups; a study group with 60 Oral Cancer patients and a control group with 30 healthy volunteers. Samples were selected based on our preformatted inclusion and exclusion criteria.

The 90 subjects included 67 males and 23 females. Comparison of the L-Fucose level between genders among Cancer and control group separately showed an

insignificant P-value (>0.05). This suggests that serum level of L-fucose cannot be correlated with gender. Among the 60 Oral Cancer patients, 50 were males and only 10 were females. Even in literatures similar results are seen probably due to the higher prevalence of tobacco usage in males when compared with females from our society who less commonly indulged in tobacco smoking. [80]

Age ranging from 25 to 75years were included in our study in both cancer and control groups. As a result, Comparison between age and Fucose levels among Cancer and control groups separately shows insignificant P-value (> 0.05). This indicates that age doesn't have any role to play in the serum level of L-Fucose in both Cancer and control groups. Similar to our study, Raj Kumar N. Parwani, Simran R. Parwani in 2011 did a study to ascertain the role of serum Fucose as a biomarker and to correlate with other studies for its effective clinical application in detecting Oral Squamous Cell Carcinoma. Their results revealed that Serum fucose levels were independent of age and sex. However, there was a significant increase in mean serum fucose level of Oral Squamous Cell Carcinoma patients compared with healthy controls. [75] Generally, Oral Squamous Cell Carcinoma (OSCC) often affects subjects above the sixth decade of life. [81] In our study also, out of the 60 cancer patients 21 were in 6^{th} decade of life.

Oral Squamous Cell Carcinoma affects the tongue in 20% - 40% of the cases and the floor of the mouth in 15% - 20% of the cases, and together these sites account for about 50% of all cases of oral SCC. The buccal mucosa, labial mucosa, gingivae, palate and retromolar area are less frequently affected oral sites. The most

commonly affected sites are the ventral surface of the tongue and the floor of the mouth and this may be because of the thin non-keratinized epithelium lining these areas. Carcinogens readily penetrate this thin epithelium and reach the progenitor cell compartment, and also, the carcinogenic agents present in tobacco and other associated products constantly accumulate in the floor of the mouth and bathe the tissues of the floor of the mouth and the ventral surface of the tongue with their noxious contents. [37] In Asian population, Oral Cancer commonly affects the buccal mucosa due to betel quid/tobacco chewing habits. [15] But in our study out of 60 cancer patients 28 presented with carcinoma of the tongue, which is quite unusual in our population. This may be due to smaller size of the sample.

According to Broders' classification, Oral Squamous cell carcinoma is histopathological graded as well differentiated, moderately differentiated, poorly differentiated and anaplastic. Among these grades, well differentiated type is common, followed by moderately differentiated. Poorly differentiated and anaplastic are rare. [81] In our study, out of 60 cancer patients, 31 were well differentiated, 27 – moderately differentiated, 2 poorly differentiated and anaplastic – nil. The Comparison between histopathological grades and Fucose levels among cancer groups shows insignificant P-value. Similarly, Rathan Shetty K.S, Satheesh Kumar Bhandary, Arunava Kali in 2013 did a study to determine the significance of serum L-fucose levels in head and neck malignancies. Comparison of glycoprotein L-fucose between the cases and control groups showed more than a two-fold rise in serum fucose levels in cases as compared to

those in controls. However, there was no relationship between serum fucose levels and Histopathological tumour differentiation. [76]

Tumor markers are produced either by the tumor itself, as a tumor byproduct, by the body in response to the presence of cancer or certain benign (noncancerous) conditions. These can aid in the diagnosis of Cancer and in the assessment of tumor burden. [53] One of them is L-fucose, which is a surface tumor marker. It is one of the essential sugars that the body requires for normal cell-to-cell communication. Physiologically, it is present in low concentrations in serum but is increased in cancer and other diseases. It has been documented that tumor cells modulate their cell surface by modifying and increasing fucosylation levels. This causes cells to escape recognition, which contributes to several abnormal characteristics of tumor cells, such as decreased adhesion and uncontrolled tumor growth. This mechanism is not specific for any particular group of malignancy but it includes Oral Carcinoma. [75]

Several studies have suggested that monitoring serum fucose levels could be a promising approach for the early detection, diagnosis and prognosis of various cancer types, including Oral Carcinoma. Clinically susceptible lesion can be analyzed with biomarker along with routine tests. [75] Thus, the present study was undertaken to estimate the serum level of L-Fucose (tumor marker) among various TNM (Tumor Node Metastasis) stages in oral cancer patients and compare them with healthy individuals.

In our study, the Mean Serum value of L-Fucose in cancer group (10.85mg/dl) and control group (3.47mg/dl) shows a wide range of difference (7.38mg/dl). Our results were in agreement with a study conducted by A K. Subhash Chandra Bose, Prerna Vyas Gokhale, Sunil Dwivedi, and Manika Singh who conducted a study on the Quantitative evaluation and correlation of serum glycoconjugates. Along with fucose, they evaluated the Protein bound hexoses and sialic acid levels in leukoplakia, oral sub mucous fibrosis and oral cancer. They concluded that serum glycoconjugates whose levels were very high in OSMF, Leukoplakia and Oral Cancer, do have a significant diagnostic and prognostic value in these diseases and this strengthens the diagnostic and prognostic value of our study.[77]

All the 60 patients in the cancer group were categorized according to TNM Staging. As a result, the largest group consisting of 30 patient were in stage IV, followed by 22 patients in stage III, 6 patients in stage II and only 2 was in stage I. Regardless of the easy access of the oral cavity for clinical examination, OSCC is usually diagnosed in advanced stages. The most common reasons are initial wrong diagnosis and ignorance of the patient or by the attending physician. [78]

Comparison of all the 4 TNM stages in cancer group showed increase in fucose levels as the stages progressed. As a result, Statistical analysis by using One way ANOVA revealed a significant P-value ($P < 0.001$) . Similarly, Wilma Delphine silvia C.R., D.M. Vasudevan and K. sudhakar Prabhu in 2001 conducted a study on thirty

untreated oral cancer patients and thirty healthy control subjects. They assessed the levels of glycoprotein-associated carbohydrates such as hexose; hexosamine, fucose and sialic acid. They found significant rise in the level in cases when compared to control subjects. Also there was a progressive rise in these markers as the stages of oral cancer advances. [73]

Unfortunately, Rise in serum fucose level is not specific for cancers, as elevated serum fucose levels have also been reported in various pathological states such as cirrhosis liver, meningitis, rickets, osteomalacia, tuberculosis, cardiovascular Disorders as well as in depressive disorders. Also, it has been observed that the serum fucose level is raised in different groups of malignancies such as breast cancer, ovarian cancer, colorectal adenocarcinomas, and leukemia as well as brain tumors. Thus, it becomes important to exclude other degenerative and proliferative diseases while evaluating serum fucose levels in oral cancer. The size of the lesion and secondary inflammation could alter these levels furthermore. [75]

In cancer patients elevated levels have been observed more so in advanced stages and in cases with metastasis than in subjects without metastasis. Serum fucose is considered one of the better biochemical markers in oral squamous cell carcinoma. Some studies have concluded that it is the most effective of the essential sugars when it comes to slowing the growth of cancer cells. Decrease in serum fucose levels after treatment has been reported in many studies, although it is emphasized that the follow-up period has to be long enough for significant serological alteration. [75]

CONCLUSION

Literature is replete with studies on fucose levels in various malignancies such as Breast Cancer, Brain Tumor, Endometrium Malignancy, Prostate Cancer and including a few on Oral Cancer. Based on the analysis of the results of our study we can conclude that there was a positive correlation between the serum L-Fucose levels and TNM stages of Oral Squamous Cell Carcinoma. Serum L-Fucose levels remained constant among healthy individuals and was raised proportionately in Oral Cancer patients, commensurate with the stage of Cancer.

In conjunction with clinic-diagnostic procedures, serum L-Fucose levels can be used as an easy, non-invasive, cost effective, biochemical indicator of Cancer detection, staging, therapeutic success, prognosis and as a post treatment evaluation tool. Further investigation on a large scale would in all probability prove serum L-Fucose as an effective tumor marker in the diagnosis and management of Oral Cancer.

SUMMARY

Despite advances in diagnostic techniques and improvement in treatment modalities, the prognosis of Oral Squamous Cell Carcinoma (OSCC) remains poor, mainly owing to the high rate of local and regional recurrence and to the development of new malignant changes within the original field of pre-cancerisation. If the health care professional does not have a high index of suspicion, additional weeks or months may elapse before a biopsy is performed. Although there has been considerable progress in the understanding of the genetic and molecular events underlying the progression of precancerous lesions to invasive carcinomas, this has yet to be translated into novel therapeutic strategies.

Glycoproteins play an important role in the cellular phenomena and alterations that occur during cancerous transformations. Although many tumor markers have been studied in Oral Cancer none of them have been shown to be specific. Significant increase in one or more of the glycoprotein constituents of serum has shown to be associated with neoplastic changes.

The carbohydrate of the glycoprotein is composed of relatively small number of different monosaccharides. One of them is L-fucose, which is usually a terminal sugar. It is one of the essential sugars that the body requires for optimum function of cell-to-cell communication. Apart from fucose being a prospective tumor marker, it is found to be a powerful immune modulator as it is distributed in macrophages, which are important for immune function. Physiologically, it is present in low concentrations in serum but is increased in cancer and other diseases. It has been documented that tumor cells modulate their surface by increasing fucosylation levels to escape recognition, which contributes to several abnormal characteristics of tumor cells,

such as decreased adhesion and uncontrolled tumor growth. This mechanism is not specific for any anatomical group of malignancy and includes oral carcinoma.

Several studies have suggested that monitoring serum/tissue fucose levels could be a promising approach for the early detection, diagnosis and prognosis of various cancer types, including oral carcinoma. Clinically susceptible lesion can be analyzed with biomarker along with routine tests. Thus, the present study was undertaken with the aim of estimating the serum level of L-Fucose in 60 Oral Cancer patients of various TNM stages and compared with 30 healthy individuals. The results showed progressive rise in the level of L-Fucose with advancement of cancer and in comparison with the controls. The increased levels of fucose have been attributed to tissue destruction and tissue proliferation. In conjunction with clinical diagnostic procedures, it holds promise as an effective biochemical indicator.

BIBLIOGRAPHY

1. Charushila Y. Kadam, Raghavendra V. Katkam, Adinath N. Suryakar, Kashinath M. Kumbar, Dipali P. Kadam. Biochemical markers in oral cancer. Biomed Res. 2011;22(1):76-80

2. Mehrotra R, Yadav S. Oral squamous cell carcinoma: Etiology, pathogenesis and prognostic value of genomic alterations. Indian J Cancer. 2006 April–June;43(2):60-65

3. Yi-Shing Lisa Cheng, Terry Rees and John Wright. A review of research on salivary biomarkers for oral cancer detection. Clin Transl Med. 2014;3:3

4. Mukesh Nandave, S K Ojha , Ranjit Kaur. Changes in levels of serum glycoproteins in major Depressive Disorders. Indian J Clin Biochem. 2005; 20(2):154-157

5. Eiji Miyoshi, Kenta Moriwaki, Naoko Terao, Cheng-Cheng Tan, Mika Terao, Tsutomu Nakagawa, Hitoshi Matsumoto, Shinichiro Shinzaki and Yoshihiro Kamada. Fucosylation Is a Promising Target for Cancer Diagnosis and Therapy. Biomolecules 2012;2:34-45

6. Kenta Moriwaki, Eiji Miyoshi. Fucosylation and gastrointestinal cancer. World J Hepatol. 2010 April 27;2(4):151-161

7. Malgorzata Borzym-Kluczyk, Iwona Radziejewska, Barbara Darewicz. Glycosylation of proteins in healthy and pathological human renal tissues. Folia Histochem Cytobiol. 2012;50(4):599–604

8. Rathan Shetty K. S and Arunava Kali. Prognostic significance of serum L-Fucose level in head and neck malignancies. Int J Pharm Bio Sci. 2014 Jan; 5(1):210–216

9. Shafer, Hine, Levy. Shafer's Text book of Oral Pathology. 5th edition. India: Elsevier publication; 2005. Chapter 29, Benign and Malignant Tumors of the Oral Cavity; p.113-308.

10. Ewa Majchrzak, Bartosz Szybiak, Anna Wegner, Piotr Pienkowski, Jakub Pazdrowski, Lukasz Luczewski, Marcin Sowka, Pawel Golusinski, Julian Malicki, Wojciech Golusinski. Oral Cavity and Oropharyngeal Squamous Cell Carcinoma in young adults: A Review of the Literature. Radiol Oncol 2014;48(1):1-10

11. Pei-Yu Li, Ling Auyeung, Shun-Chen Huang. Squamous Cell Carcinoma of the Mandibular Gingiva. Chang Gung Med J 2004;27:777-81

12. Luciana S. Marocchio, Joelma Lima, Felipe F. Sperandio, Luciana Correa, Suzana O. M. de Sousa. Oral squamous cell carcinoma: an analysis of 1,564 cases showing advances in early detection. J Oral Sci. 2010;52(2):267-273

13. Bhawna Gupta, Anura Ariyawardana, Newell W. Johnson. Oral cancer in India continues in epidemic proportions: evidence base and policy initiatives. Int Dent J. 2013;63:12–25

14. Preeti Sharma, Susmita Saxena, Pooja Aggarwal. Trends in the epidemiology of oral squamous cell carcinoma in western UP: An institutional study. Indian J Dent Res. 2010;21(3):316-319

15. Esam Ahmad Omar. The Outline of Prognosis and New Advances in Diagnosis of Oral Squamous Cell Carcinoma (OSCC): Review of the Literature. Journal of Oral Oncology. 2013

16. Ravi Mehrotra, Dwijendra K Gupta. Exciting new advances in oral cancer diagnosis: Avenues to early detection. Head Neck Oncol. 2011; 3(33)

17. Ana Livia Silva Galbiatti, Joao Armando Padovani-Junior, Jose Victor Maniglia, Clea Dometilde Soares Rodrigues, Erika Cristina Pavarino, Eny Maria Goloni-Bertollo. Head and neck cancer: causes, prevention and treatment. Braz J Otorhinolaryngol. 2013;79(2): 239-47.

18. Ernest L. Wynder, Dietrich Hoffmann. A study of tobacco carcinogenesis VIII. The Role of the Acidic Fractions as Promoters. Cancer. 1961 November-December; 14(6):1306-1315

19. Khan Z. An Overview of Oral Cancer in Indian Subcontinent and Recommendations to Decrease its Incidence. WebmedCentral Cancer. 2012;3(8)

20. Anna G Zygogianni1, George Kyrgias, Petros Karakitsos, Amanta Psyrri, John Kouvaris, Nikolaos Kelekis, Vassilis Kouloulias. Oral squamous cell cancer: early detection and the role of alcohol and smoking. Head Neck Oncol. 2011;3(2)

21. Rajeshwar N. Sharan, Ravi Mehrotra, Yashmin Choudhury, Kamlesh Asotra. Association of Betel Nut with Carcinogenesis: Revisit with a Clinical Perspective. PLoS One. 2012 August 13;7(8)

22. Wusheng Yan, Ignacio I. Wistuba, Michael R. Emmert-Buck, Heidi S. Erickson. Squamous cell carcinoma – similarities and differences among anatomical sites. Am J Cancer Res. 2011;1(3):275-300

23. P. Boscolo-Rizzo, A. Del Mistro, F. Bussu, V. Lupato, L. Baboci, G. Almadori, M.C. Da Mosto, G. Paludetti. New insights into human papillomavirus-associated Head and Neck Squamous cell Carcinoma. Acta Otorhinolaryngol Ital. 2013;33:77-87

24. Andrew G. Shuman, Gregory T. Wolf. Human Papillomavirus Status in Head and Neck Cancer. The Ethics of Disclosure. Cancer. 2010;116:4221–6.

25. Julian R. Naglik, Jonathan P. Richardson, David L. Moyes. Candida albicans Pathogenicity and Epithelial Immunity. PLoS Pathog. 2014 August;10(8)

26. Baruch Modan. Role of Diet in Cancer Etiology. Cancer. 1977 October; 40:1887-1891

27. Taia Maria Berto Rezende, Mirna de Souza Freire, Octavio Luiz Franco. Head and Neck Cancer. Proteomic Advances and Biomarker Achievements. Cancer 2010 November 1;116:4914–25

28. Vernon R. Young, David P. Richardson. Nutrients, Vitamins and Minerals in Cancer Prevention. Facts and Fallacies. Cancer. 1979;43:2125-2136

29. D Gude, DP Bansal, A Malu. Revisiting Plummer Vinson Syndrome. Ann Med Health Sci Res. 2013 Jan-Mar;3(1):119–121

30. Louise C .Strong. Genetic Etiology of Cancer. Cancer. 1977;40:438-444

31. Chris de Souza, Uday Pawar, Pankaj Chaturvedi. Precancerous Lesions of Oral Cavity. Otorhinolaryngology Clinics: An International Journal. 2009 May-September; 1(1):7-14

32. Anastasios K. Markopoulos. Current Aspects on Oral Squamous Cell Carcinoma. Open Dent J. 2012;6:126-130

33. Akhilesh Chandra, Bastian Thattil Sebastian, Archana Agnihotri. Oral Squamous Cell Carcinoma Pathogenesis and Role of p53 Protein. Universal Research Journal of Dentistry. 2013 September-December;3(3): 128-130

34. Helene Blons and Pierre Laurent-Puig. TP53 and Head and Neck Neoplasms. Hum Mutat. 2003;21:252-257

35. P Uma Devi. Basics of Carcinogenesis. Health Administrator. 17(1):16-24

36. Paula A. Oliveira, Aura Colaco, Raquel Chaves, Henrique Guedes-Pinto, Luis F. De-La-Cruz P, Carlos Lopes. Chemical carcinogenesis. An Acad Bras Cienc. 2007;79(4):593-616

37. Liviu Feller, Johan Lemmer. Oral Squamous Cell Carcinoma: Epidemiology, Clinical Presentation and Treatment. J Cancer Ther. 2012;3:263-268

38. Joao Massano, Frederico S. Regateiro, Gustavo Januario, Artur Ferreira. Oral squamous cell carcinoma: Review of prognostic and predictive factors. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2006;102:67-76

39. Shenoi R, Devrukhkar V, Chaudhuri, Sharma BK, Sapre SB, Chikhale A. Demographic and clinical profile of Oral Squamous cell Carcinoma patients: A retrospective study. Indian J Cancer. 2012 January-March; 49(1):21-26

40. Daniel Brandizzi, Mariana Gandolfo, Maria Lucia Velazco, Romulo Luis Cabrini, Hector Eduardo Lanfranchi. Clinical features and evolution of oral cancer: A study of 274 cases in Buenos Aires, Argentina. Med Oral Patol Oral Cir Bucal. 2008 Sep1;13(9):E544-8

41. M Akhter, S Hossain, Quazi B Rahman, Motiur R Molla. A study on histological grading of oral squamous cell carcinoma and its co-relationship with regional metastasis. J Oral Maxillofac Pathol. 2011 May-Aug;15(2):168–176

42. Rhayany de Castro Ribeiro Lindenblatt, Gisele Lago Martinez, Licinio Esmeraldo Silva, Paulo Silvestre Faria, Danielle Resende Camisasca, Simone de Queiroz Chaves Lourenco. Oral Squamous Cell Carcinoma Grading systems – Analysis of the best survival predictor. J Oral Pathol Med. 2012 Jan;41(1):34-9.

43. Michele Conceicao Pereira, Denise Tostes Oliveira, Gilles Landman, Luiz Paulo Kowalski. Histologic Subtypes of Oral Squamous Cell Carcinoma: Prognostic Relevance. J Can Dent Assoc 2007 May; 73(4):339-344

44. Snehal G. Patel, Jatin P. Shah, TNM Staging of Cancers of the Head and Neck: Striving for Uniformity Among Diversity. CA Cancer J Clin. 2005;55:242-258

45. Manish Gupta, Manas Gupta, Avanti Aggarwal, Ravish Ahuja. Recent advancements in the diagnosis of oral premalignant and malignant lesions: A comprehensive review. Clin Cancer Investig J. 2013 July-September; 2(3):181-184

46. E. Allegra, N. Lombardo, L. Puzzo1, A. Garozzo. The usefulness of toluidine staining as a diagnostic tool for precancerous and cancerous oropharyngeal and oral cavity lesions. Acta Otorhinolaryngol Ital. 2009;29:187-190

47. Akhtar Riaz, Balasundari Shreedhar, Mala Kamboj and S Natarajan. Methylene blue as an early diagnostic marker for Oral Precancer and Cancer. Springerplus. 2013 march 9;2:95

48. Kamarthi Nagaraju, Shiva Prasad, Ashok L. Diagnostic efficiency of toluidine blue with Lugol's iodine in oral premalignant and malignant lesions. Indian J Dent Res. 2010;21(2):218-223

49. Ravi Mehrotra, Sanjay Mishra, Mamta Sing, Mangal Singh. The efficacy of oral brush biopsy with computer assisted analysis in identifying Precancerous and Cancerous lesions. Head Neck Oncol. 2011;3:39

50. Anshul Aggarwal, Renuka Ammanagi, Vaishali Keluskar. Oral Lumenoscopy: An Adjuvant in early screening of Oral Cancer. Journal of Indian Academy of Oral Medicine and Radiology. 2011 April-June; 23(2):124-127

51. Corey J. Langer. Exploring Biomarkers in Head and Neck Cancer. Cancer. 2012;118:3882-92

52. Stephanie N. Histed, Maria L. Lindenberg, Esther Mena, Baris Turkbey, Peter L. Choyke, Karen A. Kurdziel. Review of Functional/ Anatomic Imaging in Oncology. Nucl Med Commun. 2012 April ;33(4):349–361

53. Nishat Sham Sultana, Ehtaih Sham, Rachna Kaul, Shilpa Shastry, Suraksha Bhat. Tumor Markers: A Short Overview. International Journal of Oral & Maxillofacial Pathology. 2013;4(4):07-15

54. Nishant Sharma, B.R Srivastav, Dr. Archana Shrivatav. Proteomics Tools for Oral Cancer Biomarker Discovery. Indo American Journal of Pharmaceutical Research. 2012;2(9):1039-1049

55. Thomas GT, Lewis PM, Spieght MP. Matrix metalloproteinase and oral cancer-review. Oral Oncol. 1999;35(3):227-33

56. Schliephake.H: Prognostic relevance of molecular markers of oral cancer—A review. Int J Oral Maxillofac Surg. 2003;32:233–45.

57. Iuliana Moldoveanu, Raluca-Ioana Stefan-van Staden, Constantina P. Kapnissi-Cristodoulou, Jacobus Frederick van Staden, Hassan Y. Aboul-Enein. Challenges in the enantioanalysis of fucose using stochastic and potentiometric microsensors. Sensing and Bio-Sensing Research. 2014;1:1–7

58. NG Sawke, GK Sawke. Serum fucose level in Malignant Diseases. Indian J Cancer. 2010 October-December;47(4):452-457

59. Daniel J. Becker, John B. Lowe. Fucose: biosynthesis and biological function in mammals. Glycobiology 2003;13(7):41R-53R

60. Julia Y. Chan, Ngozi A. Nwokoro, Harry Schachter. L-Fucose Metabolism in Mammals. J Biol Chem. 1979 August 10; 254(15):7060-7068

61. Petra T Vanhooren and Erick J Vandamme. L-Fucose: occurrence, physiological role, chemical, enzymatic and microbial synthesis. J Chem Technol Biotechnol 1999;74:479-497

62. Robert S. Haltiwanger. Fucose is on the TRAIL of colon cancer. Gastroenterology. 2009 July ;137(1):36–39

63. Bhairavi N. Vajaria, Kinjal R. Patel, Rasheedunnisa Begum, Franky D. Shah, Jayendra B. Patel, Shilin N. Shukla, Prabhudas S. Patel. Evaluation of serum and salivary total sialic acid and α-L-fucosidase in patients with Oral Precancerous conditions and Oral Cancer. Oral Surg Oral Med Oral Pathol Oral Radiol. 2013;115:764-771

64. Manisha Shah, Shaila Telang, Gira Raval, Pankaj Shah, Prabhudas S. Patel. Serum Fucosylation Changes in Oral Cancer and Oral Precancerous Conditions. α-L-Fucosidase as a Marker. Cancer 2008;113:336–46

65. Robert A. L. Macbeth, Garth McBride. Serum Protein-bound Fucose in Patients with Breast Masses. Cancer Res. 1965;25:1779-1780

66. Francis E. Rosato. Glycoproteins in Mammary Cancer. Ann Surg. 1968 November; 168(5):818-820

67. Adelbert S. Evans, Michael F. Dolan, Philip Z. Sobocinski, Frances A. Quinn. Utility of Serum Protein-bound Neutral Hexoses and L-Fucose for Estimation of Malignant Tumor Extension and Evaluation of Efficacy of Therapy. Cancer Res 1974 March;34:538-542

68. Toshiki Tatsumura, Hirofumi Sato, Akihiro Mon Yoshiharu Komori, Kelichi Yamamoto, Gessen Fukatani, Sigeru Kuno. Clinical Significance of Fucose Level in Glycoprotein Fraction of Serum in Patients with Malignant Tumors. Cancer Res. 1977 November;37:4101-4103

69. Ga Turner, Aw Skillen, P Buamah,T D Guthrie,T J Welsh, J Harrison, A Kowalski. Relation between raised concentrations of fucose, sialic acid, and acute phase proteins in serum from patients with cancer: choosing suitable serum glycoprotein markers. J Clin Pathol 1985;38:588-592

70. TakeshlSakal, Kazuki Yamamoto, Hiroko Yokota, Kanako Hakozakl-Usul, Fumitsugu Hino, Ikunoshln Kato. Rapid, Simple Enzymatic Assay of Free L-Fucose in Serum and Urine, and Its Use as a Marker for Cancer, Cirrhosis, and Gastric Ulcers. Clin Chem. 1990;36(3): 474-476

71. Jen-Wei Wang, Robert A. Ambros, Peter B. Weber, Thomas G. Rosano. Fucosyltransferase and α-L-Fucosidase Activities and Fucose Levels in Normal and Malignant Endometrial Tissue. Cancer Res. 1995 August 15;55:3654-3658

72. Mirsam Varkey, R. Sundara Devi, Shrinivas B. Rao. Glycoprotein Components in the serum of Patients with Cancer Breast. Indian J Clin Biochem. 1997;12(1):63-66

73. Wilma Delphine Silvia C.R, D.M. Vasudevan, K. Sudhakar Prabhu. Evaluation of Serum Glycoproteins in Oral Carcinoma. Indian J Clin Biochem. 2001; 16(1):113-115

74. Osama M. Fakri, Mufeed J. Ewadh, Oda M. Alzamily. The evaluation of alpha-L-Fucose and reduced Glutathione, a possible biomarker for prostate cancer in Iraq males. Journal of Kerbala University. 2007 September;5(3):39-51

75. Rajkumar N. Parwani, Simran R. Parwani. Quantitative evaluation of serum Fucose in Oral Squamous Cell Carcinoma Patients. J Cancer Ther Res. 2011 April-June;7(2):143-147

76. Rathan Shetty K.S, Satheesh Kumar Bhandary, Arunava Kali. Significance of Serum L-fucose Glycoprotein as Cancer Biomarker in Head and Neck Malignancies without Distant Metastasis. J Clin Diagn Res. 2013 Dec;7(12): 2818-2820

77. K. Subhash Chandra Bose, Prerna Vyas Gokhale, Sunil Dwivedi, Manika Singh. Quantitative evaluation and correlation of serum glycoconjugates: Protein bound hexoses, sialic acid and fucose in leukoplakia, Oral Sub Mucous Fibrosis and Oral Cancer. J Nat Sci Biol Med. 2013 Jan-Jun; 4(1): 122–125

78. Anastasios K. Markopoulos. Current Aspects on Oral Squamous Cell Carcinom. Open Dent J. 2012;6:126-130

79. Anant Narayan Bhatt, Rohit Mathur, Abdullah Farooque, Amit Verma, B.S. Dwarakanath. Cancer biomarkers - Current perspectives. Indian J Med Res. 2010 August;132:129-149

80. Doshi Neena P, Shah Siddharth A, Patel Keyuri B, Jhabuawala Munira F. Histological Grading of Oral Cancer: A Comparison of different systems and their relation to Lymph Node Metastasis. National Journal of Community Medicine. 2011;2(1):136-142

81. Janaina Almeida Mesquita, Alessandro Leite Cavalvanti, Cassiano Francisco Weege Nonaka, Gustavo Pina Godoy, Pollianna Muniz Alves. Clinical and histopathological evidence of Oral Squamous Cell Carcinoma in young patients: Systematized Review. J Bras Patol Med Lab. 2014 February;50(1):67-74

ANNEXURES

Sree Mookambika Institute of Medical Sciences
Kulasekharam (K.K District, TN) 629161

Phone No: 04651-280866, Fax No. 04651-280740

Institutional Human Ethics Committee

Registered under CDSCO with Reg No. ECR/446/Inst/TN/2013

Ref. No. SMIMS/IHEC/2013/C/35 **Date: 27th December 2013**

Certificate

This is to certify that the Research Protocol Ref. No. **SMIMS/IHEC/2013/C/35,** entitled "Estimation of Serum L-Fucose [Tumor Marker] Level Among Patients with Oral Cancer of Various TNM [Tumor Node Metastasis] Stages" submitted by Dr. P. Redwin Dhas Manchil, Postgraduate of Department of Oral Medicine and Radiology, SMIDS has been approved by the Institutional Human Ethics Committee at its meeting held on 19th of December 2013.

[This Institutional Human Ethics Committee is organized and operates according to the requirements of ICH-GCP/GLP guidelines and requirements of the Amended Schedule-Y of Drugs and Cosmetics Act, 1940 and Rules 1945 of Government of India.]

SREE MOOKAMBIKA INSTITUTE OF MEDICAL SCIENCES ★ KULASEKHARAM ★
IHEC
Date. 27/12/2013

Dr. Rema Menon. N
Member Secretary
Institutional Human Ethics Committee
Professor of Pharmacology and HOD
SMIMS, Kulasekharam [K.K District]
Tamil Nadu -629161

CONSENT FORM

PART 1 OF 2

INFORMATION FOR PARTICIPANTS OF THE STUDY

Dear Volunteers,

We welcome you and thank you for your keen interest in participation in this research project. Before you participate in this study, it is important for you to understand why this research is being carried out. This form will provide you all the relevant details of this research. It will explain the nature, the purpose, the benefits, the risks, the discomforts, the precautions and the information about how this project will be carried out. It is important that you read and understand the contents of the form carefully. This form may contain certain scientific terms and hence, if you

1. **Name of the Principal Investigator:**

P. Redwin Dhas Manchil
Post Graduate student
Department of Oral Medicine and Radiology,
Sree Mookambika Institute of Dental Sciences,
Kulasekharam, KanyaKumari District-629161

2. **Name of the Guide:**

Dr. Hema G. MDS.
Reader
Department of oral medicine and Radiology.
Sree Mookambika Institute of Dental Sciences.
Kulasekharam, KanyaKumari District-629161

3. Name of the Co-Guide:

Dr. J. Eugenia Sherubin MDS.

Reader

Department of oral medicine and Radiology.

Sree Mookambika Institute of Dental Sciences.

Kulasekharam, KanyaKumari District-629161

4. Institute:

Sree Mookambika Institute of Dental Sciences,

V.P.M Hospital complex, Padanilam, Kulasekharam,

Kanyakumari – 629161

Tamilnadu

5. Title of the study:

"Estimation of serum Levo–Fucose (Tumor marker) level among patients with oral cancer of various TNM (Tumor Node Metastasis) stages."

6. Background information:

Physiologically, Levo-Fucose is present in low concentrations in serum but is increased in cancer and other diseases. Several studies have suggested that monitoring serum L-Fucose levels could be a promising approach for the early detection, diagnosis and prognosis of various cancer types, including oral cancer. Clinically suspected lesions can be analyzed with such biomarker along with routine tests.

7. Aims and Objectives:

- To estimate the serum level of Levo-Fucose (tumor marker) among various TNM (Tumor Node Metastasis) stages in oral cancer patients.
- To estimate the serum level of Levo-Fucose in healthy individuals.
- To compare the serum level of Levo-Fucose among oral cancer patients and healthy individuals.

8. Scientific justification of the study:

It is always difficult to diagnose the onset of oral cancer in an initial stage. At times we fail in identifying the stage in which cancer is progressing. Therefore many diagnostic tools were taken into consideration and it is found that the difference in serum level of Levo-Fucose (tumor marker) gives an idea about the early onset of cancer. And the increased level suggests the progression of the oral cancer in various TNM (Tumor Node Metastasis) stages.

9. Procedure for the study:

- Once you are enrolled into the study a roll no will be implemented to represent the name.
- A detailed history will be taken from you in an annexure designed for that.
- Inspection and palpation will be done in a desired site.
- Photographs will be taken and recorded.
- Radiographic investigations will be done
- Routine blood examination will be done by venipuncture from which few ml will be used for our study.
- Blood will be left to clot and then centrifuged and serum will be separated and stored at 4°C.
- Serum level of L-Fucose will be estimated
- Biopsy will be taken and specimen will be sent to the lab for histopathalogical confirmation.
- After confirmation you will be sent for further treatment and management.

10. Expected risks for the participants:

- A slight pain during the needle prick.
- Multiple punctures.
- The previous study conducted did not report any complication or risk other than the slight pain and even that can be overcome by topical application of local anesthesia.

11. Expected benefits of research for the participants:

- You will not be required to pay for this lab test.

- You can enquire about the outcome of the procedures and your details.
- You will get a better treatment at the end of the procedure.

12. Maintenance of confidentiality:

a. You have the right to confidentiality regarding the privacy of your medical information (Personal details, results of physical examinations, investigations, and your medical history).

b. By signing this document, you will be allowing the research team investigators, other study Personnel, sponsors, institutional ethics committee and any person or agency required by law to view your data, if required.

c. The results of clinical tests and therapy performed as part of this research may be included in your medical record.

d. The information from this study, if published in scientific journals or presented at scientific meetings, will not reveal your identity.

13. Why have I been chosen to be in this study?

a. Chosen because of groping under the inclusion and exclusion criteria

b. Need of good sampling size

c. No invasive procedure that harm your health and helps in diagnosis and helpful for the society

14. How many people will be in the study? 90 individuals

15. Agreement of compensation to the participants (In case of a study related injury):

Patient will be taken care in case of complication and medical treatment will be provided in the institution.

16. Anticipated prorated payment, if any, to the participant(s) of the study: Not applicable

17. Can I withdraw from the study at any time during the study period?

- The participation in this research is purely voluntary and you have the right to withdraw from this study at any time during the course of the study without giving any reasons.
- However, it is advisable that you talk to the research team prior to stopping information.

18. If there is any new findings/information, would I be informed? Yes

19. Expected duration of the participant's participation in the study:

1 month

20. Any other pertinent information: No other information

21. Whom do I contact for further information?

For any study related queries, you are free to contact :

P. Redwin Dhas Manchil
Post Graduate student.
Department of Oral Medicine and Radiology,
Sree Mookambika Institute of Dental Sciences,
Kulasekharam, KanyaKumari District-629161.
Mobile No: 09962450608
redwinmanchil@gmail.com

Place:

Date: **Signature of Principal Investigator**

Signature of the participant

CONSENT FORM

PART 2 OF 2

PARTICIPANTS CONSENT FORM

The details of the study have been explained to me in writing and the details have been fully explained to me. I am aware that the results of the study may not be directly beneficial to me but will help in the advancement or medical sciences. I confirm that I have understood the study and had the opportunity to ask questions. I understand that my participation in the study is voluntary and that I am free **to** withdraw at any time, without giving any reason, without the medical care that will **normally** be provided by the hospital being affected. I agree not to restrict the use of any data or results that arise from this study provided such a use is only for scientific purpose(s). I have been given an information sheet giving details of the study. I fully consent to participate in the study titled

"Estimation of serum Levo–Fucose (Tumor marker) level among patients with oral cancer of various TNM (Tumor Node Metastasis) stages."

Serial no / Reference no:

Name of the participant: Address of the participant:

Contact number of the participant:

Signature / thumb impression of the participant / Legal guardian

Witnesses:

1.

2.

Date:

Place:

ஒப்புதல் படிவம்

பகுதி I - 2

ஆய்வில் பங்கேற்பவருக்கான தகவல் படிவம்

மதிப்பிற்குரிய தன்னார்வலரே,

தாங்கள் இந்த ஆய்வில் பங்கேற்க வந்தமைக்கு நன்றி. இந்த ஆய்வில் பங்கேற்பதற்கு முன் இந்த ஆய்வின் முக்கியத்துவம் மற்றும் தன்மை அறிந்து கொள்ள வேண்டும். இந்த படிவம் ஆய்வின் முழுவிபரங்களையும் உங்களுக்கு தெரிவிக்கும். ஆய்வில் பங்கேற்பதற்கு முன் இந்த படிவத்தை முழுவதும் படித்து புரிந்து கொண்ட பின் பங்கேற்க வேண்டும். இந்த படிவத்தில் சில மருத்துவ மற்றும் அறிவியல் சொற்கள் உள்ளன. ஆதலால் எதேனும் சந்தேகங்கள் ஏற்பட்டால் இந்த படிவத்தின் இறுதியில் குறிப்பிட்டுள்ள நபரிடம் ஆய்வில் பங்கேற்பதற்கு முன் தெளிவு பெற வேண்டும்

1. ஆய்வாளர்

மரு. பி. ரெட்வின் தாஸ் மன்சில்
முதுகலை பட்டதாரி
ஓரல் மெடிசின் மற்றும் ரேடியோலஜி
ஸ்ரீ மூகாம்பிகா பல் மருத்துவமனை கல்லூரி, குலசேகரம்,
கன்னியாகுமரி

2. வழிகாட்டி

மரு. ஹேமா. ஜி.
ரீடர் (துணை பேராசிரியர்)
ஓரல் மெடிசின் மற்றும் ரேடியோலஜி
ஸ்ரீ மூகாம்பிகா பல் மருத்துவமனை கல்லூரி, குலசேகரம்,
கன்னியாகுமரி

3. துணை வழிகாட்டி

மரு. ஜெ. யூஜினியா செருபின்
ரீடர் (துணை பேராசிரியர்)
ஓரல் மெடிசின் மற்றும் ரேடியோலஜி
ஸ்ரீ மூகாம்பிகா பல் மருத்துவமனை கல்லூரி, குலசேகரம்,
கன்னியாகுமரி

4. ஆய்வு நிலையம்

ஸ்ரீ மூகாம்பிகா பல் மருத்துவமனை கல்லூரி, குலசேகரம்,
கன்னியாகுமரி மாவட்டம், தமிழ்நாடு.

5. தலைப்பு : டி.என்.எம். அளவுகோல் முலம் பிரிக்கப்பட்ட புற்றுநோயளிகளின் சீரத்தில் உள்ள எல்பியுகோஸின் அளவை கண்டறிதல்.

6. ஆய்வின் பின்பலம் :

இயல்பாக எல்லா நபர்களிடமும் எல் பியுக்கோவின் அளவு குறைவாகவே காணப்படும். பல ஆய்வுகளில் எல் பியுக்கோவின் அளவு புற்றுநோயாளிகளிடம் அதிகமாக காணப்படும் என நிரூபிக்கப்பட்டுள்ளது. இதன் அளவைக் கொண்டு புற்று நோயின் நிலையை ஆரம்ப நிலையிலே கண்டுபிடிக்க உதவும்.

7. குறிக்கோள் :

- பல்வேறு டி.என்.எம். நிலைகளில் உள்ள புற்றுநோயாளிகளின் சீரம் எல்.பியுக்கோலின் அளவை மதிப்பிடுதல்.
- புற்றுநோயாளிகளின் சீரத்தில் உள்ள எல்.பியுக்கோலின் அளவை மதிப்பிடுதல்.
- ஆரோக்கியமான நபர்களின் எல். பியுக்கோலின் அளவை மதிப்பிடுதல்.
- திசுக்களின் கூறுகளின் மூலம் அறியப்பட்ட புற்றுநோயாளிகளின் நிலையை எல்.பியுக்கோலின் சீரத்தை கொண்டு மதிப்பிடுதல்.
- பல்வேறு வயதினில் உள்ள புற்றுநோயாளிகளின் எல். பியுக்கோலின் மதிப்பிடுதல்.
- புற்றுநோயாளிகள் மற்றும் ஆரோக்கியமான நபர்களின் எல் பியுக்கோலின் அளவை ஒப்பிடுதல்.
- எல். பியுக்கோலின் அளவு ஆண் மற்றும் பெண் பாலின புற்றுநோயாளிகளிடம் ஒப்பிடுதல்.

8. அறிவியல் சான்று :

புற்றுநோயின் ஆரம்ப நிலையை கண்டறிதல் கடினமானது. எனவே அதனை கண்டறிய பல அளவுகோல்களை பயன்படுத்தப்படுகிறது. ஆகவே எல்.பியுக்கோல் பயன்படுத்தி புற்றுநோயின் சரியான நிலையை கண்டறிய முடியும் என பல ஆய்வுகள் வழிகாட்டுகின்றது.

9. செயல்முறை :

- ✧ ஆய்வில் பங்கேற்பின் தங்களுக்கான தனி இலக்க எண் வழங்கப்படும்.
- ✧ விரிவான மருத்துவ வரலாறு மற்றும் குறிப்புகள் பெறப்படும்.
- ✧ நோயின் பாதிக்கப்பட்ட பகுதியை சோதனை செய்து அதன் குறிப்புகள் பதிவு செய்யப்படும்.
- ✧ புகைப்படங்கள் எடுக்கப்படும்.
- ✧ ஊடுகதிர் ஆய்வு செய்யப்படும்.
- ✧ இரத்த பரிசோதனைக்காக இரத்தமாதிரி எடுக்கப்படும்
- ✧ இரத்தத்தின் எல். பியுக்கோல் அளவு கண்டுபிடிக்கப்படும்.
- ✧ பரிசோதனைக்காக சதை கூறுகள் அறுவை சிகிட்சை மூலம் பெறப்படும்.

10. எதிர்மறை விளைவுகள் :

- ஊசி போடும் போது சிறிதாக வலி ஏற்பட வாய்ப்பு உள்ளது.
- இரத்த தேவைக்காக சில நேரங்களில் பலமுறை ஊசி செலுத்தப்பட வாய்ப்பு உள்ளது.

11. ஏற்படும் நன்மைகள் :

- ஆய்விற்காக பணம் எதுவும் தர தேவையில்லை
- ஆய்வின் முடிவுகளை நீங்கள் அறிந்து கொள்ளலாம்.

12. தகவலின் பாதுகாப்பு :

- உங்களுடைய தகவல்கள் அனைத்தும் பாதுகாக்கப்படும் எந்த நிலையிலும் வெளியிடப்படாது.
- நீங்கள் இந்த ஆய்வில் பங்கேற்றபின் எங்களது ஆய்வாளர் உங்களுடைய தகவல்களை தெரிந்து கொள்ள முடியும். மேலும் தேவை ஏற்பட்டால் எத்திக்கல் கமிட்டியிடமும் காண்பிக்கப்படும்.
- ஆய்வின் முடிவுகள் உங்களுடைய மருத்துவ தகவல் படிவத்தில் (கோப்பில்) பதிவு செய்யப்படும்.
- இந்த ஆய்வு முடிவு வெளியிடப்படும் பொழுது உங்களது தகவல்கள் வெளியிடப்படாது.

13. இந்த ஆய்வில் நீங்கள் சேர்வதற்கான காரணம்?

- நீங்கள் இந்த ஆய்வின் சேர்ப்பு மற்றும் விடுப்பு வரம்புகளின் உள் அமையப்படுவதால்
- ஆய்வின் தேவையான மாதிரிக்காகவும்
- சமூகத்தின் மற்றும் மருத்துவ முன்னேற்றத்திற்காக

14. ஆய்வின் மாதிரி எண்ணிக்கை : 90 நபர்கள்

15. நஷ்டயீடு :

ஆய்வில் ஏற்படும் மாற்றங்களுக்கான சிகிட்சை ஆய்வு நிலையத்தில் வழங்கப்படும்.

16. நஷ்டயீடு பணம் : பணம் எதுவும் வழங்கபடாது.

17. ஆய்வில் இருந்து எந்த நேரத்திலும் எந்த காரணமும் இன்றி விலகலாம்

18. ஆய்வின் முன்னேற்றத்தில் ஏற்படும் மாற்றங்கள் தெரிவிக்கப்பட வேண்டும்

19. ஆய்வின் கால அவகாசம் : ஒரு ஆண்டு

20. வேறு எந்த தகவல்களும் தேவை இல்லை

21. தொடர்பு கொள்ள வேண்டிய நபர் :

மரு. பி. ரெட்வின் தாஸ் மன்சில்
முதுகலை பட்டதாரி,
ஒரல் மெடிசின் மற்றும் ரேடியோலஜி,
ஸ்ரீ முகாம்பிகா பல் மருத்துவமனை கல்லூரி, குலசேகரம்.
தொலைபேசி எண் : 9962450608
மின் அஞ்சல் :

இடம் :

ஆய்வாளர் கையொப்பம்

தேதி :

பங்குபெறுபவர் கையொப்பம்

ஒப்புதல் படிவம்

பாகம் : 2

பங்குபெறுபவரின் ஒப்புதல் படிவம்

இந்த ஆய்வின் முழு விபரங்களும் எழுத்து மூலமாகவும், விரிவாகவும், என்னிடம் கூறப்பட்டது. எனக்கு இந்த ஆய்வின் விளைவுகள் எந்த பயனும் தரவில்லை என்றாலும், மருத்துவ அறிவியலின் மேம்பாட்டிற்காக பயன்படுத்தப்படும் என்று அறிவேன். நான் இந்த ஆய்வில் எந்த நிர்பந்தமும் இல்லாமல் பங்குபெறுகிறேன். மேலும் இந்த ஆய்வின் எல்லா விதிகள் மற்றும் விதிமுறைகளையும் அறிவேன். எந்த சந்தோகத்திற்கும் கேள்வி கேட்கவோ அல்லது ஆய்வில் இருந்து விலகவோ எல்லா உரிமைகளும் எனக்கு உண்டு என்று அறிவேன். இந்த ஆய்வின் விளைவுகள் எந்த அறிவியல் பயன்பாட்டிற்கும் பயன்படுத்த எனக்கு தடையில்லை. எனக்கு இதற்கு முன்பாக ஆய்வின் தகவல் படிவம் கொடுக்கப்பட்டது. எனக்கு இந்த ஆய்வில் பங்குபெற முழு சம்மதம்.

ஆய்வின் பெயர் :

டி.என்.எம். அளவுகோல் மூலம் பிரிக்கப்பட்ட புற்றுநோயணுக்களின் சீரத்தில் உள்ள எல்.பியுகோலின் அளவை கண்டறிதல்.

குறிப்பு எண் :

பெயர் :

முகவரி :

தொலைபேசி எண் : கையொப்பம்

சாட்சி : 1

2

இடம் :

தேதி :

സമ്മതപത്രം - 1

പഠനവുമായി സഹകരിക്കുന്ന വ്യക്തികളുടെ അറിവിലേയ്ക്ക്

പ്രിയപ്പെട്ട സന്നദ്ധ സേവകൻ/സേവക,

ഞങ്ങൾ നിങ്ങളെ സ്വാഗതം ചെയ്യുന്നു. അതോടൊപ്പം ഈ പഠനവുമായി സഹകരിക്കാനുള്ള സന്നദ്ധതയോട് നന്ദി രേഖപ്പെടുത്തുന്നു. നിങ്ങൾ ഈ പഠനത്തിൽ പങ്കെടുക്കുന്നതിന് മുൻപ് ഈ പഠനം എന്തിനാണ് നടത്തപ്പെടുന്നത് എന്ന് അറിയേതു്. അതിനാൽ ഈ ഫോറത്തിൽ ഗവേഷണപഠനത്തിന്റെ വിവരങ്ങളും മറ്റും വിശദമായി രേഖപ്പെടുത്തിയിരിക്കുന്നു. ഈ പഠനത്തിന്റെ രീതി, ഉദ്ദേശം, പ്രയോജനം, അപകടസാദ്ധ്യത, ക്ലേശം, മുൻകരുതൽ, എങ്ങനെ ഈ പഠനം മുൻപോട്ടു കൊുപോകുന്നു എന്നിങ്ങനെ എല്ലാ വിവരങ്ങളും ഫോറത്തിൽ രേഖപ്പെടുത്തിയിരിക്കുന്നു. സദയം ഈ വിവരങ്ങൾ വായിച്ചു മനസ്സിലാക്കുവാൻ അഭ്യർത്ഥിക്കുന്നു. ഈ വിവരങ്ങളിൽ ശാസ്ത്രപരമായ പദങ്ങൾ ഉള്ളതിനാൽ സംശയനിവാരണത്തിനു പ്രധാന പഠനകർത്താവിനോടോ താഴെ രേഖപ്പെടുത്തിയിരിക്കുന്ന വ്യക്തികളോടോ ഫോറം ഒപ്പിടുന്നതിനു മുൻപോ അല്ലെങ്കിൽ ഈ പഠനത്തിന്റെ കാലാവധി തീരുന്നവരെയോ സമീപിക്കാവുന്നതാണ്.

1. **പ്രധാന പഠനകർത്താവ്/ഗവേഷകൻ**

ഡോ. റെഡ്‌വിൻ ദാസ് മൻസിൽ പി.
ബിരുദനാന്തര ബിരുദ വിദ്യാർത്ഥി
ഡിപ്പാർട്ട്മെന്റ് ഓഫ് ഓറൽ മെഡിസിൻ ആന്റ് റേഡിയോളജി,
ശ്രീ മൂകാംബിക ഇൻസ്റ്റിറ്റ്യൂട്ട് ഓഫ് ഡെന്റൽ സയൻസസ്,
വി.പി. എം. ഹോസ്പിറ്റൽ കോംപ്ലക്സ്, പടനിലം
കുലശേഖരം, കന്യാകുമാരി - 629 161

2. **പ്രധാന മാർഗ്ഗദർശി**

ഡോ. ഹേമ.ജി
റീഡർ
ഡിപ്പാർട്ട്മെന്റ് ഓഫ് ഓറൽ മെഡിസിൻ ആന്റ് റേഡിയോളജി,
ശ്രീ മൂകാംബിക ഇൻസ്റ്റിറ്റ്യൂട്ട് ഓഫ് ഡെന്റൽ സയൻസസ്,
വി.പി. എം. ഹോസ്പിറ്റൽ കോംപ്ലക്സ്, പടനിലം
കുലശേഖരം, കന്യാകുമാരി - 629 161

3. **സഹമാർഗ്ഗദർശി**

ഡോ. ജെ.യൂജ്നീനാ ഷെറുബിൻ
ഡിപ്പാർട്ട്മെന്റ് ഓഫ് ഓറൽ മെഡിസിൻ ആന്റ് റേഡിയോളജി,
ശ്രീ മൂകാംബിക ഇൻസ്റ്റിറ്റ്യൂട്ട് ഓഫ് ഡെന്റൽ സയൻസസ്,
വി.പി. എം. ഹോസ്പിറ്റൽ കോംപ്ലക്സ്, പടനിലം
കുലശേഖരം, കന്യാകുമാരി - 629 161

4. **പഠനകേന്ദ്രം**

ശ്രീ.മൂകാംബിക ഇൻസ്റ്റിറ്റ്യൂട്ട് ഓഫ്
ഡെന്റൽ സയൻസ്, വി.പി.എം.
ഹോസ്പിറ്റൽ കോംപ്ലക്സ്, പടനിലം,
കുലശേഖരം, കന്യാകുമാരി 629 161

5. **പഠനത്തിന്റെ ശീർഷകം**

വായിൽ അർബുദം ബാധിച്ച രോഗികളിൽ ടി.എൻ.എം. സ്റ്റേജനുസരിച്ച് രക്തത്തിൽ എൽഫ്യൂക്കോസിന്റെ (ട്യൂമർ മാർക്കർ) അളവ് നിർണ്ണയം

6. **പശ്ചാത്തല വിവരം:** സാധാരണയായി ചെറിയ തോതിൽ രക്തത്തിൽ കാണപ്പെടുന്ന എൽ-ഫ്യൂക്കോസ്, പക്ഷെ അർബുദ രോഗികളിൽ ഉയർന്ന തോതിൽ കാണപ്പെടുനനു. അതിനാൽ എൽ- ഫ്യൂക്കോസിന്റെ വ്യതിയാനങ്ങൾ പ്രാരംഭഘട്ടത്തിലെ രോഗനിർണ്ണയത്തിനും, തുടർചികിത്സയ്ക്കും സഹായകരമാകുമെന്ന് പല പഠനങ്ങളിലും തെളിയിക്കപ്പെട്ടിട്ടു്. അർബുദം ബാധിച്ച രോഗികളിൽ ഇത്തരം ബയോമാർക്കറുകളുടെ സഹായത്തോടെ അർബുദം നേരത്തെ കുപിടിക്കാൻ സാധിക്കുന്നതാണ്.

7. **ലക്ഷ്യങ്ങളും ഉദ്ദേശങ്ങളും:** 1) വിവിധ ടി.എൻ.എം. സ്റ്റേജുകളിലുള്ള അർബുദ രോഗികളുടെ രക്തത്തിൽ എൽ-ഫ്യൂക്കോസിന്റെ അളവ് നിർണ്ണയിക്കുക

2) അർബുദ രോഗികളിൽ രക്തത്തിൽ എൽ-ഫ്യൂക്കോസിന്റെ അളവ് നിർണ്ണയിക്കുക

3) ആരോഗ്യമുള്ള വ്യക്തികളുടെ രക്തത്തിൽ എൽ-ഫ്യൂക്കോസിന്റെ അളവ് നിർണ്ണയിക്കുക

4) ഹിസ്റ്റോപത്തോളജിക്കൽ തലത്തിൽ വിവിധ വിഭാഗങ്ങളായി തരം തിരിക്കപ്പെട്ട അർബുദ രോഗികളുടെ രക്തത്തിൽ എൽ-ഫ്യൂക്കോസ് അളവ് നിർണ്ണയം

5) രക്തത്തിലുള്ള എൽ ഫ്യൂക്കോസിന്റെ അളവ് വിവിധ പ്രായത്തിലുള്ളവരിൽ നിർണ്ണയിക്കുക

6) ആരോഗ്യമുള്ള വ്യക്തികളുടെയും, അർബുദം ബാധിച്ച രോഗികളുടെയും രക്തത്തിലുള്ള എൽ-ഫ്യൂക്കോസിന്റെ അളവ് താരതമ്യമപ്പെടുത്തുക

7) അർബുദം ബാധിച്ച സ്ത്രീകളുടെയും, പുരുഷൻമാരുടെയും രക്തത്തിലുള്ള എൽ-ഫ്യൂക്കോസിന്റെ അളവ് താരതമ്യപ്പെടുത്തുക.

8. **ഗവേഷണം നടത്തുവാനുള്ള ന്യായീകരണം:** അർബുദ രോഗനിർണ്ണയവും, അതിന്റെ വ്യാപ്തി കുപിക്കുന്നതും പ്രാരംഭഘട്ടത്തിൽ പ്രയാസമുള്ളതാകുന്നു. അതിനായി രക്തത്തിലുള്ള പലതരം ട്യൂമർ മാർക്കറുകളുടെ അളവ് സഹായകമാണ്. ഇതിൽ തന്നെ എൽ-ഫ്യൂക്കോസിന്റെ അളവും, വ്യതിയാനങ്ങളും രോഗത്തിന്റെ വിവിധ ഘട്ടങ്ങളുടെ വ്യാപ്തിയേയും സൂചിഷിക്കുന്നു.

9. **പഠനരീതി:**

1) പഠനത്തിന്റെ ഭാഗമായി വ്യക്തിക്ക് ഒരു നമ്പർ നൽകുന്നതാണ്.
2) നിങ്ങളുടെ മുഴുവൻ വിവരങ്ങളുമടങ്ങിയ കേസ് ഹിസ്റ്ററി രേഖപ്പെടുത്തുന്നതാണ്.
3) ആവശ്യമെങ്കിൽ പരിശോധന നടത്തപ്പെടുത്തുന്നതാണ്
4) ആവശ്യാനുസരണമായി ചിത്രങ്ങൾ എടുക്കാവുന്നതാണ്.
5) എക്സ്റേ പരിശോധന നടത്തുന്നു.
6) എൽ-ഫ്യൂക്കോസിന്റെ അളവ് നിർണ്ണയത്തിനായി രക്ത സാംപിളുകൾ എടുക്കുന്നു.
7) രോഗവ്യാപ്തി നിർണ്ണയിക്കുന്നതിനായി കലകലോ, ദ്രവമോ നീക്കി പരിശോധനയ്ക്ക് എടുക്കുന്നതാണ്.
8) പരിശോധനകൾക്കൊടുവിൽ നിങ്ങളെ തുടർചികിത്സയ്ക്കായി അയക്കുന്നതാണ്.

10) **പ്രതീക്ഷിക്കുന്ന അപകടസാദ്ധ്യതകൾ**

1) രക്ത സാംപിൾ എടുക്കുമ്പോഴുള്ള ചെറിയ വേദന
2) ശരിയായ അളവിൽ രക്തം കിട്ടിയില്ലെങ്കിൽ മാത്രം വീണ്ടും സാംപിൾ എടുക്കേണ്ടി വരുന്നു.

11) **പ്രതീക്ഷിക്കാവുന്ന പ്രയോജനങ്ങൾ**

1) ലാബ് ടെസ്റ്റുകൾക്ക് നിങ്ങൾ തുക നൽകേണ്ടതില്ല
2) നിങ്ങൾക്ക് ഈ ഗവേഷണത്തിന്റെ വിവരങ്ങൾ തിരക്കാവുന്നതാണ്.
3) നിങ്ങളുടെ ചികിത്സ കൂടുതൽ മെച്ചപ്പെടുത്താൻ ഈ പഠനം സഹായിക്കുന്നു.

12) **വ്യക്തിവിവരങ്ങളുടെ സ്വകാര്യത:**

1) രോഗവിവരങ്ങളും മറ്റ് വ്യക്തിവിവരങ്ങളും സ്വകാര്യമായി സൂക്ഷിക്കപ്പെടുന്നതായിരിക്കും
2) ഈ ഫോറത്തിൽ ഒപ്പിടുന്നത് വഴി നിയമം അനുശാസിക്കുന്ന രീതിയിൽ പഠനത്തിൽ ഉൾപ്പെടുന്ന വ്യക്തികൾക്ക് നിങ്ങളുടെ വിവരങ്ങൾ പരിശോധിക്കാവുന്നതാണ്.
3) ഈ പഠനത്തിന്റെ വിവരങ്ങൾ ശാസ്ത്രാനുപാധികളായ പ്രസിദ്ധീകരണങ്ങളിലോ, കൂടി ആലോചനകളിലോ വെളിപ്പെടുത്തുമ്പോൾ നിങ്ങളുടെ സ്വകാര്യത സൂക്ഷിക്കപ്പെടുന്നതാണ്

13) **എന്തുകൊ് നിങ്ങൾ തിരഞ്ഞെടുക്കപ്പെട്ടു?**

1) പഠനത്തിന് നല്ല ശതമാനം ആളുകൾ ആവശ്യമാണ്.

2) പല കൂട്ടിക്കുറച്ചിലുകൾക്കൊടുവിൽ നിങ്ങൾ ഉൾപ്പെടുന്ന വിഭാഗത്തെ തിരഞ്ഞെടുത്തു

3) നിങ്ങളുടെ സഹകരണം മൂലം സമൂഹത്തിന് സഹായവും നന്മയും ഉാകുന്നു.

14) **എത്ര ആളുകൾ ഈ പഠനത്തിൽ ഉൾപ്പെടുന്നു?**

90

15) **നഷ്ടപരിഹാര ഉടമ്പടി? (പഠനവുമായി ബന്ധപ്പെട്ട് എന്തെങ്കിലും പരിക്കുായാൽ)**

പഠനവിധേയമായി ഏതെങ്കിലും തരത്തിൽ രോഗം സങ്കീർണ്ണമായാൽ രോഗിയെ ഈ സ്ഥാപനത്തിൽ വിദഗ്ദ്ധ ചികിത്സക്കു വിധേയനാക്കുന്നതാണ്.

16) **ഏതെങ്കിലും വിധത്തിൽ വേതനം ലഭിക്കുമോ?**

ഇല്ല

17) **എപ്പോൾ വേണമെങ്കിലും എനിക്ക് ഈ പഠനത്തിൽ നിന്ന് പിൻമാറാമോ?**

കാരണം വ്യക്തമാക്കാതെ എപ്പോൾ വേണമെങ്കിലും നിങ്ങൾക്ക് ഈ പഠനത്തിൽ നിന്നും പിന്മാറാവുന്നതാണ്. എങ്കിലും അതിന് മുൻപായി ഗവേഷകരുമായി സംസാരിക്കുന്നത് നല്ലതാണ്.

18) **പഠനവുമായി ബന്ധപ്പെട്ട എന്തെങ്കിലും പുതിയ വിവരങ്ങൾ ഉങ്കിൽ എന്നെ അറിയിക്കുന്നതാണോ?**

അതെ

19) **പ്രതീക്ഷിക്കുന്ന പഠനകാലാവധി ?**

ഒരു വർഷം

20) **മറ്റെന്തെങ്കിലും വിവരം ?**

ഇല്ല

21) **വിവരങ്ങൾക്ക് ബന്ധപ്പെടേത് ആരെ?**

ഡോ. റെഡ്വിൻ ദാസ് മൻസിൽ.പി
ബിരുദാനന്തര ബിരുദ വിദ്യാർത്ഥി/നി
ഡിപ്പാർട്ട്മെന്റ് ഓഫ് ഓറൽ മെഡിസിൻ ആന്റ് റേഡിയോളജി,
ശ്രീ മൂകാംബിക ഇൻസ്റ്റിറ്റ്യൂട്ട് ഓഫ് ഡെന്റൽ സയൻസസ്,
വി.പി. എം. ഹോസ്പിറ്റൽ കോംപ്ലക്സ്, പടനിലം
കുലശേഖരം, കന്യാകുമാരി - 629 161
ഡോ. ഡോ. റെഡ്വിൻ ദാസ് മൻസിൽ.പി - 09962450608
ഇ.മെയിൽ,ഐ.ഡി.: redwinmanchil@gmail.com

സ്ഥലം: **പ്രധാന ഗവേഷകന്റെ ഒപ്പ്**

തീയതി **സന്നദ്ധ സേവകൻ/ സേവക**

സമ്മതപത്രം - 2

പഠനത്തിലുള്ള എന്റെ പങ്കാളിത്തം സ്വന്തം താൽപര്യപ്രകാരം മാത്രമാണെന്നും, എപ്പോൾ വേണമെങ്കിലും ചോദ്യങ്ങൾ ബന്ധപ്പെട്ടവരോട് ചോദിക്കാമെന്നും, ഈ പഠനത്തിൽ നിന്നും കാരണം രേഖപ്പെടുത്താതെ എപ്പോൾ വേണമെങ്കിലും എനിക്ക് പിൻവാങ്ങാമെന്നും ഞാൻ മനസ്സിലാക്കുന്നു. ഈ പഠനാവസാനം വെളപ്പെടുന്ന അറിവുകളും, രേഖകളും, ശാസ്ത്രപരമായ ഉദ്ദേശങ്ങൾക്കു ഉപയോഗിക്കാൻ ഞാൻ സമ്മതിക്കുന്നു. പഠനോദ്ദേശം വിവരിക്കുന്ന വിശദാംശങ്ങൾ നൽകിയിട്ടു്. "വായിൽ അർബുദം ബാധിച്ച രോഗികളിൽ ടി.എൻ.എം. സ്റ്റേജുനുസരിച്ച് രക്തത്തിൽ എൽഫ്യൂക്കോസിന്റെ (ട്യൂമർ മാർക്കർ) അളവ് നിർണ്ണയം" എന്ന ഈ പഠനവുമായി സഹകരിക്കാൻ എന്റെ പരിപൂർണ്ണ സമ്മതം അറിയിക്കുന്നു.

ഡോ.റെഡ്വിൻ ദാസ് മൻസിൽ.പി

പേര് (സന്നദ്ധ സേവകൻ/സേവക)
ബന്ധപ്പെടാനുള്ള നമ്പർ

സന്നദ്ധ സേവകന്റെയോ/
സേവകയുടെയോ വിലാസം

സാക്ഷികൾ

1.

2.

ഒപ്പ്/വിരൽ അടയാളം
(സ്വന്തം/നിയമപരായ
സംരക്ഷകൻ)

തീയതി:

സ്ഥലം:

CASE RECORD

S.no:

Date:

I. DEMOGRAPHIC DATA:

a. Name
b. Age
c. Sex
d. Address
e. Occupation

II. CHIEF COMPLAINT:

III. HISTORY OF PRESENTING ILLNESS:

IV. HISTORY OF PAST ILLNESS:

a. Medical
b. Dental
c. Family

V. PERSONAL HISTORY:

VI. CLINICAL EXAMINATION:

VII. GENERAL EXAMINATION:

VIII. VITAL SIGNS:

a. Pulse
b. Temperature
c. Blood pressure
d. Respiratory rate

IX. LYMPH NODE EXAMINATION:

X. LOCAL EXAMINATION:

a. Extra oral examination
    i. Inspection
    ii. Palpation

XI. INTRAORAL EXAMINATION:

a. Soft tissue examination
    i. Inspection
    ii. palpation
b. Hard tissue examination
    i. Inspection
    ii. Palpation

XII. PROVISIONAL DIAGNOSIS:

XIII. INVESTIGATIONS:

a. Hematological
b. Biopsy
c. Serum L-Fucose Estimation

XIV. CONFIRMATORY DIAGNOSIS WITH TNM STAGING:

CONTROL GROUP

CONTROLS	AGE	SEX	FUCOSE LEVEL in mg/dl
1	28	MALE	3.31
2	29	MALE	3.84
3	29	MALE	3.67
4	27	MALE	3.52
5	30	MALE	3.41
6	42	FEMALE	3
7	44	MALE	3.96
8	35	FEMALE	3.82
9	47	MALE	3.94
10	50	FEMALE	3.51
11	55	MALE	3.02
12	60	FEMALE	3.09
13	63	MALE	3.17
14	70	FEMALE	3.59
15	61	MALE	2.89
16	32	MALE	4
17	30	FEMALE	2.99
18	41	MALE	4
19	48	FEMALE	3.06
20	37	MALE	3.09
21	39	FEMALE	3.81
22	30	MALE	3.41
23	41	MALE	3.8
24	44	MALE	3.62
25	46	MALE	3.73
26	38	FEMALE	3.64
27	43	FEMALE	3.77
28	51	FEMALE	3.33
29	44	FEMALE	3.05
30	34	FEMALE	3.21

CANCER GROUP

PATIENTS	AGE (years)	SEX	SITE	TNM STAGE	HISTOPATHALOGICAL GRADING	FUCOSE LEVEL in mg/dl
1	44	MALE	BUCCAL MUCOSA	1	WELL DIFFERENTIATED	8.26
2	65	MALE	BUCCAL MUCOSA	1	MODERATELY DIFFERENTIATED	8.01
3	40	MALE	BUCCAL MUCOSA	2	WELL DIFFERENTIATED	8.99
4	67	MALE	HARD PALATE	2	MODERATELY DIFFERENTIATED	9.05
5	42	MALE	BUCCAL MUCOSA	2	MODERATELY DIFFERENTIATED	9.2
6	48	MALE	ALVEOLUS	2	MODERATELY DIFFERENTIATED	9.03
7	55	MALE	HARD PALATE	2	MODERATELY DIFFERENTIATED	8.97
8	72	MALE	BUCCAL MUCOSA	2	WELL DIFFERENTIATED	9.87
9	50	MALE	BUCCAL MUCOSA	3	WELL DIFFERENTIATED	10.02
10	60	MALE	TONGUE	3	MODERATELY DIFFERENTIATED	10.54
11	56	MALE	TONGUE	3	WELL DIFFERENTIATED	10.77
12	60	MALE	ALVEOLUS	3	WELL DIFFERENTIATED	10.88
13	55	MALE	TONGUE	3	MODERATELY DIFFERENTIATED	10.27
14	34	MALE	TONGUE	3	WELL DIFFERENTIATED	10.48
15	61	MALE	ALVEOLUS	3	WELL DIFFERENTIATED	10.89
16	49	MALE	TONGUE	3	MODERATELY DIFFERENTIATED	10.66
17	60	MALE	TONGUE	3	WELL DIFFERENTIATED	10.88
18	74	MALE	TONGUE	3	MODERATELY DIFFERENTIATED	11.43
19	72	MALE	TONGUE	3	WELL DIFFERENTIATED	10.85
20	55	MALE	BUCCAL MUCOSA	3	WELL DIFFERENTIATED	10.34
21	70	MALE	BUCCAL MUCOSA	3	WELL DIFFERENTIATED	10.25
22	65	FEMALE	BUCCAL MUCOSA	3	WELL DIFFERENTIATED	10.25
23	42	MALE	ALVEOLUS	3	WELL DIFFERENTIATED	10.25
24	41	FEMALE	ALVEOLUS	3	MODERATELY DIFFERENTIATED	10.72

25	39	FEMALE	ALVEOLUS	3	WELL DIFFERENTIATED	10.23
26	40	MALE	BUCCAL MUCOSA	3	MODERATELY DIFFERENTIATED	10.12
27	54	MALE	BUCCAL MUCOSA	3	WELL DIFFERENTIATED	10.29
28	60	FEMALE	HARD PALATE	3	WELL DIFFERENTIATED	10.84
29	28	FEMALE	TONGUE	3	WELL DIFFERENTIATED	10.29
30	62	FEMALE	HARD PALATE	3	WELL DIFFERENTIATED	10.43
31	47	MALE	TONGUE	4	WELL DIFFERENTIATED	11.34
32	62	MALE	TONGUE	4	WELL DIFFERENTIATED	11.89
33	67	MALE	ALVEOLUS	4	MODERATELY DIFFERENTIATED	11.42
34	50	MALE	TONGUE	4	WELL DIFFERENTIATED	11.9
35	65	MALE	TONGUE	4	MODERATELY DIFFERENTIATED	11.25
36	55	MALE	BUCCAL MUCOSA	4	MODERATELY DIFFERENTIATED	11.99
37	76	MALE	TONGUE	4	WELL DIFFERENTIATED	11.13
38	55	MALE	TONGUE	4	WELL DIFFERENTIATED	11.87
39	65	MALE	BUCCAL MUCOSA	4	MODERATELY DIFFERENTIATED	11.23
40	57	MALE	TONGUE	4	WELL DIFFERENTIATED	11.45
41	55	MALE	TONGUE	4	MODERATELY DIFFERENTIATED	11.98
42	65	MALE	TONGUE	4	MODERATELY DIFFERENTIATED	12.54
43	62	MALE	TONGUE	4	POORLY DIFFERENTIATED	12.11
44	40	MALE	TONGUE	4	MODERATELY DIFFERENTIATED	12.09
45	57	MALE	BUCCAL MUCOSA	4	POORLY DIFFERENTIATED	11.96
46	61	FEMALE	BUCCAL MUCOSA	4	MODERATELY DIFFERENTIATED	11.29
47	48	MALE	TONGUE	4	MODERATELY DIFFERENTIATED	11.11
48	35	MALE	HARD PALATE	4	WELL DIFFERENTIATED	11.93
49	55	FEMALE	TONGUE	4	MODERATELY DIFFERENTIATED	12.11

50	61	MALE	HARD PALATE	4	MODERATELY DIFFERENTIATED	11.14
51	35	MALE	TONGUE	4	MODERATELY DIFFERENTIATED	12.29
52	61	MALE	TONGUE	4	MODERATELY DIFFERENTIATED	11.55
53	60	FEMALE	BUCCAL MUCOSA	4	MODERATELY DIFFERENTIATED	11.26
54	49	FEMALE	TONGUE	4	WELL DIFFERENTIATED	11.87
55	70	MALE	TONGUE	4	WELL DIFFERENTIATED	11.09
56	68	MALE	ALVEOLUS	4	MODERATELY DIFFERENTIATED	11.26
57	45	MALE	BUCCAL MUCOSA	4	MODERATELY DIFFERENTIATED	11.98
58	62	MALE	ALVEOLUS	4	WELL DIFFERENTIATED	10.55
59	39	MALE	TONGUE	4	WELL DIFFERENTIATED	11.21
60	55	MALE	TONGUE	4	WELL DIFFERENTIATED	11