# "THE DIAGNOSTIC USES OF SERUM ADENOSINE DEAMINASE, 5à- NUCLEOTIDASE AND RETINOL IN ORAL AND LARYNGEAL CANCERS - A CROSS-SECTIONAL STUDY"


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# LIST OF ABBREVIATIONS USED

| | | |
|---|---|---|
| ACE | - | Angiotensin converting enzyme |
| ADA | - | Adenosine deaminase |
| 5′ - AMP | - | 5′- Adenosine monophosphate |
| ATP | - | Adenosine triphosphate |
| Brd U | - | Bromo deoxyuridine |
| CEA | - | Carcinomaembryonic antigen |
| CT | - | Computed tomography |
| DNA | - | Deoxyribo nucleic acid |
| DOA | - | Date of admission |
| D/W | - | Distilled water |
| ECM | - | Extra cellular matrix |
| EGFR | - | Epidermal growth factor Receptor |
| EHNA | - | Erythro-9-(2-hydroxy-3-nonyl) Adenine |
| FNAC | - | Fine needle aspiration cytology |
| FNCB | - | Fine needle cutting biopsy |
| FC | - | Flow cytometry |
| HIV | - | Human immuno deficiency virus |
| HNSCC | - | Head and neck squamous cell Carcinoma |
| HPV | - | Human papilloma virus |

| | | |
|---|---|---|
| IHC | - | Immuno histochemistry |
| ISH | - | In situ hyridisation |
| LOH | - | Loss of heterozygosity |
| MRI | - | Magnetic resonance imaging |
| OP No. | - | Out patient department number |
| 5′-NT | - | 5 ′- Nucleotidase |
| PALB | - | Prealbumin |
| PCNA | - | Proliferating cell nuclear antigen |
| PCR | - | Polymerase chain reaction |
| PET Scan | - | Positron emission tomography scan |
| RBP | - | Retinol binding protein |
| ROS | - | Reactive oxygen species |
| S | - | Standard |
| SB | - | Standard blank |
| SCCA | - | Squamous cell carcinoma antigen |
| SCID | - | Severe combined immuno Deficiency |
| S.D | - | Standard deviation |
| SI. No. | - | Serial number |
| SMF | - | Submucosal fibrosis |
| T | - | Test |
| TA-4 | - | Tumor antigen – 4 |
| U/L | - | Units/Liter |
| USG | - | Ultrasound |

# ABSTRACT

## BACKGROUND AND OBJECTIVES:

Oral and laryngeal cancers are the most common type of head and neck cancers in India. Oral cancers account for 9.4% of all head and neck cancers whereas laryngeal cancer account for 4% of all head and neck cancers. The disease process has better prognosis if it is diagnosed early. In view of this the objective of the study was to assess the reliability of non-enzymatic anti-oxidant retinol and serum enzyme markers adenosine deaminase (ADA) and 5′-Nucleotidase (5′-NT) in oral and laryngeal cancer patients as supportive parameters for diagnostic purpose.

## METHODOLOGY:

The present cross-sectional study comprised of 50 healthy subjects and 50 clinically and histopathologically confirmed patients of oral and laryngeal cancer age and sex matched attending the outpatient department of the Otorhinolaryngology and Head and Neck Surgical Oncology Services of K.L.E.S. Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum during February 2009 to January 2010. Serum enzyme markers ADA, 5′-NT and non-enzymatic antioxidant retinol were evaluated.

## RESULTS:

In the present study, mean serum ADA level in controls was 19.8 ± 4.25 U/L while in patients with oral cancer and laryngeal cancer patients it was 41.1 ± 8.77 U/L and 48.4 ± 10.78 U/L respectively. The mean serum 5′-NT level in controls was 8.7 ±

4.15 U/L while in patients with oral cancer and laryngeal cancer patients it was 49 ± 10.45 U/L and 62.6 ± 13.96 U/L respectively. The mean serum retinol levels in controls was 41.1 ± 9.37 μg/dl while in patients with oral and laryngeal cancer it was 22.3 ± 5.81 μg/dl and 20.1 ± 6.53 μg/dl respectively.

## INTERPRETATION AND CONCLUSION:

The present study showed a significant increase in serum Adenosine deaminase and 5′- Nucleotidase levels in oral and laryngeal cancer patients as compared to controls with p value < 0.000 and a significant decrease in serum retinol levels in oral and laryngeal cancer cases in comparison to controls with p value < 0.000. When various stages of oral and laryngeal cancer (Stage I, II, III and IV) were compared with controls, serum ADA and 5′-NT levels were significantly increased whereas serum retinol levels were significantly decreased.

The study concludes that serum ADA, 5′-NT and retinol may be used as supportive biochemical parameters for diagnostic purpose and may add further for prognostic information in oral and laryngeal cancers.

## KEYWORDS:

Oral cancer; Laryngeal cancer; Adenosine deaminase; 5′-Nucleotidase; Retinol.

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# INTRODUCTION

These days the world is heading towards various types of non-communicable diseases which are also known as the modern day epidemics. Amongst these cancers are the most prevalent whose etiopathogenesis still remains obscure[1].

Tumors of the head and neck comprise an important group of neoplasia, the incidence of which is high and increasing despite all advances in modern medicine[2].

It is the 6$^{th}$ most common cancer in the world and 640,000 people are diagnosed with the disease each year[3]. This malignancy is more prevalent in the developing world and unfortunately has not received satisfactory attention as the other more prevalent cancers of the developed world like lung, breast, colon etc[2].

In India, head and neck cancers account for one third of all cancers and constitute 23% of all cancers in males and 6% in females[4]. The disproportionately higher prevalence of head and neck cancer in relation to other malignancies in India may be due to the use of tobacco in various forms, consumption of alcohol and low socioeconomic condition related to poor hygiene, poor diet or infections of viral origin. The age-standardized incidence rates are in excess of 30/100,000 per annum in males and 10/100,000 per annum in females[4].

In tobacco users, the oral cavity bears the brunt of the carcinogens and nearly 80,000 oral cancers are diagnosed every year in the country. Since, the betel juice produced by chewing the tobacco and other ingredients of the betel quid subsequently come in contact with the supraglottis and the pyriform sinus, the incidence of laryngeal cancers is also considerably higher in the Indian population[5]. In India oral cancers account for 9.4% of all head and neck cancers whereas laryngeal cancer account for 4% of all head and neck cancers[5,6]. Early detection of cancer offers the best chance for cure. The goal is to diagnose cancer as early as possible so as to

reduce the morbidity and mortality. Since, the oral cavity is more accessible to complete examination it could be used in early detection of precancerous and cancerous lesions. But either due to ignorance or inaccessibility of medical care, the disease gets detected in the later stages (stage III and IV)[2]. The 5-year survival rate of early-stage oral cancer (stage I and II) is approximately 80% while survival drops to 19% for late stage disease[7]. For laryngeal cancers early detection is only possible for the glottic cancers as they present with early hoarseness of voice, whereas supraglottic and sub glottic cancers are mostly diagnosed in advanced stages[8].

Thus, there is a need for improvement in early detection of oral and laryngeal carcinomas, because in the initial stages, treatment is more effective resulting in better survival rate and also the morbidity is reduced.

A majority of the initial alterations in these lesions are not readily recognizable on clinical or histopathological examination. The serum levels of a variety of substances shows significant changes in head and neck cancer patients. Such substances are collectively called as biochemical tumor markers[9]. To date, most markers lack a high degree of specificity and sensitivity. However, serial measurement of markers showing elevated pre-treatment levels may help in the diagnosis and monitoring response to therapy. Other potential uses of tumor markers include earlier detection of recurrence and or metastasis and possible prediction of prognosis. Measurement of more than one marker seems to enhance the diagnostic accuracy of the test[9].

Enzymes are biological catalysts produced by the living cells and play an important role in the metabolic reaction of cells. They are present in much higher concentration inside cells and are released into the systemic circulation due to tumor necrosis or changes in the membrane permeability of cancer cells. With a few

exceptions most enzymes are not unique for a specific organ, therefore enzymes are the most suitable as non specific tumor markers. Elevated enzyme levels may signal the presence of a malignancy[10]. Latest serum markers emerging for detection of patients with squamous cell carcinoma of various head and neck cancers is SCC antigen and Cyfra 21-1. Although not tumor specific they are useful for monitoring follow-up, detecting recurrence and determining prognosis[11,12]. Other markers include EGFR, p53, NSE, ERA (MOC-31), Ki-67, Cyclin D1, bcl-2 these may help differentiate squamous from other tumors and also be useful in prognostication[13].

However the analytical methods involved for these are tedious and unapproachable for the general population since they are available only at sophisticated, well equipped centers and are expensive. Therefore there is need for simple biochemical investigations which can be easily assayed and are less expensive and can detect metastasis.

In view of this the present study has been undertaken to assess the clinical utility of some promising enzyme markers namely adenosine deaminase, 5′-nucleotidase and retinol which are accurate, easily identifiable and may be of some diagnostic and prognostic significance.

# OBJECTIVES

The objectives of the present study were:-

1. To estimate serum adenosine deaminase (ADA), 5′- nucleotidase (5′-NT) and retinol.

2. To assess the reliability of serum adenosine deaminase (ADA), 5′-nucleotidase (5′-NT) and retinol in oral and laryngeal cancer patients as supportive parameters for diagnostic purpose.

# REVIEW OF LITERATURE

The origin of the word cancer is credited to Hippocrates who used the terms "*carcinos*" and "*carcinoma*". In Greek these words refer to a "crab", because the finger-like spreading projections from a cancer call to mind the shape of a crab. Celsus later translated the Greek term into cancer, the Latin word for crab[14].

The most merciless and dreaded disease of the human body is cancer. As defined by the British Oncologist Willis "Cancer is an abnormal mass of tissue, the growth of which exceeds and is in-coordinated with that of the normal tissue and persists in the same excessive manner even after cessation of the stimuli which evoked the change"[15]. Cancer is one of the major threats to public health in the developed world and increasingly in the developing world. In developed countries cancer is the second most common cause of death[16].

There is a disproportionately higher incidence of head and neck cancer in relation to other malignancies in India. Recent studies confirm that oral cancer forms a large part of the cancer load in parts of India and is a serious public health problem. Oral cancer is any cancerous tissue growth located in the mouth and most commonly involves the lower lip, lateral borders of the tongue, floor of the mouth and the buccal mucosa[17].

The overall mortality rate for oral cancer remains high at approximately 50%, even with modern medical services, probably due to the advanced stage of the disease at presentation[2]. It is the most disfiguring type of cancer and often requires surgery that can grossly distort the patient's physical appearance. Moreover, patients who are treated with chemotherapy and or radiation often lose much or all of their sense of taste and develop

conditions such as mucositis or dysphagia that cause great difficulty in eating and maintaining their appropriate weight[18]. Oral cancer's high mortality rate is due to the fact that the disease is usually discovered in late stages, where the 5-year survival rate is only 30%-to-40%. But when discovered in early stages, the survival rate leaps to 80%-to-90%[18]. Earlier detection of oral lesions would therefore greatly improve the prognosis of these patients.

The incidence of laryngeal carcinoma is relatively low in comparison to that of carcinomas of all organs and makes up one quarter to one third of all cancers of the throat. Laryngeal cancer can be further classified into the glottic cancers when it involves vocal cords, supraglottic cancers when it involves areas of larynx above the vocal cords and subglottic cancers when areas of larynx below the level of vocal cords are involved[8]. It is a disease that usually affects older men. Most patients are diagnosed in their fifties or sixties, and it only rarely occurs in younger people. Screening of high risk patients (chronic smokers) may prove beneficial in early detection though it is not advocated by most physicians[19].

## HISTORY

Human cancer is probably as old as the human race. Evidence of head and neck carcinomas has been found in ancient skulls. The oldest available specimen of a human cancer was found in the remains of skull of a female who lived during the Bronze Age (1900-1600 BC). The tumor in the woman's skull was suggestive of head and neck cancer[14]. The oldest known tumor is contained in a fossil found in east Africa by Leaky that dates back more than 500,000 years. Some historians speculate that there might have been a high incidence of nasopharyngeal cancer in some ancient populations because of the inhalation of wood smoke in poorly ventilated huts. In approximately 400 BC, Hippocrates described a common chronic ulcer at the edge of the tongue that he attributed to the presence of sharp teeth rubbing against the tongue.

Some of the oldest written documents with reference to cancer are the papyri documents in which ancient Egyptians treated oral cancer with a mixture of cinnamon, gum, honey & oil[20]. The ancient Indian physician Sushruta described the removal of tumors and developed great skill in plastic surgery, partly by defects created by frequent amputations of the nose and ears for punishment[14].

In ancient Rome, Aurelius Cornelius Celsus (30 A.D) treated cancer of the face and the lip by excision. Galen (200 A.D) advocated the humoral theory of disease and postulated that neoplasms were due to an excess of black bile, which solidified in certain sites like the lips and the tongue[20]. Little medical advancement was made for head and neck cancers until the advent of anaesthesia and surgical excision in the 11$^{th}$ century.

John Hill of London was the first to recognize the dangers of tobacco. In 1761, he wrote a book entitled "Cautions Against the Immoderate Use of Snuff"[14]. Cancer of the

larynx was first described by Boerhave in 1769 and removal of cancerous mass was done by Gurdon Buck in 1851[21].

The earliest, albeit crude, laryngoscopes gave the opportunity to remove tissue, and the first "polyp" is said to have been removed in 1860. A biopsy was taken from the larynx of Crown Prince Frederick in 1887[22].

In 1893, President Grover Cleveland was found to have a squamous cell carcinoma of the hard palate that required surgical excision. The operation was performed secretly on a yacht so that he could manage the “financial panic of 1893”. He was known for his heavy cigar smoking and social drinking[23].

## EPIDEMIOLOGY OF ORAL AND LARYNGEAL CANCERS

### Oral Cancer:-

#### *Global Scenario*

Oral cancer is the sixth leading cancer worldwide[24]. Incidence rates vary in men from 1 to 10 cases per 100,000 population in many countries. According to Globocan 2002, oral cancers accounted for 2,74,000 cases in the year 2002 with almost two-third occurring in men[25]. Rates in men are high in Western Europe, Southern Europe, South Asia, South Africa, Australia and New Zealand. In females incidence is higher in South Asia. In South-Central Asia, it ranks among the three most common type of cancer. This pattern reflects the prevalence of specific risk factors such as tobacco and alcohol use in Western Europe, Southern Europe and Southern Africa and the chewing of betel quid in

South Central Asia. The high rate of lip cancer in Australia is due to exposure to sunlight[25].

### *Indian Scenario*

Oral cancer is a serious public health problem in India and makes up 9.4% of all head and neck cancers[5]. The disease ranks number one among all cancers in male patients and number three among cancers in female patients. India has the highest rate of oral cancer in the world, caused by tobacco consumption. Of all cancer cases, oral cancer constitutes 12% in men and 8% among women. WHO research indicates a 500% increase in cancer by 2025, of which 220% will be due to tobacco use. There is already a 60% increase in India in the last three decades[26]. Annual incidence rate is 52,008 in males and 30,906 in females. The male to female ratio of occurrence varies from 2-15:1. The overall incidence and mortality attributed to oral carcinoma is increasing, with current estimates of age-standardized incidence rate being 12.8 per 100,000 in males and 7.5 per 100,000 in females[27].

## Laryngeal cancers:-

### *Global Scenario*

Laryngeal cancer is the 14$^{th}$ most common cancer in the world[19.] More than 160,000 new cases occur worldwide annually. It is predominantly a cancer of men in whom it comprises 2.4% of cases and 2.1% of deaths. The sex ratio is almost 7:1 which is greater than for any other site. There is large geographical variability in the disease frequency. High risk countries are found in Southern and Eastern Europe, South America

and Western Asia. Populations at high risk are those who indulge in habits of tobacco smoking and alcohol consumption[25].

## *Indian Scenario*

Laryngeal cancer comprises 4% of all head and neck cancers[5]. In India the incidence rate is 24,216 in males and 3,157 in females. The peak incidence of laryngeal cancer is highest in men aged between 55 to 65 years. Age-standardized incidence rates are 6.2 per 100,000 in males and 0.8 per 100,000 in females[27]. The male-to-female ratio varies from 12:1; however in the last decade there has been a decrease in this ratio, because of an increase of laryngeal cancer in women. There is a notable social class difference, in that laryngeal cancer is twice as common in men with low socioeconomic status. It is also more common in people residing in cities than in rural areas. Most studies show that inhabitants of the most industrialized cities have an incidence of laryngeal cancer 2 to 3 times higher than that of rural inhabitants. These racial, social and urban variations may reflect the different lifestyle and habits and also confirm the harmful effects of tobacco and alcohol.

# GEOGRAPHICAL DISTRIBUTION OF ORAL AND LARYNGEAL CANCERS

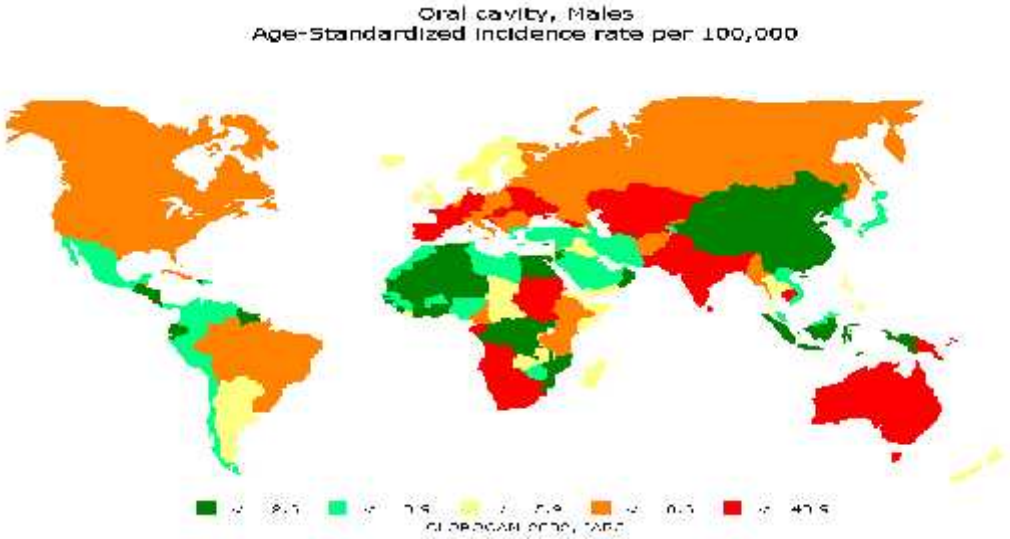
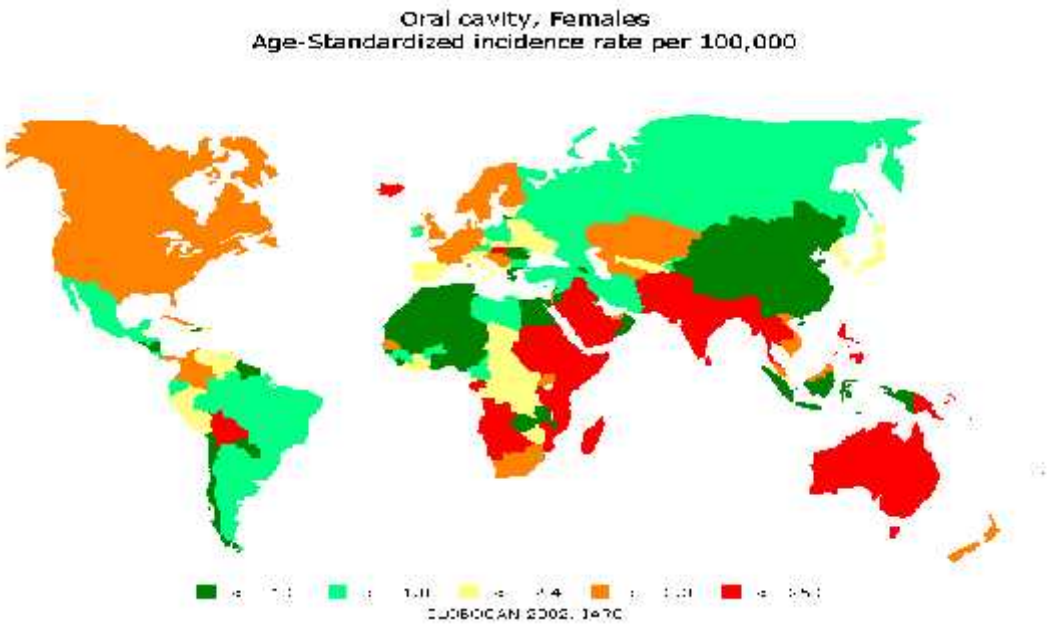



**FIGURE 1:- Age-Standardized incidence rates of Oral cancer per 100,000 in males and females.**



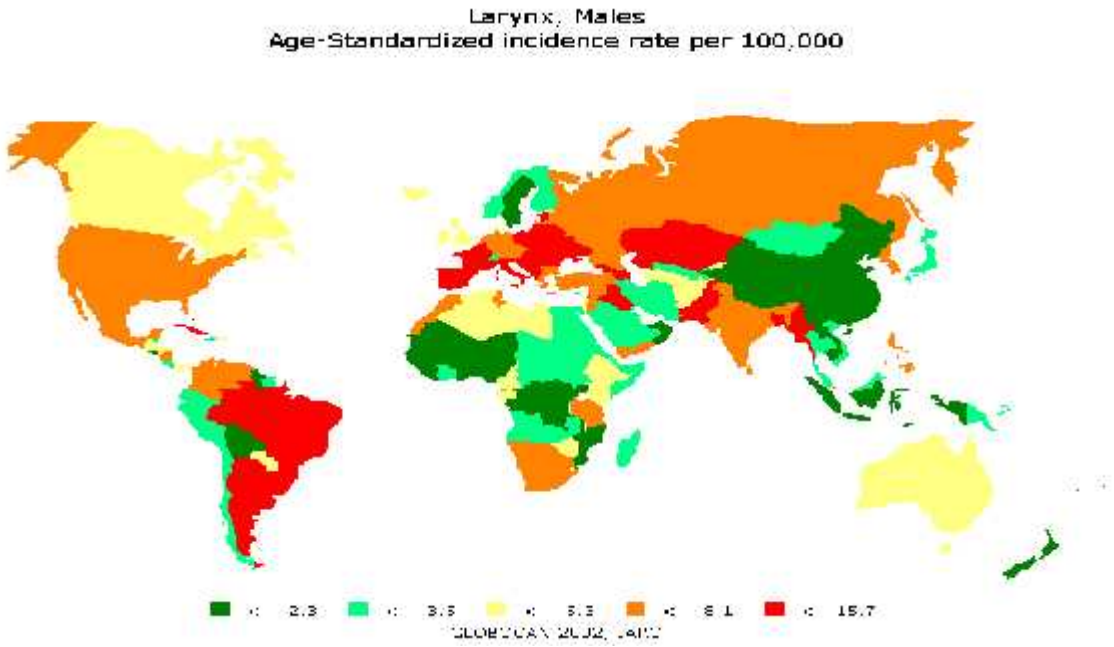
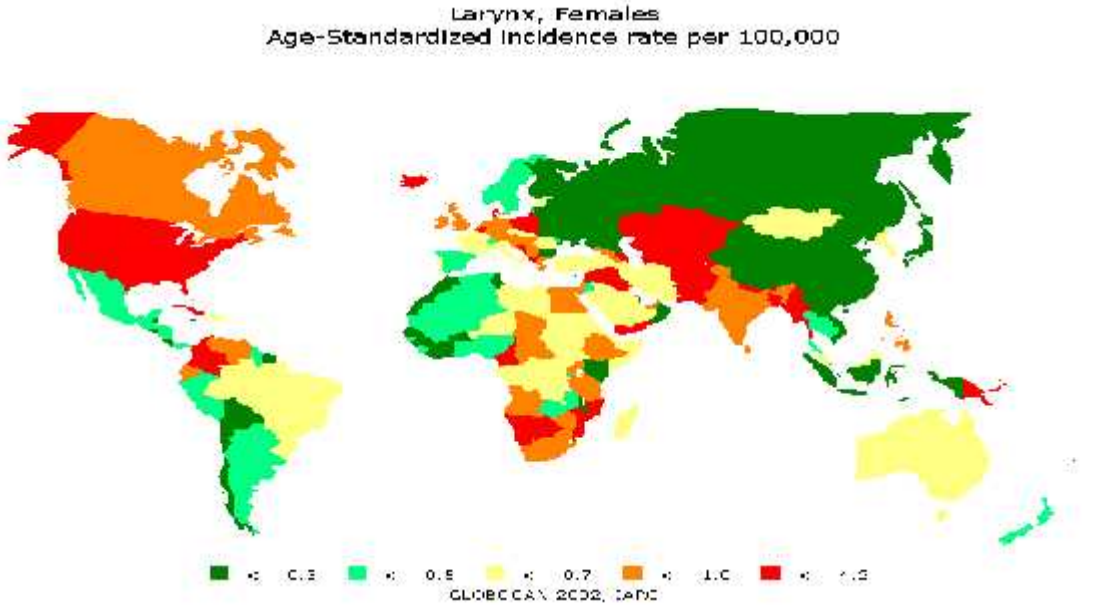



**FIGURE 2:- Age-Standardized incidence rates of Laryngeal cancer per 100,000 in males and females.**



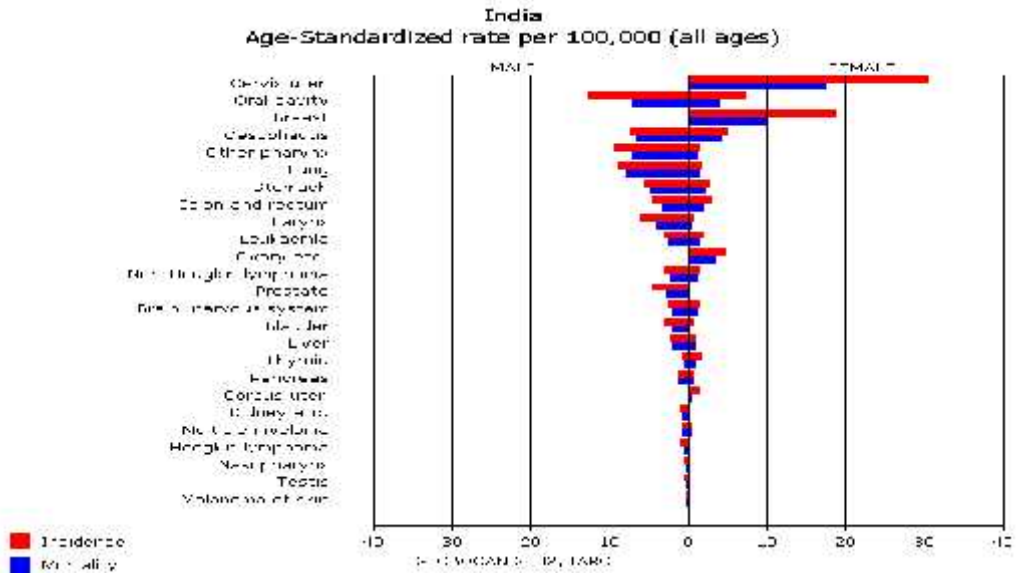



**FIGURE 3:- Age-Standardized incidence and mortality rates of oral and laryngeal cancer compared to other cancers in men and women in India.**

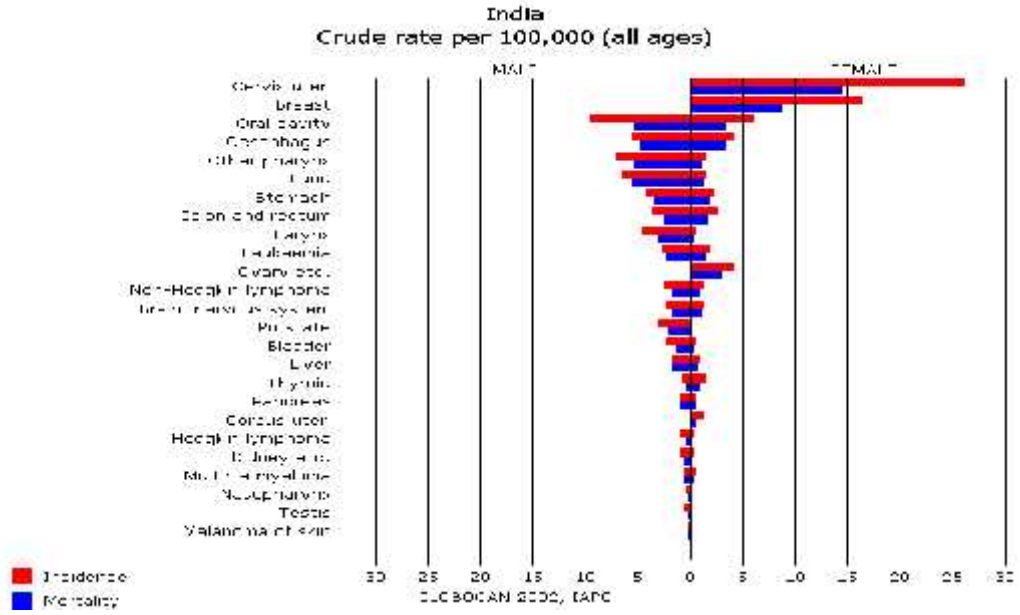



**FIGURE 4:- Crude incidence and crude mortality rates in oral and laryngeal cancer compared to other cancers in men and women India.**

## ETIOLOGY

**Risk factors for oral cancer include:-**

a. Tobacco, betel quid & Areca nut (gutkha).
b. Alcohol.
c. Viruses.
d. Diet and nutrition.
e. Family history.
f. Immunodeficiency.
g. Ultraviolet radiation.
h. Oral and dental factors
i. Other factors

***Tobacco, Betel Quid & Areca Nut***

Large epidemiological studies continue to confirm the correlation between the use of tobacco and cancer of the head and neck. Tobacco consumption is one of the most common preventable factors associated with the development of oral cancer. Almost one half of all cancer cases in men and one-quarter in women in India are believed to be tobacco related. More than 300 carcinogens have been identified in tobacco smoke. Of these, the aromatic hydrocarbons benzpyrene and tobacco specific nitrosamines act locally on keratinocyte stem cells. Once absorbed, these compounds produce DNA adducts that interfere with DNA replication. Smoking is considered to be an independent risk factor in 80% to 90% of patients with cancer of the oral cavity. The relative risk of developing cancer of the oral cavity is six to eight times greater among smokers than among non-smokers. Exposure to tobacco is thought to result in morphologic changes

within the mucosa of the oral cavity that culminate in malignant transformation. These changes are reversible with the elimination of tobacco use. All forms of tobacco use are associated with cancer of the oral cavity. In India and other parts of South-East Asia, the use of betel quid is very common. Quid is the mixture of dried and cured tobacco leaf, betel nut and slaked lime. Even without tobacco, the swallowing of betel nut juice or unripened betel fruit is seemed to enhance the risk for cancer of the oral cavity. Smoking is the other common mode of consumption of tobacco. Cancer of lip has been associated with pipe smoking. Cigar smokers are four to twenty times more likely to develop cancer of the oral cavity than are non-smokers. Reverse smoking (that is where the lighted portion of the tobacco product is kept within the mouth during inhalation) is associated with 47 time's greater risk in developing carcinoma of the hard palate when compared to non-smokers[2].

***Alcohol***

Alcohol acting both independently as well as synergistically with smoking has been implicated in carcinogenesis. More importantly, alcohol may act as a solvent and enhance the penetration of carcinogens into target tissues. Acetaldehyde, which is the alcohol metabolite, has been identified recently as a tumor promoter[2]. People who consume alcohol have an increased risk of oral cancer by 6 times[28]. In a person who smokes and drinks the risk of oral cancer is 15 times as compared to a non-smoker and a person who does not drink[29]. Various studies report that three fourth of oral cancer cases can be prevented if exposure to tobacco and alcohol are controlled.

***Viruses***

Patients with recurrent herpes stomatitis develop cancer of the oral cavity even in the absence of other risk factors. Human papilloma virus (HPV) positivity is higher in tumors from the oral cavity (50%). Among those, only a small fraction of HPV-infected lesions rarely proceed to malignant transformation. HPV subtypes 6 and 16 are the most common types associated with cancer of the oral cavity[30].

***Diet and nutrition***

The importance of diet and nutrition in cancer has been indicated in several epidemiological studies. Fruits and vegetables (rich in vitamins A and C) are described to have a protective effect (20-60%), whereas meat and red chilli powder are thought to be risk factors[2,31]. Although the individual micronutrients responsible have not been formally identified, vegetables and fruits that protect against cancer and precancerous lesions, are rich in beta-carotene, vitamin C and vitamin E, with anti-oxidant properties. Supporting this is a study by Ibrahmin et al. reporting that oral cancer patients at the time of diagnosis had lower plasma vitamin A and beta- carotene levels than controls[32].

***Family History***

Epidemiological evidence from case-control studies of HNSCC, indicates that a family history of head and neck cancer is a risk factor. The ability to repair DNA damaged by tobacco carcinogens, such as benz-pyrene diol epoxide, is defective in some patients with head and neck cancer. Head and neck cancer patients show an increased susceptibility to chromosome damage by mutagens[33].

***Immunodeficiency***

A defective immune response, as seen in HIV infected individual, may predispose to cancer. The commonest oral malignancy in HIV-infected patients is Kaposi's sarcoma and the human herpes virus-8[34]. Oral squamous cell carcinomas of the lip are more common in transplant recipients receiving immunosuppressive therapy. Candida albicans can induce epithelial proliferation and can produce carcinogens from pro-carcinogens in vitro. Chronic hyperplastic candidiasis presents as nodular or speckled-white mucosal plaques. They are potentially malignant oral epithelial lesions[35]. Other oral lesions like lichen planus, dyskeratosis congenita, submucosal fibrosis are also important risk factors.

***Ultraviolet radiation***

Chronic exposure to actinic radiation such as the sun light is a significant factor in the development of cancer of the lower lip. Prolonged exposure to sunlight has been shown to cause hyperkeratosis and atrophy of fat and glandular elements within skin. The lips are especially vulnerable to ultraviolet radiation because they lack a pigmented layer for protection and are constantly exposed to sunlight. Blacks have some pigment in their lips, which may explain the fact that cancer of the lip is a rare occurrence in this population[36].

***Occupational exposure***

Occupational exposure to isopropyl oils, sulphuric acid, nickel chemicals in leather, metal processing and metal working, textile fiber processing, wood dust are all risk factors for oral cancer[36].

***Oral and dental factors***

Poor oral and dental hygiene is often associated with cancer of the oral cavity. Poor oral hygiene has been correlated with higher levels of oral micro flora and a two fold increase in salivary acetaldehyde (a known carcinogen in the oral cavity) production. Oral sores from ill fitting dentures were associated with a two fold increase in cancer of the tongue[36].

***Other risk factors***

Other important independent risk factors include excessive consumption of hot spicy foods, usage of alcoholic mouth washes, syphilis infection etc[36].

**Risk factors for laryngeal cancer:-**

a. Smoking.

b. Alcohol.

c. Diet.

d. Viral infections.

e. Gastro-esophageal reflux.

f. Occupational exposure.

g. Other risk factors.

***Smoking***

Smoking increases the risk of laryngeal cancer by 5-25 times. Studies show that people who smoked 40 or more cigarettes daily had an age- adjusted death rate of 15/100,000 compared with 0.6/100,000 person-years among non-smokers. The relative risk of laryngeal cancer between smokers and non-smokers is 15.5 in men and 12.4 in women[37]. International studies have confirmed that the risk of laryngeal cancer developing decreases with time after the cessation of smoking.

***Alcohol***

Alcohol increases the risk of laryngeal cancer by 2-6 times. In a person who smokes and drinks the risk of laryngeal cancer is 40 times as compared to a non-smoker and drinker. Heavy alcohol intake significantly increases the risk to the supraglottis[38].

***Diet***

There is evidence that high dietary carotenoids, vitamin C and Zinc may possibly decrease the risk of laryngeal cancer[39]. Other studies have shown that increased iron and zinc consumption is associated with a reduced risk of laryngeal cancer[40]. It has been estimated that among smokers and drinkers, the low intake of fruits and vegetables may contribute to 25-50% of laryngeal cancers[41].

***Viral infections***

Human papilloma virus positivity is higher in tumors from the larynx (33%)[30]. Abramson et al demonstrated HPV-16 related sequences in patients with verrucous

carcinoma of the larynx and has suggested human papilloma virus in the etiology of verrucous carcinoma[42].

***Gastro-esophageal reflux***

Biliary reflux is frequent after gastric surgery and may reach the proximal segment of the esophagus and the larynx. It is possible that duodenal content (consisting of bile acids, trypsin etc) together with pepsin and acid residues in partial gastric resection cases, may cause harmful action on the multistratified epithelium of the larynx[43].

***Occupational Exposure***

Chemical carcinogens in the work place that relate to laryngeal cancer include exposure to nickel refining, wood dust, rubber products, chromium, mustard gas, radium, formaldehyde, asbestos, organic solvents, mineral oils and tanning by-products[44].

***Exposure to radiation***

Exposure to radiation has also been implicated as an etiological factor in laryngeal cancer. Studies show that the occurrence of laryngeal cancers increases in patients previously irradiated for benign disease[45]. Radiation induced DNA breaks may facilitate the integration of the HPV genes into host DNA, thus transforming cells to malignant ones[30].

***Others***

A form of tea known as “mate” in Latin America and Brazil has been recognized as a risk factor for aero-digestive sites. De Stefani and others calculated a relative risk of

4.9 for laryngeal cancer in those who drink this beverage compared to those who consume none[46].

## BASIC CONCEPTS OF TUMOR BIOLOGY

### Clonal Evolution

This theory was proposed by Nowell in 1976. This model is built on the theory of natural selection and states that cancer cells develop strategies to survive a hostile host environment (e.g. oxygen, nutrients) to grow and proliferate. In this model, repeated carcinogenic insults or events occur within a cell, usually at the genetic or epigenetic level. When enough events occur, a selective growth advantage is conferred on the affected cell. As this cell proliferates, mutant progeny arise as a result of further insults and genomic instability. Most of the offspring do not survive because of immunological surveillance, apoptosis or metabolic derangement. Eventually, however a dominant clonal population of cells is produced that not only survives, but flourishes. As evolution occurs within this clonal population, new clones are produced with acquired additional characteristics, such as capacity for invasion, that define cancer. Thus, within the resulting malignancy, a vast majority of cells have developed from a single clone, they therefore are genotypically similar or clonal[47].


**FIGURE 5:- When normal cells are damaged beyond repair, they are eliminated by apoptosis (A). Cancer cells avoid apoptosis and continue to multiply in an unregulated manner (B).**

**Field Carcinogenesis**

Carcinogens in tobacco are responsible for the vast majority of HNSCC and can induce molecular changes throughout the entire upper aero-digestive tract. Slaughter first describes these changes, originating the concept of field carcinogenesis or "condemned mucosa" in 1953. He hypothesized that because of constant carcinogenic pressure the entire upper aero-digestive tract is at a risk of developing multiple primary tumors.

The original hypothesis was that multiple genetic events occurred throughout the involved mucosa, allowing the development of multiple molecularly distinct lesions. In an alternative hypothesis, a single lesion is thought to form multiple upper aero-digestive tract lesions through the process of intra epithelial migration i.e. rather than several molecularly similar transformed progenitor cells migrate to distant sites, thus explaining the appearance of multiple primary lesions, second lesions and recurrent lesions[47].

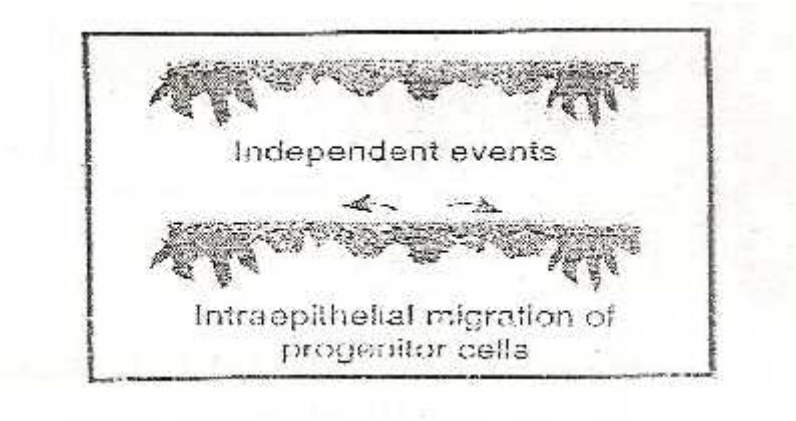



**FIGURE 6:- Field Carcinogenesis theory**

## Molecular Progression Model

Epidemiological analysis indicates that the development of head and neck cancer is a multi-step process involving the activation of oncogenes and inactivation of tumor suppressor genes in a clonal selection of cells. These genetic alterations include gene amplification and over-expression of oncogenes such as c-myc, erbB-2, Epidermal Growth Factor Receptor (EGFR), cyclin D1 and mutations, deletions and hypermethylation leading to p16 and p53 tumor suppressor gene inactivation.

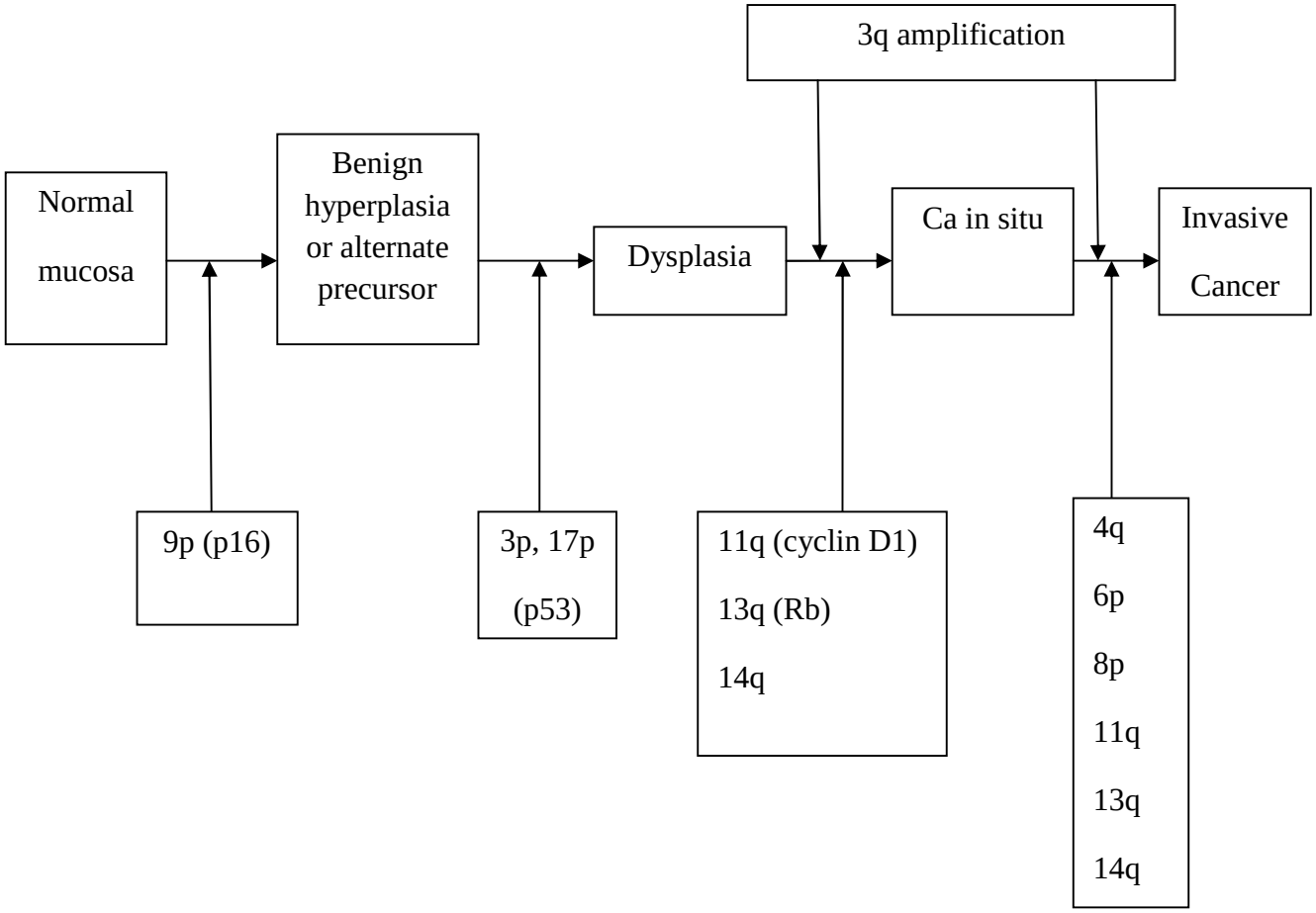
The first reproducible change is the loss of chromosomal regions of 3p and 9p21. Loss of heterozygosity (LOH) in conjunction with promoter hypermethylation at this locus results in the inactivation of the p16 gene, an inhibitor of cyclin-dependent kinase. This alteration is associated with the transition from normal to hyperplasia or hyperkeratosis and occurs prior to the development of histological atypia. Subsequent LOH at 17p with mutation of the p53 tumor suppressor gene is associated with progression to dysplasia.

Recently it has been demonstrated that gross genomic alterations as well as deletions on 4q, 6p, 8p, 11q, 13q and 14q may act as predictors of progression to frank malignancy. Ultimately amplification and over expression of the cyclin D1 gene (located on chromosome 11q13), which constitutively activates cell cycle progression, is a common late event. Data suggest that alterations of this gene confer the ability to invade into certain clones[2,47].

The progression model is based on association rather than a direct causation. The genetic events need not occur at the precise time points depicted. The subclinical

accumulation of genetic events continuously occurs throughout the progression to invasive cancer.

**Summary of Molecular Progression model of head and neck carcinogenesis.**[2,15]




## Premalignant lesions of the oral cavity

Premalignant lesions of the oral cavity are leukoplakia, erythroplakia and submucosal fibrosis (SMF).

Leukoplakia is a whitish patch or plaque that cannot be characterized clinically or pathologically as any other disease and which is not associated with any physical or chemical causative agent except the use of tobacco. Pindborg studied the natural history of leukoplakia. He reported that 20% disappear, 18% decrease in size without treatment, 46% increase in size and 4% show malignant transformation. Leukoplakia of lateral border of tongue is the worst lesion (44% malignant conversion) [36].

Erythroplakia is a chronic red mucosal macule, 80% of which may harbor micro invasive carcinoma. Without therapy 60-90% of erythroplakia may turn into cancer in 5-10 years[36].

SMF is a collagen disorder that is characterized by extreme sensitivity to temperature and spices, whitening of mucosa, progressive trismus and bleeding. It is usually associated with habit of areca chewing in tropical countries like India, but in the west 90% have association with HPV and 50% with Candida albicans. It is commonly seen in Indian subcontinent and 50-70% individuals develop cancer within a decade[36.]

**Malignant Tumors of the Oral Cavity**[48]

*Squamous cell carcinoma or Epidermoid carcinoma*.

*Variants of Squamous cell carcinoma*

- In situ squamous cell carcinoma
- Invasive squamous cell carcinoma
- Verrucous carcinoma
- Spindle cell carcinoma.
- Adenoid squamous cell carcinoma and adenosquamous carcinoma.

- Basaloid squamous cell carcinoma.
- Small cell carcinoma (oat cell carcinoma)
- Undifferentiated squamous cell carcinoma.

**Site Incidence of Oral Squamous Cell Carcinoma[49]**

| Site of cancer | Incidence % |
|---|---|
| Tongue | 35 |
| Floor of Mouth | 30 |
| Lower alveolus | 15 |
| Buccal Mucosa | 10 |
| Upper alveolus and hard palate | 8 |
| Retromolar region | 2 |

**Staging of Oral Cancer**

Tumor size and the extent of metastatic spread are the best indicators of patient's prognosis. Quantifying these clinical parameters is called staging the disease[50].

The stage of cancer at presentation is a reasonable surrogate indicator of rate of growth, extension of disease to adjacent distant structures, type of tumor and tumor host relationship. The most widely used staging system is that developed by the American Joint Committee for Cancer staging and End Results Reporting (AJCCS) known as the TNM system (T- primary tumor; N- regional lymph nodes; M- distant metastasis). Once the three clinical parameters are determined, they are tallied together to determine the appropriate stage. The higher the stage classification, the worse is the prognosis[17].

**TNM Clinical Classification**[17,49]

**Primary Tumor (T):**

TX : Primary tumor cannot be assessed

T0 : No evidence of primary tumor

Tis : Carcinoma in situ

T1 : Tumor 2cm or less in greatest dimension.

T2 : Tumor more than 2cm but not more than 4 cm in greatest dimension.

T3 : Tumor more than 4cm in greatest dimension.

T4 (lip) : Tumor invades through cortical bone, inferior alveolar nerve, floor of mouth, or skin of face, i.e chin or nose.

T4a (oral cavity) : Tumor invades adjacent structures (e.g through cortical bone, into deep [extrinsic] muscle of tongue [genioglossus, hyoglossus, palatoglossus and styloglossus], maxillary sinus, skin of face).

T4b : Tumor invades masticator space, pterygoid plates, or skull base and or encases internal carotid artery.

**Regional Lymph Nodes (N):**

NX : Regional lymph nodes cannot be assessed.

N0 : No regional lymph node metastasis

N1 : Metastasis in a single ipsilateral lymph node, 3cm or less in greatest dimension.

N2 : Metastasis in a single ipsilateral lymph node, more than 3cm but not more than 6cm in greatest dimension; or in multiple ipsilateral lymph nodes, none more than 6cm in greatest dimension; or in bilateral or contralateral lymph nodes, none more than 6cm in greatest dimension.

N2a : Metastasis in a single ipsilateral lymph node, more than 3cm but not more than 6cm in greatest dimension.

N2b : Metastasis in multiple ipsilateral lymph nodes, none more than 6cm in greatest dimension.

N2c : Metastasis in bilateral or contralateral lymph nodes, none more than 6cm in greatest dimension.

N3 : Metastasis in a lymph node more than 6cm in greatest dimension.

**Distant Metastasis (M):**

Mx : Distant metastasis cannot be assessed.

M0 : No distant metastasis.

M1 : Distant metastasis.

**Stage Grouping**

| | | | |
|---|---|---|---|
| Stage 0 | Tis | N0 | M0 |
| Stage I | T1 | N0 | M0 |
| Stage II | T2 | N0 | M0 |
| Stage III | T3 | N0 | M0 |
| | T1 | N1 | M0 |
| | T2 | N1 | M0 |
| | T3 | N1 | M0 |
| Stage IV A | T4a | N0 | M0 |
| | T4a | N1 | M0 |
| | T1 | N2 | M0 |
| | T2 | N2 | M0 |
| | T3 | N2 | M0 |
| | T4a | N2 | M0 |
| Stage IV B | Any T | N3 | M0 |
| | T4b | Any N | M0 |
| Stage IV C | Any T | Any N | M1 |

**Diagnosis of Oral Cancer**

The common methods used to detect oral cancer[49]:-

1) History and clinical examination of the head and neck region:-It should include examination of the accessible oral tissues, posterior 1/3 of tongue and indirect examination of the nasopharynx and larynx. Persistent white and red patches, ulcers, lumps, loose teeth, and bony abnormalities all require investigation.

Palpation of the neck should be performed. Cervical lymphadenopathy may indicate malignant disease and should always be further investigated.

2) Examination of tumor under anaesthesia and biopsy:-

   a) Brush cytology.

   b) Excision biopsy.

   c) Incision biopsy.

   d) Punch biopsy.

   e) Fine needle aspiration cytology (FNAC).

   f) Fine needle cutting biopsy (FNCB).

3) Radiological Examination:-

- Orthopantogram /dental occlusal view/CT Scan:-For evaluating bone erosions in mandible
- CT/MRI:- For site and extent of primary tumor.
- CT/MRI/USG:- To assess tumor depth.
- Chest X ray/CT Scan/ USG abdomen:- To assess chest and abdomen.
- PET Scan:- For evaluation of post treatment recurrent or residual disease.

**Malignant tumors of the larynx**[48]

**Epithelial Cancer**

*Squamous cell carcinomas (SCC)*

*Variants of squamous cell carcinoma*:-

-In situ squamous cell carcinoma.
-Invasive squamous cell carcinoma.
-Verrucous carcinoma.
-Spindle cell carcinoma.
-Basaloid squamous cell carcinoma.
-Lymphoepithelial squamous cell carcinoma.
-Adenoid squamous carcinoma.
-Pseudovascular squamous cell carcinoma.
-Papillary squamous cell carcinoma.

*Neuroendocrine carcinomas*

- Neuroendocrine carcinoma, small cell type.
- Neuroendocrine carcinoma, large cell type.

*Adenocarcinoma*

*Mucoepidermoid carcinoma*

*Adenoid cystic carcinoma*

*Non specific adenocarcinoma*

*Cartilaginous tumors*

**Mesenchymal Neoplasms**

- *Sarcomas*
- *Fibrosarcomas*
- *Rhabdomyosarcoma*
- *Osteosarcoma*
- *Synovial sarcoma*
- *Liposarcoma*
- *Malignant Schwannoma*
- *Leiomyosarcoma*
- *Chondrosarcoma*
- *Angiosarcoma*
- *Giant cell sarcoma*
- *Malignant hemangiopericytoma*
- *Malignant fibrous histiocytoma*

**Others**

- *Malignant lymphomas*
- *Plasmacytomas*
- *Metastatic Malignancies (from skin, kidneys, breast, lung etc)*

Carcinoma of larynx is further divided according to tumor location into three subtypes:

1. Supraglottic carcinoma:-Confined to the supraglottic area (epiglottis, false vocal cords, arytenoid, aryepiglottic fold and laryngeal ventricles).
2. Glottic carcinoma:-involves the true vocal folds.
3. Subglottic carcinoma:-extend or arise more than 10mm below the free margin of the true vocal fold up to the inferior border of the cricoid cartilage.

Depending on the extent of the lesion another subsite which can be mentioned is transglottic carcinoma.

Glottic carcinomas represent the majority of laryngeal cancers (50%-60%), followed by the supraglottic carcinomas (30%-40%), while the subglottic carcinomas are uncommon (5% or less).

**Staging of Laryngeal Cancer.**[48,49]

The most widely used staging system developed by the American Joint Committee for Cancer staging and End Results Reporting (AJCCS) known as the TNM system and is based on the clinical observation related to the primary tumor (T), involvement of regional lymph nodes (N) and distant metastasis (M).

**TNM Clinical Classification**

**Primary Tumor (T):**

TX : Primary tumor cannot be assessed.

T0 : No evidence of primary tumor.

T1s : Carcinoma in situ.

**<u>Supraglottis</u>**

T1 : Tumor limited to one subsite of supraglottis, with normal vocal cord mobility.

T2 : Tumor invades mucosa of more than one adjacent subsite of supraglottis or

glottis or region outside the supraglottis (e.g mucosa of base of tongue, vallecula, medial wall of pyriform sinus) without fixation of the larynx.

T3 : Tumor limited to larynx with vocal cord fixation and or invades any of the following: post-cricoid area, pre-epiglottic tissues, paraglottic space, and/or minor thyroid cartilage erosion (e.g, inner cortex).

T4a : Tumor invades through the thyroid cartilage and/or invades tissues beyond the larynx (e.g, trachea, soft tissues of neck including deep extrinsic muscle of the tongue, strap muscles, thyroid, or oesophagus).

T4b : Tumor invades prevertebral space, encases carotid artery, or invades mediastinal structures.

**Glottis**

T1 : Tumor limited to vocal cord(s) (may involve anterior or posterior commissures) with normal mobility.

T1a : Tumor limited to one vocal cord.

T1b : Tumor involves both vocal cords.

T2 : Tumor extends to supraglottis and or subglottis with impaired vocal cord mobility.

T3 : Tumor limited to the larynx with vocal cord fixation and or invades paraglottic space, and or minor thyroid cartilage erosion (e.g, inner cortex).

T4a : Tumor invades through the thyroid cartilage and/or invades tissues beyond the larynx (e.g, trachea, soft tissues of neck including deep extrinsic muscle of the tongue, strap muscles, thyroid, or oesophagus)

T4b : Tumor invades prevertebral space, encases carotid artery, or invades mediastinal structure.

**Subglottis**

T1 : Tumor limited to the subglottis.

T2 : Tumor extends to vocal cord(s) with normal or impaired mobility.

T3 : Tumor limited to the larynx with vocal cord fixation.

T4a : Tumor invades cricoid or thyroid cartilage and/or invades tissues beyond the larynx (e.g, trachea, soft tissues of neck including deep extrinsic muscle of the tongue, strap muscles, thyroid, or oesophagus)

T4b : Tumor invades prevertebral space, encases carotid artery, or invades mediastinal structures.

**Regional Lymph Nodes (N):**

NX : Regional lymph nodes cannot be assessed.

N0 : No regional lymph node metastasis.

N1 : Metastasis in a single ipsilateral lymph node, 3cm or less in greatest dimension.

N2 : Metastasis in a single ipsilateral lymph node, more than 3cm but not more than 6cm in greatest dimension, or in multiple ipsilateral lymph nodes, none more than 6cm 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 3cm but not more than 6cm in greatest dimension.

N2b : Metastasis in multiple ipsilateral lymph nodes, none more than 6cm in greatest dimension.

N2c : Metastasis in bilateral or contralateral lymph nodes none more than 6cm in greatest dimension.

N3 : Metastasis in a lymph node more than 6cm in greatest dimension.

**Distant Metastasis (M):**

Mx : Distant metastasis cannot be assessed.

M0 : No distant metastasis.

M1 : Distant metastasis.

## Stage Grouping

| | | | |
|---|---|---|---|
| Stage 0 | Tis | N0 | M0 |
| Stage I | T1 | N0 | M0 |
| Stage II | T2 | N0 | M0 |
| Stage III | T3 | N0 | M0 |
| | T1 | N1 | M0 |
| | T2 | N1 | M0 |
| | T3 | N1 | M0 |
| Stage IV A | T4a | N0 | M0 |
| | T4a | N1 | M0 |
| | T1 | N2 | M0 |
| | T2 | N2 | M0 |
| | T3 | N2 | M0 |
| | T4a | N2 | M0 |
| Stage IV B | T4b | Any N | M0 |
| | Any T | N3 | M0 |
| Stage IV C | Any T | Any N | M1 |

**Diagnosis of Laryngeal Cancer**

The common methods used to detect laryngeal cancer[49]:-

1) History and clinical examination of the head and neck region.

2) Indirect laryngoscopy/Hopkins telescopy/flexible laryngoscopy:- To assess cord mobility and extent of mucosal disease.

3) Direct laryngoscopy:- To define the exact extent of the disease, assess areas not well seen by indirect laryngoscopy/Hopkins telescopy namely anterior commissure, pyriform fossa and post cricoid region and obtain a biopsy.

4) Microlaryngoscopy:- To detect early cord lesions and obtain biopsy from vocal cord.

5) Videostroboscopy

6) Chest X-ray

7) CT Scan/MRI:- To assess the size and extent of the disease, cartilage invasion, extra laryngeal spread, para or pre-epiglottic space involvement, tumor volume and nodal disease.

8) Bone Scan and PET Scan:- For evaluating post treatment residual or recurrent disease or metastasis.

**TUMOR MARKERS IN ORAL AND LARYNGEAL CANCERS**

Despite recent advances in surgery and multimodal treatment regimens, the prognosis of head and neck cancers is still relatively poor and has shown only slow progress. A large number of serological, molecular or genetic markers have been examined in carcinomas of the head and neck. All of these are however controversial regarding their prognostic value and clinical effectiveness[51].

Serum SCCA, CYFRA 21-1, and CEA are the most common tumor markers for head and neck squamous cell carcinoma (HNSCC), although their diagnostic sensitivity still requires improvement, especially at early stages[52]. Squamous cell carcinoma antigen (SCCA), a member of the ovalbumin serine proteinase inhibitor family, serves as a circulating marker of squamous cell carcinoma[53]. It was first isolated by conventional protein purification methods from cervical squamous cell carcinoma in 1977. Biochemical characterization of the original protein fraction (TA-4) shows that it is comprised of a group of proteins with a molecular weight of approximately 45 kDa[54]. SCC antigen is a serological marker for squamous cell carcinomas of the uterine cervix, lung, head and neck and oesophagus[54,55]. A study of the distribution of antibody binding sites showed that anti-SCCA recognizes metaplastic, dysplastic and neoplastic squamous epithelium[56].

Various studies show that serum SCC antigen levels correlate with clinical stage and treatment outcome in various carcinomas. High pre-treatment levels indicate extensive disease and poor prognosis for patients with squamous cell carcinoma histiotype in the head and neck region, but not for the adenocarcinomas[54]. A good correlation has been reported between serum SCC antigen levels and the extent of disease. In accordance some studies have shown elevated SCC antigen levels as a poor prognostic marker[57,58,59]. It is a more sensitive marker than Cyfra21-1[60].

Further studies are however still required to establish the effectiveness of this marker.

Molecular and Genetic Markers for oral and laryngeal cancers[13,17].

| Marker | Detection Method | Target |
|---|---|---|
| Proliferation PCNA, Ki67, BrdU Histone AgNORs | IHC mRNA ISH Silver stain | Cycling cells |
| Genetic Ploidy | FC | Aneuploid cells |
| Oncogenes C-myc | IHC | Cycling cells |
| Tumour Suppressor mutations | IHC, PCR | Cycling cells |
| Cytokeratin 8/19 | IHC | Anaplasia |
| Blood Group Antigens | IHC | Anaplasia |
| Integrins/ECM Ligands | IHC | Invasion and metastatic potential |

**Abbreviations:**

| | |
|---|---|
| **IHC** | Immunohistochemistry |
| **FC** | flow cytometry |
| **PCR** | polymerase chain reaction |
| **ISH** | in-situ hybridization |

Further studies are still required for establishment of reliable diagnostic markers for the early detection of oral and laryngeal cancers.

## Role of enzyme markers in oral and laryngeal cancer

The cytoplasm contains most of the enzymes that catalyze all biosynthetic and degradative metabolism. Under normal circumstances enzymes are retained within their cells of origin by the plasma membrane surrounding the cell and tissue .They are released into the systemic circulation as a result of tumor necrosis or a change in the membrane permeability of the cancer cells. Elevated enzyme levels may signal the presence of a malignancy[10]. The plasma membrane is metabolically the active part of the cell and its integrity depends on the cell's production of adenosine triphosphate (ATP). Any process that impairs ATP production, either by depriving the cell of oxidizable substrates or by reducing the efficiency of energy production by restricting the access of oxygen (anoxia),

promotes deterioration of the cell membrane. The membranes become leaky and membrane constituents are shed into the surrounding milieu at an increased rate when cells replicate more rapidly[61].

Small molecules are the first to leak from damaged or dying cells, followed by larger molecules, such as enzymes. Cytosolic enzymes appear early in the serum, followed much later by mitochondrial and membrane bound enzymes. Ultimately the contents of the cells are discharged leading to raised enzyme activity in malignancy[10].

**ADENOSINE DEAMINASE: (Adenosine aminohydrolase EC 3.5.4.4)**

Adenosine deaminase was identified by Spencer et al[62] and is involved in the catabolism of purine bases capable of catalysing the deamination and hydrolytic cleavage of adenosine irreversibly converting it into inosine and ammonia[63] (Figure 8). The enzyme contains reactive thiol groups. It is widely distributed in human tissues, highest being in the lymphoid tissue. This enzyme is considered as an ectoenzyme[64], since it is found on the surface of many cells and is located in the cell membrane[65].

**ISOENZYME FORMS OF ADA**

Human ADA exists in at least three molecular forms ADA1, ADA1+CP, ADA2.

ADA1 - Spleen, lymphocytes, monocytes and neutrophils.

ADA1+CP - Liver, lung, muscle, pancreas, kidney. (This is modified form of ADA1).

ADA2 - It could be detected only in monocytes[66].

These different forms of ADA are separated by polyacrylamide gel electrophoresis[67].

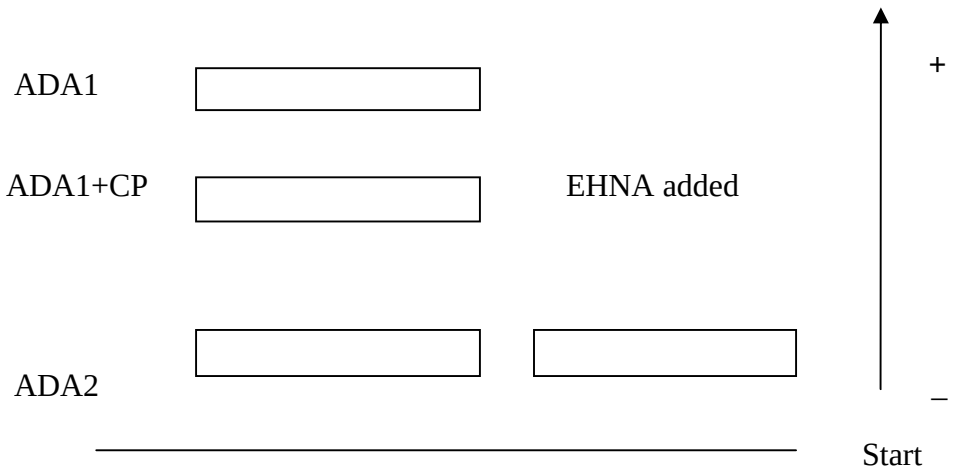



**FIGURE 7:- Separation of isoenzymes of ADA by electrophoresis**.

But only two principle isoenzymes are important.

- ADA1 (ADA1 & ADA1+CP) and
- ADA2

These have different optimal pH, Michaelis constant, relative specificity patterns and also gene located on different loci[68]. The structural gene for ADA is encoded as a single 32 Kb locus containing 12 exons on the long arm of chromosome 20 (20q 13.2).

ADA1 has roughly equal affinities for adenosine and 2'deoxyadenosine and is found in many tissues[69]. Decreased ADA1 in the lymphocytes and erythrocytes causes severe combined immunodeficiency syndrome (SCID). In this condition there is dysfunction of both B and T lymphocytes with impaired cellular immunity and decreased production of immunoglobulins. ADA deficiency accounts for about 50% of causes of autosomal recessive SCID[70].

**Molecular Defect**

Specific point mutation in exon 11 accounts for a high proportion of these, while a few mutations are homozygous for large deletions encompassing exon1.

Most of the mutations in the gene to ADA so far detected have been base substitutions, though deletions have also been detected.

↓

Reduced activity of ADA

↓

Accumulation of deoxyadenosine and dATP

↓

Increased toxicity

↓

Injury to lymphocytes

↓

Decrease in cell mediated and humoral immunity[70]

ADA2 is the major component of the activity of total ADA in the serum of healthy persons. ADA2 has much greater affinity for adenosine and is found only in macrophages and monocytes, which release it when stimulated in the presence of live organisms.

**Tissue distribution of ADA**

The study of distribution of ADA in various organs of rabbit was first studied by Convey and Cooke in 1939[71]. ADA distribution in humans is ubiquitous[65]. In humans the

activity of ADA was found relatively higher in lymphoid tissues and thymus (~ 800 IU/mg)[66]. The bulk of the activity is localized in the cytosol of the lymphocytes[72]. The activity is also 10 times greater in lymphocyte than in erythrocytes[73]. It is 5-20 times higher in T-lymphocytes than B-lymphocytes[74], as it is related to the differentiation of lymph nodes[75].

Among non lymphoid tissues in humans relatively high levels of ADA is found in the villi of epithelial cells lining the duodenum (570 IU/mg) whereas more than 500-fold lower activity was reported in liver and the lowest in erythrocytes (~ 1 IU/mg) ; levels are lower in the other portions of the GI tract. Tissues such as muscle, lung, liver, kidney, brain and blood have low activity in most species[69].

**Functions of ADA**

**1)** This enzyme deaminates adenosine, deoxyadenosine and certain synthetic ribosides.

**2)** ADA in purine salvage pathway: ADA catalyses the irreversible hydrolytic deamination of adenosine and deoxyadenosine to inosine and deoxyinosine respectively[76].

**3)** The physiological roles of ADA can be seen in connection with adenosine, the concentration of which can be modulated by enzymatic action of ADA. An increase in adenosine leads to coronary vasodilation, reduction in heart rate and contractile force, inhibition of platelet aggregation, mast cell granulation, inactivation of eosinophil migration, renal vasoconstriction, regulation of ion channel activity, membrane potential and release of neurotransmitter hormone[77].

**4)** ADA is essential for differentiation and proliferation of lymphoid cells particularly T-cells[75].

**5)** ADA plays a role in maturation of monocytes to macrophages[79].

**6)** ADA activity is increased during cellular activation for energy demand to detoxify toxic metabolites[63].

**7)** ADA for gene therapy: Adenosine deaminase deficiency has become important because it is the first disease to be treated by somatic gene therapy. One reason for selecting ADA deficiency as a suitable condition for somatic gene therapy was that cells which express the gene for ADA would have a selective advantage for growth over uncorrected cells. The trial began in 1996 using retroviral medicine to transfer ADA gene into their T-cells. The number of T-cells normalized as did many cellular and humoral immune response. Gene treatment ended after 2 years, but integrated vector and ADA gene expression persisted and it was concluded that gene therapy can be safe and effective addition to the treatment of this condition[70].

**Role of serum ADA activity in oral and laryngeal cancer**

The purine nucleoside adenosine is produced at increased levels in the tissues of solid cancers as a result of local hypoxia. Adenosine inhibits the cell-mediated anti-tumor immune response, promotes tumor cell migration and angiogenesis, and stimulates the proliferation of tumor cells. As a result serum ADA activity is also increased to detoxify the high amounts of toxic adenosine and deoxyadenosine substrates produced from accelerated purine metabolism in the cancerous tissues. It has also been suggested that

increased ADA activity might be a physiological attempt of the cancer cells to provide more substrates needed by cancer cells to accelerate the salvage pathway activity[79].

Several studies have been conducted to know the levels of serum ADA activity in head and neck cancer patients.

In a study conducted by Harbans Lal and his co-investigators, the activity of ADA was estimated in various head and neck cancer patients and compared to controls. Serum ADA levels was increased by more than 150% in patients with head and neck cancer. ADA activity was found to be directly related to the stage of cancer, indicating that the increase was directly proportional to the primary tumor mass. On comparing the serum ADA levels in relation to the type of lesion the serum ADA levels were higher in patients with ulcerative growths than in those with proliferative growth. Serum ADA activities were also observed in patients of head and neck cancer following radiotherapy. After the completion of radiotherapy the decrease in mean ADA level was 85% and the decrease correlated well with a decrease in the tumor mass and improvement in the patients clinical condition[80].

Ashok K.J and co-investigators assessed the ADA activities in head and neck cancer patients as well as normal subjects. Serum ADA levels were significantly elevated in head and neck cancer patients and a highly significant increase in levels were noted as the disease progressed from stage I to stage IV[81].

R. Mishra and his co-workers also assessed serum ADA levels in head and neck cancer patients and found an increase in levels of ADA with increase in stage of the cancer. ADA activity was also significantly higher in squamous cell carcinoma patients

in comparison to other histopathological subtypes and more in poorly differentiated squamous carcinoma in comparison to undifferentiated squamous carcinoma. The activity of ADA was also found to decrease following surgery and radiotherapy[82].

Orhan Canbolat and his fellow searchers studied serum ADA and total SOD activities before and after surgical removal of cancerous laryngeal tissue. Activities of both enzymes were found to be higher in cancerous patients as compared to controls. No significant differences were found however between pre- and post-operative values for enzymes in the patient group. These findings were not in agreement with previous studies. They concluded that high levels of serum ADA and SOD after surgical operation might be as a result of previous enzyme leakage from cancerous tissues since half life spans of these enzymes in serum might be long enough to continue high enzyme activity for more than one month[83].

Kalcioğlu MT and his co-workers estimated the activities of adenosine deaminase, xanthine oxidase, superoxide dismutase, glutathione peroxidase and malondialdehyde levels in the sera of 35 patients with head and neck cancers and compared them to those of healthy control subjects. Serum adenosine deaminase activity was found to be significantly increased in the patient group ($p<0.001$). The results of their study indicate that serum adenosine deaminase activity may be helpful in the diagnosis and follow-up of head and neck cancers[84].

**5à- NUCLEOTIDASE: (5à-ribonucleotide phosphorylase E.C 3.1.3.5)**

It is an alkaline phosphomonoesterase that hydrolyses nucleotides with a phosphate group on carbon atom 5' of the ribose, for example adenosine 5′-monophosphate (5′-AMP) to adenosine and inorganic phosphate (Figure 8)[70]. These nucleotides are also hydrolyzed by non-specific phosphatases such as alkaline phosphatase present in the serum. However 5′-NT is inactivated by nickel, hence if hydrolysis is carried out with and without added nickel, the difference gives the 5′-NT activity[85]. It is an intrinsic membrane glycoprotein present as an ectoenzyme in a wide variety of mammalian cells having a pH optimum of 7.8. It was first discovered by Reis in 1934[85].

**Tissue Distribution**

5′-Nucleotidase is widely distributed in living systems including human placental tissues. The posterior lobe of the pituitary contains the most, with relatively modest amounts in thyroid, testis and aorta and smaller quantities in lung, kidney, liver and pancreas. The above nucleotides are also hydrolyzed by non-specific alkaline phosphatases[85]. Reis (1951) found that 5′-NT was markedly activated by Mn ions, only slightly by Mg and strongly inactivated by Ni and Zn ions[85].

**Isoenzyme forms of 5à-NT**

Human isonucleotidases as separated by electrophoresis exist in three 5′-nucleotidase forms[86] :- NTP-1:- mean 12% of total 5′-nucleotidase.

NTP-2:- mean 30% of total 5′-nucleotidase.

NTP-3:- mean 58% of total 5′-nucleotidase.

The increase in total 5′-nucleotidase in patients with hepatobiliary disease is mainly due to the NTP-1 iso-form.

**Functions of 5à-NT**

1) 5'-NT is an ectoenzyme in many tissues concerned with the conversion of AMP into adenosine. This activity is probably part of a metabolic pathway for removing extracellular adenine nucleotides released during processes such as neuro-transmission, strenuous exercise, platelet thrombus formation and shock[87].

2) In turn, besides being further metabolized, the product adenosine has effects at A1- or A2- type adenosine receptors on various tissues including vasculature. A possible role of 5'-nucleotidase in the regulation of blood flow has been proposed for several years[87].

3) It is a cell surface glycoprotein and it undergoes various stages of posttranslational processing and appears to circulate between the cell surface and an intracellular pool and possibly interacts with elements of the cytoskeleton. It is attached to the plasma membrane either as a short-stalked integral membrane protein or through a glycosyl-phosphatidyl inositol lipid anchor thereby being involved in varied functions like cell-cell communication, signal transduction, membrane transport etc[87].

4) It helps in nucleic acid repair[87].

5) In addition, in white adipose tissue 5'-nucleotidase activity shows sex differences and adaptivity in several pathophysiological states[87].

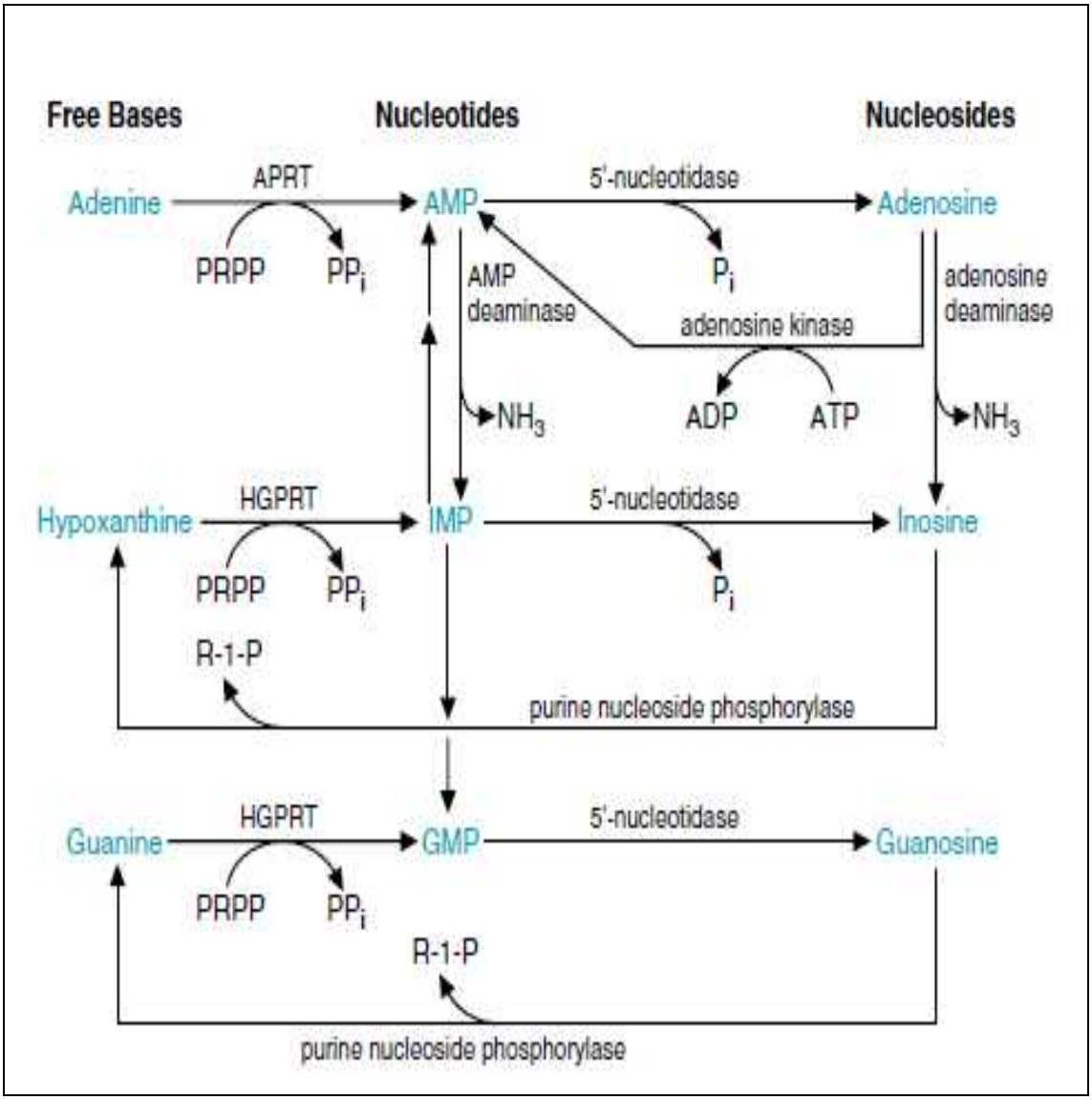



**FIGURE 8:- Purine Salvage Pathway**

**Clinical significance**

Despite its ubiquitous distribution, serum 5′-NT activities are thought to reflect hepatobiliary disease especially obstructive jaundice with considerable specificity. Dixon and Purdom first observed that serum 5′-NT was clinically useful for differential diagnosis of hepatobiliary and osseous diseases, the enzyme activity being increased only in hepatobiliary diseases[88].This finding has been confirmed by several authors. The intracellular location of liver and bile ductile 5′-NT is almost exclusively the plasma membrane and a detergent is required for solubilization of the enzyme. In patients with hepatobiliary disease, altered concentrations of bile salts or modified tissue perfusion in some way solubilises 5′-NT from the cells of the bile ducts and liver causing an increase in the serum enzyme activity. This mechanism could account for the increased serum activity in hepatobiliary disease and relative infrequent elevation in those diseases that affect other tissues with similar 5′-NT content[88].

In Paget's disease and other bone diseases however 5′-NT usually remains within normal range. These results have been confirmed by later investigators using newer techniques. 5′-NT is useful as alkaline phosphatase as a liver function test, since it is not significantly raised in bone diseases[89]. It is suggested that high levels encountered in liver disease are caused by cholestasis rather than hepatocellular damage. High levels also occur in cirrhosis, especially in early cases, but in advanced cases values may be only marginally elevated due to deficient enzyme synthesis[88]. Schwartz and Bodansky serially determined serum 5′-nucleotidase activities in patients with metastasis of the liver and found the test useful in following the course of the disease process[90]. Serum 5′-NT

activity in normal children and adolescents is similar to that in adult[91]. Values are unchanged by pregnancy[92].These findings are in contrast with those of alkaline phosphatase, which is elevated during pregnancy and also in children during periods of rapid growth as in early infancy or in adolescence[92]. 5′-NT values can therefore be used to assess whether the increase is attributable to hepatobiliary disease or bone disease in these subjects.

**Role of serum 5å-NT activity in oral and laryngeal cancer.**

5′-NT is an important enzyme participating in the purine and DNA metabolism. 5′-NT activities were found increased (Ozturk et al), unchanged (Durak et al) or decreased (Camici et al) in some cancerous tissues and cell systems. Decreased activity was evaluated as an attempt of the cancer cell to preserve the mononucleotide pool, and an increased activity was mostly evaluated as an attempt to supply salvage pathway activity.

There are very few studies available on altered levels of 5′-NT in oral and laryngeal cancer patients.

In a study conducted by H Lal and co-workers the 5′-NT levels were significantly increased in head and neck cancer patients (4-5 fold) as compared to controls. It was also noted that levels of 5′-NT were significantly higher from stage I to stage IV. Patients with cervical metastasis showed higher enzyme levels than those without cervical metastasis. The rise in serum 5′-NT activity in relation to the stage of cancer suggests that the increase in tumor mass may be directly responsible for the increase levels of the enzyme. In the present study also most of the patients were of squamous cell carcinoma and

showed a comparatively higher serum 5′-NT levels than those with other types of cancer[93].

H Lal and co-investigators also studied 5′-NT activity in head and neck cancer patients before and after treatment and concluded that there was a gradual fall in serum 5′-NT activity after chemotherapy ,while after radiotherapy there was a faster decrease. The decrease in enzyme activity may be as a result of treatment and is suggestive of tumor inactivity[93].

**Retinol (Vitamin A)**

The term retinoid first coined by Sporn in 1976 refers to naturally occurring and synthetic vitamin A (retinol) metabolites and analogues[94]. It is a member of fat-soluble vitamin and studies have shown that vitamin A is an important physiological regulator of embryonic development, vision, reproduction, bone formation, haematopoesis, differentiation, proliferation and apoptosis[95]. Several in vitro and in vivo studies have examined the positive and negative effects of vitamin A analogues in premalignant and malignant lesions.

Epidemiological and experimental studies have implicated reactive oxygen species (ROS) induced lipid per-oxidation in the development of pre-cancer and cancer. The extent of oxidative damage caused by ROS can be exacerbated by a decreased efficiency of antioxidant defense mechanisms of the body. The deteriorative effects of free radicals are counteracted by the antioxidant vitamins A, C, and E that prevent tissue damage by trapping organic free radicals and deactivating excited oxygen molecules[96]. Fruits and vegetables are excellent sources of antioxidant vitamins. Vitamin A has a

stabilizing effect on the mucous membranes. It functions as a non-enzymatic antioxidant and a free radical scavenger. By interfering with action of pro-carcinogens, it prevents binding of carcinogens to DNA, inhibits chromosome aberrations, restrains replication of transformed cell and suppresses action of cancer promoters. Retinol also acts within the cell to control gene expression[97]. The deficiency of vitamin A causes loss of mucous secreting cells and epithelial atrophy resulting in mucosal irritation and metaplasia. It therefore has been implicated as a biological factor in reducing the incidence of cancer.

Comstoc et al observed low levels of retinol and b-carotene in patients with cancer[98]. Stone and Troll reported that retinol and its analogues, retinyl acetate and retinoic acids are effective inhibitors of superoxide radical production in polymorphonuclear leukocytes[99].

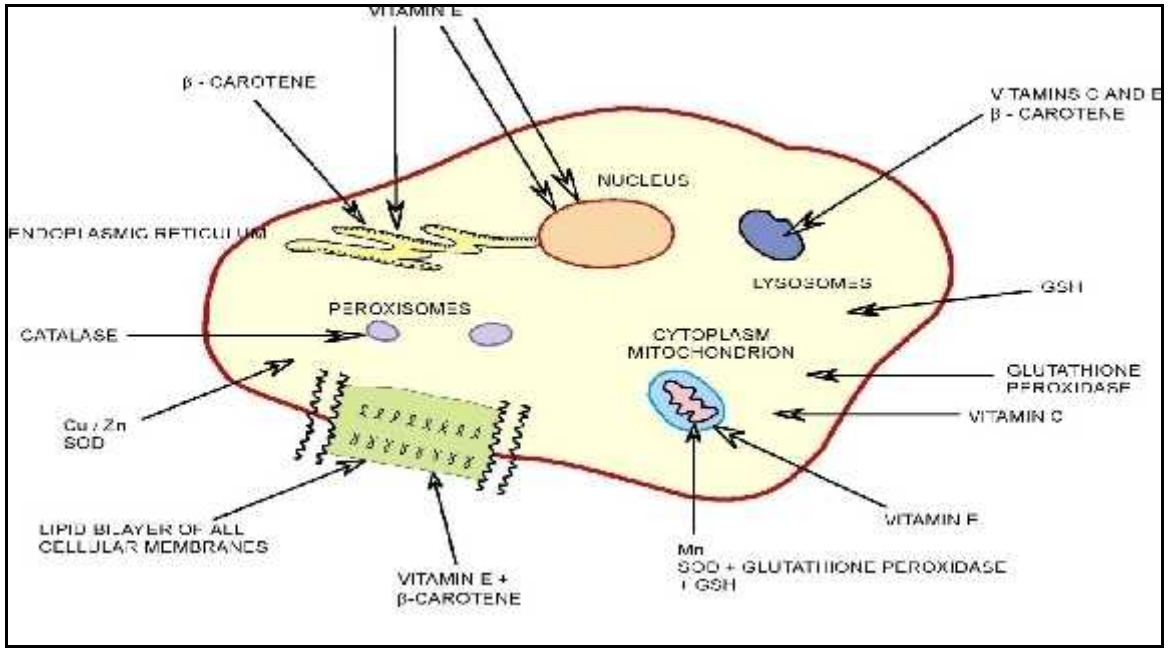



**FIGURE 9:-Anti-oxidant protection inside the cell**

The chemo preventive role of retinoids, minerals and vitamins in carcinogenesis is increasingly being appreciated. Several epidemiologic studies conducted worldwide show that a low intake of fruits and vegetables of high carotenoid content is directly proportional with the increased risk of cancer especially of head and neck cancer[100]. In a study oxidative DNA damage was assessed in 24 vegetarians and compared to 24 non-vegetarians. The study showed that DNA strand breaks and oxidized purine were significantly lower in vegetarians. Sufficient anti-oxidative status is crucial in free radical defense. Intake of protective food commodities were significantly higher in vegetarians; thus less risk to oral cancer[100].

Umesh Kapil et al estimated vitamin A, C and zinc levels in laryngeal cancer patients. The mean serum vitamin A, zinc and plasma vitamin C levels were significantly lower in laryngeal cancer patients as compared to the controls indicating a strong association of these micronutrients with laryngeal cancer[39].

Kandpal N et al evaluated oxidant stress, vitamin A and angiotensin-converting enzyme (ACE) levels in cases of laryngeal carcinoma and found a decrease in mean serum vitamin A levels with an increase in oxidative stress and ACE levels[101].

Olmedilla B et al evaluated retinol, alpha-tocopherol, and carotenoids in serum of men with cancer of the larynx before and after commercial enteral formula feeding. The levels of all compounds analyzed were significantly lower in men with laryngeal cancer than in the control group. Retinol and tocopherol levels increased significantly after enteral formula feeding, although they continued to be significantly lower than those of the controls[102].

Ettore Bidoli and his co-workers investigated various micronutrients in laryngeal cancer. Significant inverse relationships were observed between laryngeal cancer and vitamin C, beta-carotene, vitamin E, thiamine etc supporting that some aspects of diet are also linked to laryngeal carcinogenesis[103].

In a study by Elio Riboli and his co-workers it was postulated that the presence of tobacco and alcohol with low intake of fruit and vegetables accounted for 25 to 50 percent of the laryngeal cases among men.[41]

Khanna R et al supplemented 20 patients suffering from leukoplakia with Vitamins A, C and E for 3 months. At the end of this period there was a significant elevation of serum catalase levels ($p<0.05$) while levels of MDA, SOD and GP were almost same. The study showed a positive benefit of vitamin (A,C,E) and nutrition supplementation on the antioxidant enzyme defense system hence prevention of oral carcinogenesis in patients with leukoplakia[104].

In a study conducted by G. Ramaswamy and his co-workers, a significant decrease in the serum levels of vitamin A, beta-carotene and vitamin C, were found in tobacco chewers compared to normal non-chewers suggesting the anti-oxidant role of these vitamins[105].

In a study conducted by Hans F. Stich et al, vitamin A supplementation decreased the frequency of micronuclei in cells scraped from inside the human cheek which are a measure of chromosome breakage in earlier cell divisions leading to carcinogenesis. The study suggests a possibility that an increase in the dietary intake of retinol and or carotene

may reduce the incidence of oral cancer, which is an important neoplasm in many parts of Asia[106].

In a study conducted by Ramaswamy PG and his co-workers, vitamin A levels were estimated in various epithelial cancers. In cancer of the oral cavity, the males showed significantly lower levels of retinol compared with their female counterparts[107].

In a study conducted by N.De Vries et al, low levels of serum retinol and beta carotene were found in head and neck cancer patients with and without second primary tumor[108].

Bichler E et al studied the serum levels of retinol, RBP (retinol-binding protein) and PALB (prealbumin), which were found to be significantly lower in patients with malignant tumors of the head and neck region than in controls. The reduced serum levels could be considered as a possible factor in tumor development and growth[109].

Drozdz M and his fellow workers studied vitamin A, E zinc and retinol binding protein in serum of patients with laryngeal cancer. Serum vitamin A concentrations in laryngeal cancer patients was found to be significantly lower than those of patients of similar age with either non-malignant laryngeal or other than laryngeal diseases[110].

# METHODOLOGY

The present study was conducted on patients with oral and laryngeal cancer attending the outpatient department of the Otorhinolaryngology and Head and Neck Surgical Oncology Services of KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum during a period one year.

**Study Design**

Cross sectional study.

**Study Period**

The present study was conducted during the period of February 2009 to January 2010.

**Sample size**

The present study comprised of total 50 clinically and histologically confirmed patients of oral and laryngeal cancer and 50 healthy subjects age and sex matched. Cases were grouped into stage I, II, III and IV.

**Sample procedure**

Sample size was calculated by taking 80% of the average of the previous three years.

**Selection Criteria**

***Inclusion Criteria***

- Patients who are newly diagnosed and clinically and histopathologically confirmed with oral and laryngeal cancer.

***Exclusion Criteria***

Patients suffering from

- Tuberculosis
- Liver disorders
- Diabetes mellitus
- Coronary heart disease
- Renal disorders
- Pancreatic disorders
- Pulmonary disorders

**PROCEDURE**

All cases were evaluated and selected by simple random technique after fulfilling the selection criteria. The study was approved by the Ethical and Research committee of J.N. Medical College, Belgaum.

Clinically and histopathologically confirmed cases of oral and laryngeal cancer reported to the department of the Otorhinolaryngology and Head and Neck Surgical Oncology services of K.L.E.S. Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum and were screened. After finding the suitability as per inclusion and exclusion criteria they were requested to participate in the study and briefed about the nature of the study, the interventions used and written consent was obtained (Annexure-I). The consented patients were enrolled in the present study. Further descriptive data of the participants like name, age, sex, detailed history, were obtained by interviewing the participants and were recorded on a predesigned and pretested performa (Annexure-II).

**Collection of Blood Samples**

About 5 ml of venous blood was collected from the antecubital vein under aseptic precautionary measures using disposable syringe. The whole blood was allowed to clot and serum was separated by centrifugation and stored at $4^0$ C. The estimation of the parameters was carried out immediately. The following methods were used for the assay of the parameters.

1) Serum ADA: Galanti and Giusti Method.
2) Serum 5′-Nucleotidase: Method of Campbell.
3) Serum retinol: Bessey OA, Lowry OH, Brock MJ, Lopez JA.

**ESTIMATION OF ADENOSINE DEAMINASE IN SERUM:-**BY GALANTI AND GIUSTI METHOD[111]

**Principle**

Adenosine deaminase hydrolyses adenosine to ammonia and to inosine. The ammonia formed further reacts with a phenol and hypochlorite in an alkaline medium to form a blue indophenol complex with sodium nitroprusside acting as a catalyst. Intensity of the blue coloured indophenol complex formed is directly proportional to the amount of ADA present in the sample.

$$\text{Adenosine} + H_2O \xrightarrow{\text{ADA}} \text{Ammonia} + \text{Inosine}$$

$$\text{Ammonia} + \text{Phenol} + \text{hypochlorite} \xrightarrow{\text{Alkaline Medium}} \text{Blue indophenol complex}$$

**Reagents**

The following reagents are supplied by the company Microexpress – A division of Tulip Diagnostics (P) Ltd, Goa.

a) ADA- MTB reagent (L1):- Buffer reagent, ready to use.

b) ADA- MTB reagent (L2):- Adenosine reagent, ready to use.

c) ADA- MTB reagent (L3):- Phenol reagent.

d) ADA- MTB reagent (L4):- Hypochlorite reagent.

e) ADA- MTB standard (S):- ADA standard, ready to use.

**Reagent preparation**

Reagents L1, L2 and standard (S) are ready to use. Both the phenol reagent (L3) and hypochlorite reagent (L4) need to be diluted 1:5 with distilled water before use (1 part of reagent + 4 parts of distilled water).

**Storage and Stability**

**1.** Store the reagents at 2 to 8°C away from light when not in use.

**2.** Stability of the reagents is as per the expiry date mentioned on the label.

**3.** The working phenol reagent and working hypochlorite reagent are stable for 6 months when stored at 2 to 8°C in tightly closed bottles.

**4.** ADA is reported to be stable in serum for three days at 2 to 8°C as after this, ammonia may be released in the samples even without any microbial contamination.

**Conditions to be maintained to get accurate result:-**

1) Water bath temperature should be maintained at 37°C.

2) Do not use haemolysed, contaminated or turbid sample specimens. Adenosine reagent may form crystals at 2 to 8°C. Dissolve the crystals gently by warming the reagent for sometime before use at 37°C to 50°C.

3) The kit components from the same lot should be used for achieving accurate and reproducible results. Do not intermix reagents from different lots.

4) The sequence of addition of reagents should be followed meticulously.

**Test Procedure**

**1.** Bring all the reagents and samples to room temperature before use.

**2.** Prepare the working phenol reagent and working hypochlorite reagent.

**3.** Set the spectrophotometer filter at 570-630 nm (Hg 578 or 623nm) at 37°C.

**4.** Pipette into clean dry test tubes labelled Blank (B), Standard (S), Sample Blank (SB) and Test (T) as follows**:**

| **Additional Sequence** | **B (ml)** | **S (ml)** | **SB (ml)** | **T(ml)** |
|---|---|---|---|---|
| **Buffer reagent** | 0.20 | 0.20 | --- | --- |
| **Adenosine reagent** | --- | --- | 0.20 | 0.20 |
| **Deionised water** | 0.02 | --- | --- | --- |
| **Standard** | --- | 0.02 | --- | --- |
| **Sample** | --- | --- | --- | 0.02 |

**5.** Mix well and incubate at 37°C for exactly 60 minutes and then add the following.

| **Additional Sequence** | **B (ml)** | **S (ml)** | **SB (ml)** | **T (ml)** |
|---|---|---|---|---|
| **Working phenol reagent** | 1.00 | 1.00 | 1.00 | 1.00 |
| **Sample** | --- | --- | 0.02 | --- |
| **Working hypochlorite reagent** | 1.00 | 1.00 | 1.00 | 1.00 |

**6.** Mix well and incubate at 37°C for 15 minutes or at room temperature for 30 minutes.

**7.** Measure the absorbance of the blank (Abs. B), standard (Abs. S), sample (Abs. SB) and test (Abs. T) against distilled water.

**Calculation:-**

$$\text{Total ADA activity in U/L} = \frac{\text{Abs. T} - \text{Abs.SB}}{\text{Abs.S} - \text{Abs.B}} \times 50$$

**REFERENCE VALUES**:-

| **Sample** | **Prediction** | **Values** |
|---|---|---|
| **Serum, Plasma, Pleural, Pericardial and Ascitic Fluids** | Normal | < 30 U/L |
| | Suspect | 30 U/L to 40 U/L |
| | Strong Suspect | >40 U/L to 60U/L |
| | Positive | > 60 U/L |
| **CSF** | Normal | < 10 U/L |
| | Positive | >10 U/L |

**ESTIMATION OF 5å-NUCLEOTIDASE IN SERUM:** BY METHOD OF CAMPBELL[85]

**Principle:-**

5′-Nucleotidase enzyme catalyses the dephosphorylation of nucleotides that have a phosphate group attached to C-5 of the ribose radical. The method involves two parallel enzyme activity determinations with adenosine-5-phosphate as substrate. In one the presence of nickel specifically inhibits 5′-nucleotidase and therefore estimates the hydrolysis of the substrate by non-specific alkaline phosphatase. In the second the absence of nickel allows the estimation of total phosphatase activity. The difference in activity (in terms of inorganic phosphate liberated) gives the 5′-nucleotidase activity.

**Determination of serum 5å-Nucleotidase**

**Method of Campbell**

**Reagents:-**

1. Barbitone Buffer: 40mM, pH 7.5, 4.8 grams diethyl barbituric acid and 2.90 grams sodium diethyl barbiturate per litre.
2. Adenosine-5′-phosphate: 10mM, 0.174 gram per 50 ml. To dissolve add 9.0 ml of 0.1N sodium hydroxide and make to 50 ml with water. Keep in refrigerator.
3. Nickel chloride: 0.1 M, 23.77 grams per litre.
4. Manganous Sulphate: 0.02 M, 4.66 grams per litre.
5. Trichloroacetic acid: 10 per cent solution in water.

6. Acetate buffer: 2M, pH 4.0, 46 grams of trihydrate of sodium acetic acid (to prepare 2N acetic acid make 115 ml glacial acetic acid to a litre with water and standardized against sodium hydroxide using phenolphthalein as indicator).
7. Ammonium molybdate: 5 per cent in water.
8. Metol and Sodium sulphite: 2 grams of metol and 10 grams of sodium sulphite in water and made to 100 ml. Keep in the dark at 4°C.
9. Phosphate standard: Prepare a stock solution containing 6 mM per litre. Dissolve 0.204 gram anhydrous potassium dihydrogen phosphate in water and make to 250 ml and add a drop of chloroform.

Dilute standard 1 in 100 for use. This contains 60 µM per litre.

**Technique:-**

Set up two tubes as follows:

**A.** Total activity:-Serum 0.2 ml, manganous sulphate 0.1 ml and buffer 1.5 ml.

**B.** Non-specific alkaline phosphatase activity:-Serum 0.2 ml, manganous sulphate 0.1 ml, buffer 1.3 ml and nickel chloride 0.2 ml.

Warm to 37°C then add 0.2 ml adenosine-5′-phosphate to each and incubate at 37°C for thirty minutes. Add 2.0 ml of 10% trichloroacetic acid, mix well and let it stand briefly, than centrifuge. Take 2 ml of the supernatants (=0.1 ml serum) for estimation of inorganic phosphorous. For the blank and standard add 1ml of water and 1ml of the standard for use, each with 1 ml trichloroacetic acid added.

To all four add 3ml acetate buffer, 0.5 ml of molybdate and 0.5 ml metol. Mix and allow it to stand for ten minutes, and then read using a red filter or at 680 nm.

**Calculation:-**

5′-nucleotidase activity in I.U per litre

$$= \frac{\text{Reading of A - Reading of B}}{\text{Reading of standard - Reading of blank}} \times \frac{1000}{0.1} \times \frac{60}{1000} \times \frac{1}{30}$$

$$= \frac{\text{Reading of A – Reading of B}}{\text{Reading of standard – Reading of Blank}} \times 20.$$

**Reference Values:**

Serum:-2-17 U/L.

**ESTIMATION OF VITAMIN A (RETINOL) IN SERUM:** BY METHOD OF BESSEY OA ET AL[112]

**Principle:**

Proteins get precipitated on addition of ethanol and concentration of retinol can be determined by reading extinction of heptane extract of retinol at 327nm.

**Reagents:**

**1.** Absolute Ethanol.

**2.** N- Heptane.

**3.** Retinol stock standard: 10-mg %:- 10 mg of retinol palmitate (Sigma) was dissolved in 100 ml of n- heptane.

4. Retinol working standard, 100 μg %:- 1 ml of stock standard retinol solution was diluted to 100 ml with n- heptane.

**Procedure:**

In a clean dry test tube, 2.0 ml plasma was taken. To this 1.0 ml of distilled water (D/W), 4.0 ml of each ethanol and n – heptane were added. Contents of tube were mixed for 15 minutes using a cyclo mixer and then centrifuged at 3000 rpm for 5 minutes. Upper heptane layer was then separated and read at 327 nm against heptane blank using a double beam spectrophotometer. Working standard of retinol was directly read at 327 nm.

**Calculations:**

Concentration of retinol is expressed as μg % of retinol palmitate

$$= \frac{\text{OD of sample}}{\text{OD of standard}} \times \text{concentration of standard (μg \%)}$$

**Reference Values**

Serum:- 25-75 μg/dl

## STATISTICAL ANALYSIS

Statistical analysis of all the obtained parameters in patients with oral and laryngeal cancer and control groups were done using student's unpaired't' test. The mean and standard deviation (S.D) for each of the outcome was computed. The comparison between controls and various stages of oral and laryngeal cancer subjects was done by analysis of variance (ANOVA) followed by Bonferroni multiple comparison test.

# RESULTS

## CLINICAL OBSERVATION

The present study comprises of 100 participants, 50 healthy controls and 50 cases of oral and laryngeal cancer, which was confirmed by clinical and histopathological examination and the mean age and sex is matched in two groups. The findings are tabulated as below.

**Table No. 1:- MEAN AGE IN CASES AND CONTROLS**

| Group | Mean Age | Standard deviation |
|---|---|---|
| Control (n = 50) | 54.52 | 8.48 |
| Oral cancer cases (n = 25) | 56.0 | 8.35 |
| Laryngeal cancer cases (n = 25) | 57.2 | 8.46 |
| F value for 2, 97 = 0.885 | | p = 0.416 |

The mean age for controls was 54.52 ± 8.48 years and in oral and laryngeal cancer patients it was 56.0 ± 8.35 years and 57.2 ± 8.46 years respectively with p value of 0.416. Hence age is matched between the two groups of cancers and control.

**Table No. 2:- SEX DISTRIBUTION IN CASES AND CONTROLS**

| Group | Male | Female |
|---|---|---|
| Control ( n= 50) | 35 | 15 |
| Oral cancer cases (n = 25) | 22 | 3 |
| Laryngeal cancer cases ( n = 25) | 18 | 7 |
| $x^2 = 3.04$ | df = 2 | p = 0.219 |

Out of 50 controls, 35 are male and 15 females. Out of 25 oral cancer cases 22 are males and 3 are females, whereas out of 25 laryngeal cancer cases 18 are males and 7

are females with p value of 0.219. Hence sex is matched between the two groups of cancers and control.

## ORAL CANCER

**TABLE No. 3:- TYPES OF HABITS IN ORAL CANCER**

| Habits | No. of Cases | Percentage |
|---|---|---|
| Tobacco and betel quid chewing | 7 | 28 |
| Smoking | 4 | 16 |
| Alcohol | 0 | 0 |
| Combined | 14 | 56 |
| No habits | 0 | 0 |
| Total | 25 | 100 |

Maximum number of cases- 14 (56 %) had combined habits.

**TABLE No. 4:- SITE OF LESION IN ORAL CANCER**

| Site | No. of cases | Percentage |
|---|---|---|
| Buccal mucosa | 8 | 32 |
| Tongue | 7 | 28 |
| Hard palate | 4 | 16 |
| Lip | 3 | 12 |
| Floor of mouth | 2 | 8 |
| Alveolus | 1 | 4 |
| Total | 25 | 100 |

Maximum number of cases – 8 (32%) had lesions on the buccal mucosa.

**TABLE No. 5:- CLINICAL (TNM) STAGING OF ORAL CANCER CASES**

| Clinical Stage | No. of cases | Percentage |
|---|---|---|
| Stage I | 1 | 4 |
| Stage II | 11 | 44 |
| Stage III | 8 | 32 |
| Stage IV | 5 | 20 |
| Total | 25 | 100 |

Maximum number of cases – 11 (44%) belonged to TNM Stage II.

## LARYNGEAL CANCER.

**TABLE No. 6:- TYPES OF HABITS IN LARYNGEAL CANCER**

| Habits | No. of Cases | Percentage |
|---|---|---|
| Tobacco smoking | 8 | 32 |
| Alcohol consumption | 0 | 0 |
| Combined | 10 | 40 |
| No habits | 7 | 28 |
| Total | 25 | 100 |

Maximum number of cases- 10 (40 %) had combined habits.

**TABLE No.7:- SITE OF LESION IN LARYNGEAL CANCER**

| Site | No. of cases | Percentage |
|---|---|---|
| Supra glottic cancer | 14 | 56 |
| Glottic cancer | 11 | 44 |
| Sub glottic cancer | 0 | 0 |
| Total | 25 | 100 |

Maximum number of cases – 14 (56%) had supraglottic cancer.

**TABLE No. 8:- CLINICAL (TNM) STAGING OF LARYNGEAL CANCER**

| Clinical Stage | No. of cases | Percentage |
|---|---|---|
| Stage I | 3 | 12 |
| Stage II | 3 | 12 |
| Stage III | 11 | 44 |
| Stage IV | 8 | 32 |
| Total | 25 | 100 |

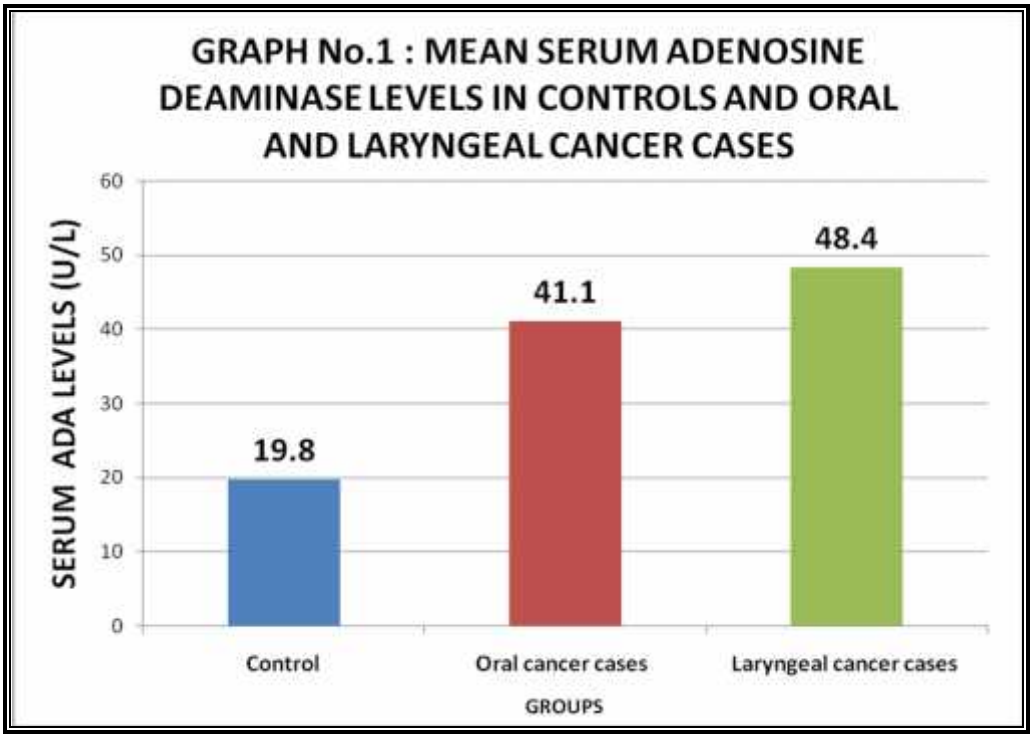
Maximum number of cases – 11 (44%) belonged to TNM Stage III.

## BIOCHEMICAL OBSERVATIONS

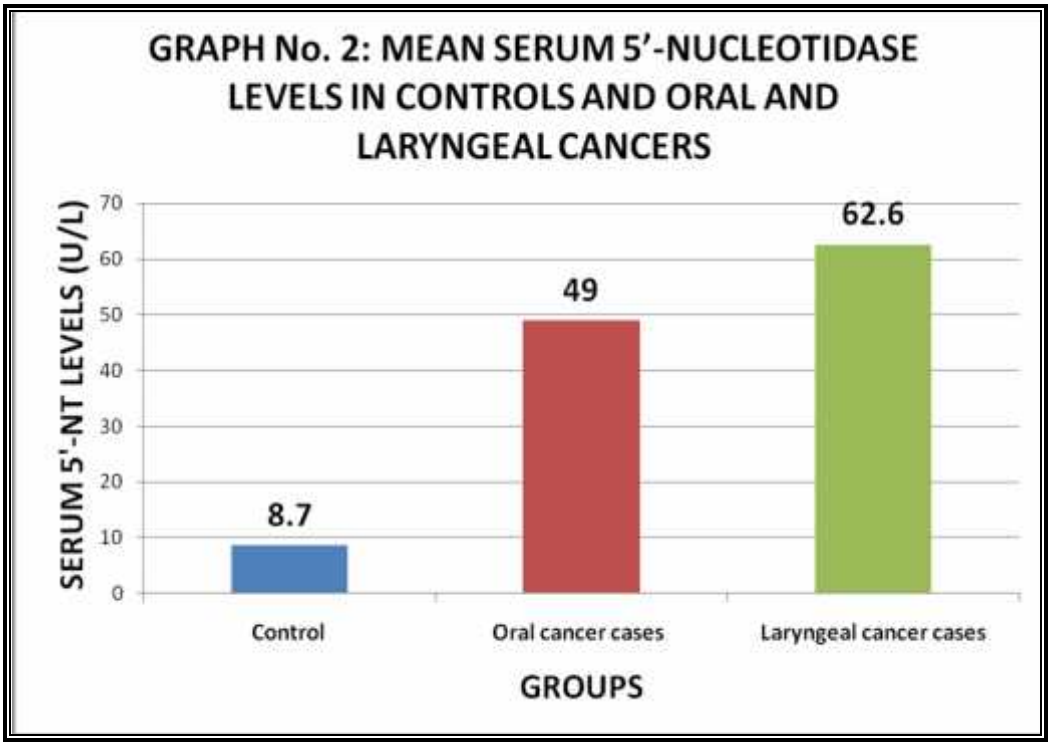
**TABLE No. 9:- SERUM ENZYMES AND RETINOL LEVELS IN CONTROL AND ORAL AND LARYNGEAL CANCERS**

| GROUPS | ADA (U/L) | 5à-NT (U/L) | Retinol (µg/dl) |
|---|---|---|---|
| Control (n = 50) | 19.8 ± 4.25 | 8.7 ± 4.15 | 41.1 ± 9.37 |
| Oral cancer cases (n = 25) | 41.1 ± 8.77 | 49 ± 10.45 | 22.3 ± 5.81 |
| Laryngeal cancer cases (n = 25) | 48.4 ± 10.78 | 62.6 ± 13.96 | 20.1 ± 6.53 |

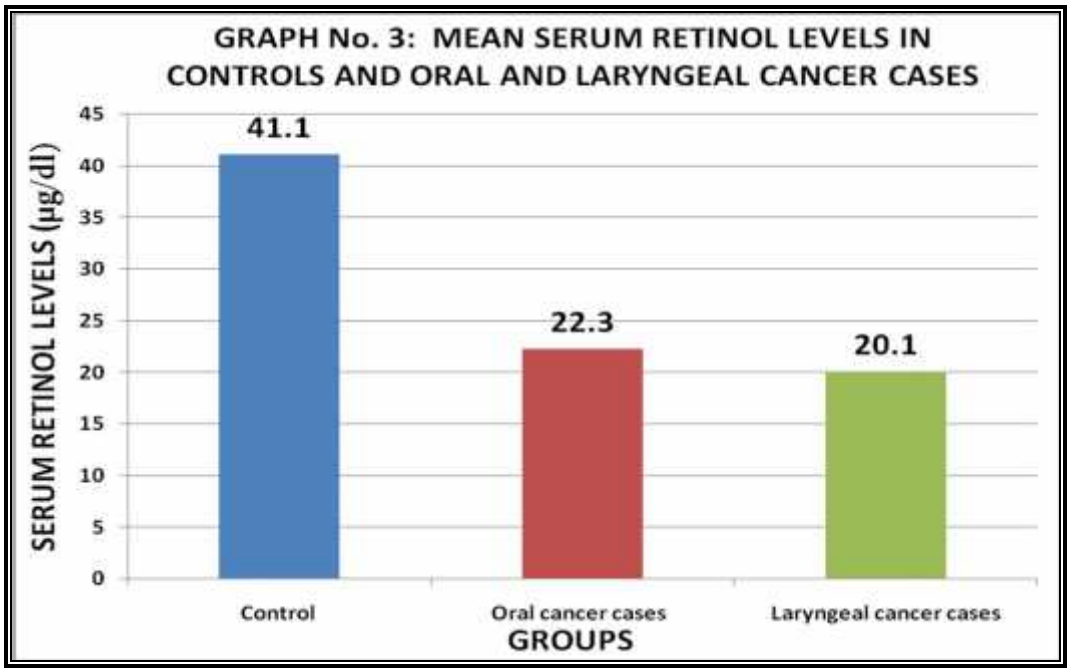
F value for 2, 97 for ADA = 141.952 $p < 0.000$
F value for 2, 97 for 5′-NT = 343.197 $p < 0.000$
F value for 2, 97 for retinol = 78.570 $p < 0.000$




The mean serum adenosine deaminase level in the oral cancers was 41.1 ± 8.77 U/L and in laryngeal cancers 48.4 ± 10.78 U/L with p value less than 0.000 which was statistically highly significant (TABLE No. 9 and GRAPH No.1).



The mean serum 5′- nucleotidase level in oral cancers was 49 ± 10.45 U/L while in the laryngeal cancers it was 62.6 ± 13.96 U/L with p value < 0.000 which was significant (TABLE No. 9 and GRAPH No. 2)



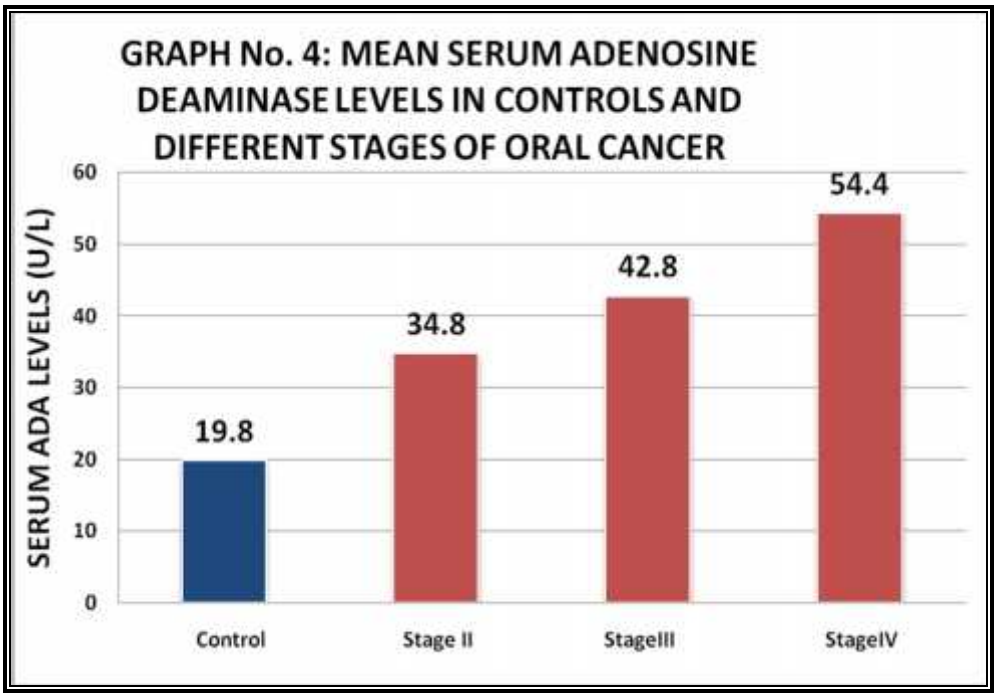

The mean serum retinol level in oral cancers was 22.3 ± 5.81 µg/dl while in laryngeal cancers it was 20.1 ± 6.53 µg/dl with a significant p value < 0.000 (TABLE No. 9 and GRAPH No. 3)

**TABLE No.10:- SERUM ENZYMES AND RETINOL LEVELS IN CONTROLS AND DIFFERENT STAGES OF ORAL CANCER**

| **Enzymes and Retinol** | **Control** | | **Stage ff** | | **Stage fff** | | **Stage fV** | |
|---|---|---|---|---|---|---|---|---|
| | **Mean** | **S.D.** | **Mean** | **S.D.** | **Mean** | **S.D.** | **Mean** | **S.D.** |
| ADA(U/L) | 19.8 | 4.25 | 34.8* | 3.31 | 42.8* | 4.61 | 54.4* | 4.87 |
| 5'NT(U/L) | 8.7 | 4.15 | 41.7* | 3.32 | 51.0* | 7.69 | 64.6* | 4.27 |
| Retinol (µg/dl) | 41.1 | 9.37 | 25.7* | 3.76 | 20.3* | 3.25 | 15.4* | 2.31 |

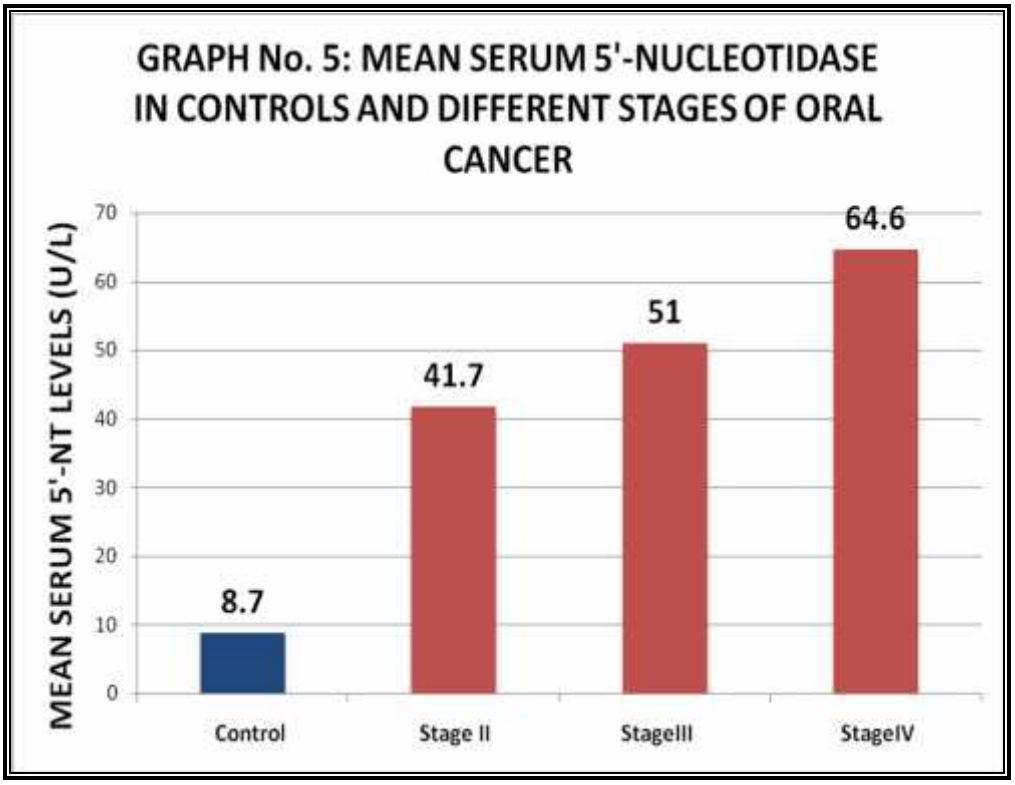
*p < 0.000 statistically significant

Only one case of oral cancer was found in Stage I of the disease (TABLE No. 5) due to which it was not included in the statistical analysis.



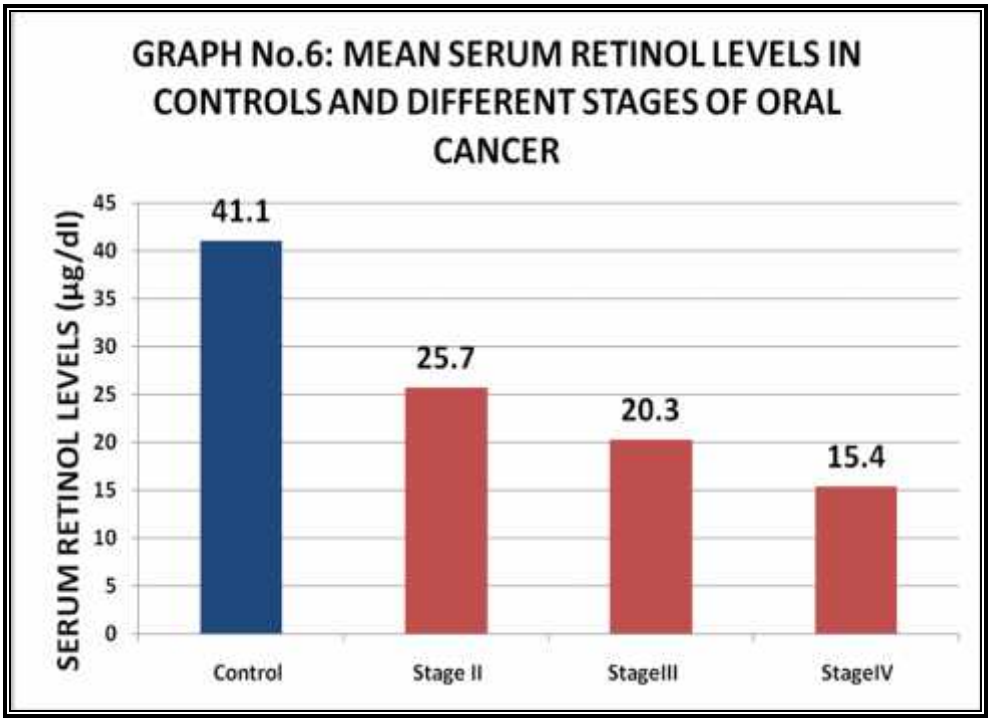

Serum ADA levels in various stages of oral cancer Stage II (34.8 ± 3.31 U/L), Stage III (42.8 ± 4.61 U/L) and Stage IV (54.4 ± 4.87 U/L) were also estimated and compared with controls (19.8 ± 4.25 U/L). A significant increase in serum ADA was observed in Stage II, III and IV when compared to controls with p value < 0.000 (TABLE No. 10 and GRAPH No.4).

An inter-stage comparison of serum ADA levels showed a significant increase in levels of ADA when compared between stage II and stage III with p value of 0.001, between stage II and stage IV with p value of 0.000 and between stage III and IV with a p value of 0.000.




Serum 5′-NT levels in various stages of oral cancer Stage II (41.7 ± 3.32 U/L), Stage III (51.0 ± 7.69 U/L) and Stage IV (64.6 ± 4.27 U/L) was also estimated and compared with control (19.8 ± 4.25 U/L). A significant increase in serum 5′-NT was observed in Stage II, III and IV when compared to controls with p value < 0.000 (TABLE No. 10 and GRAPH No. 5).

An inter-stage comparison of serum 5′-NT levels showed a significant increase in levels of 5′-NT when compared between stage II and stage III with p value of 0.004, stage II and stage IV with p value of 0.000 and between stage III and IV a p value of 0.001.



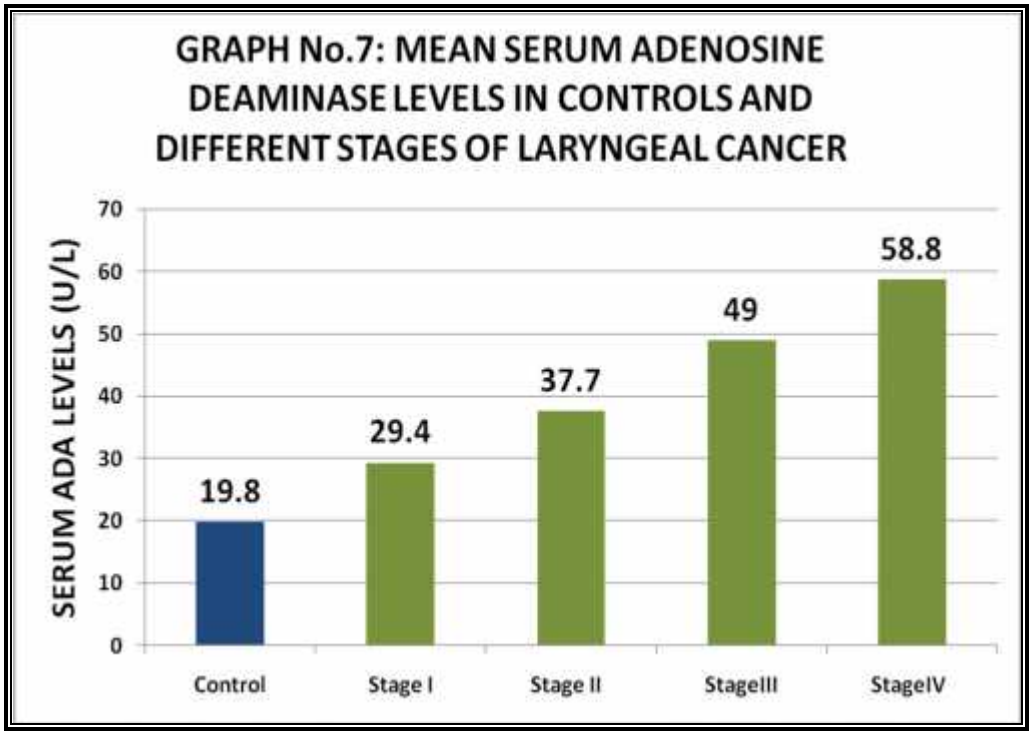

Serum retinol levels in various stages of oral cancer Stage II (25.7 ± 3.76 µg/dl), Stage III (20.3 ± 3.25 µg/dl) and Stage IV (15.4 ± 2.31 µg/dl) was estimated and compared with controls (41.1 ± 9.37 µg/dl). A significant decrease in serum retinol was observed in Stage II, III and IV when compared to controls with p value < 0.000.

An inter-stage comparison of serum retinol levels showed a significant decrease in levels of retinol when compared between stage II and stage III with p value of 0.006, between stage II and IV with p value of 0.000 and between stage III and IV the p value of 0.056 (TABLE No. 10 and GRAPH No. 6).

**TABLE No. 11:- SERUM ENZYMES AND RETINOL LEVELS IN CONTROL AND DIFFERENT STAGES OF LARYNGEAL CANCER**

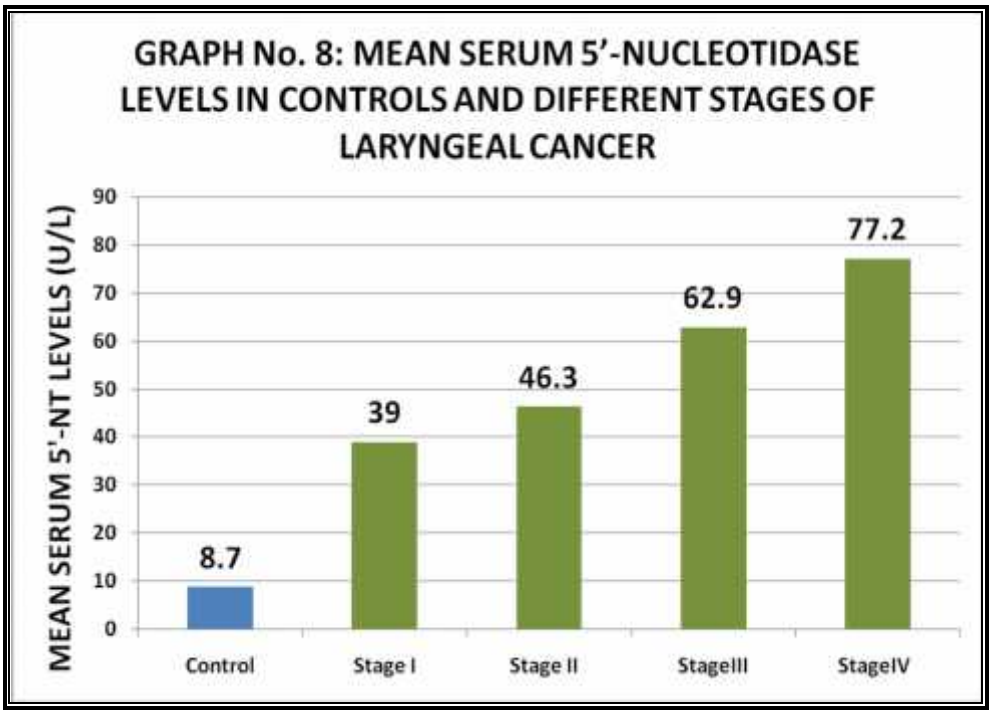
| Enzymes and retinol | Control | | Stage f | | Stage ff | | Stage fff | | Stage fV | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| ADA(U/L) | 19.8 | 4.25 | 29.4* | 3.14 | 37.7* | 4.05 | 49.00* | 4.67 | 58.8* | 5.72 |
| 5å-NT(U/L) | 8.7 | 4.15 | 39.0* | 4.00 | 46.3* | 5.51 | 62.90* | 5.33 | 77.2* | 4.16 |
| Retinol (µg/dl) | 41.1 | 9.37 | 33.3* | 6.79 | 23.2* | 3.26 | 18.7* | 3.77 | 16.1* | 2.95 |

*p value < 0.000 statistically significant




Serum ADA levels was estimated in various stages of laryngeal cancer Stage I (29.4 ± 3.14 U/L), Stage II (37.7 ± 4.05 U/L), Stage III (49.0 ± 4.67 U/L) and Stage IV (58.8 ± 5.72 U/L) and compared with controls (19.8 ± 4.25 U/L). A significant increase in serum ADA was observed in Stage I, II, III and IV when compared to controls with p value < 0.000 (TABLE No.11 and GRAPH No 7).

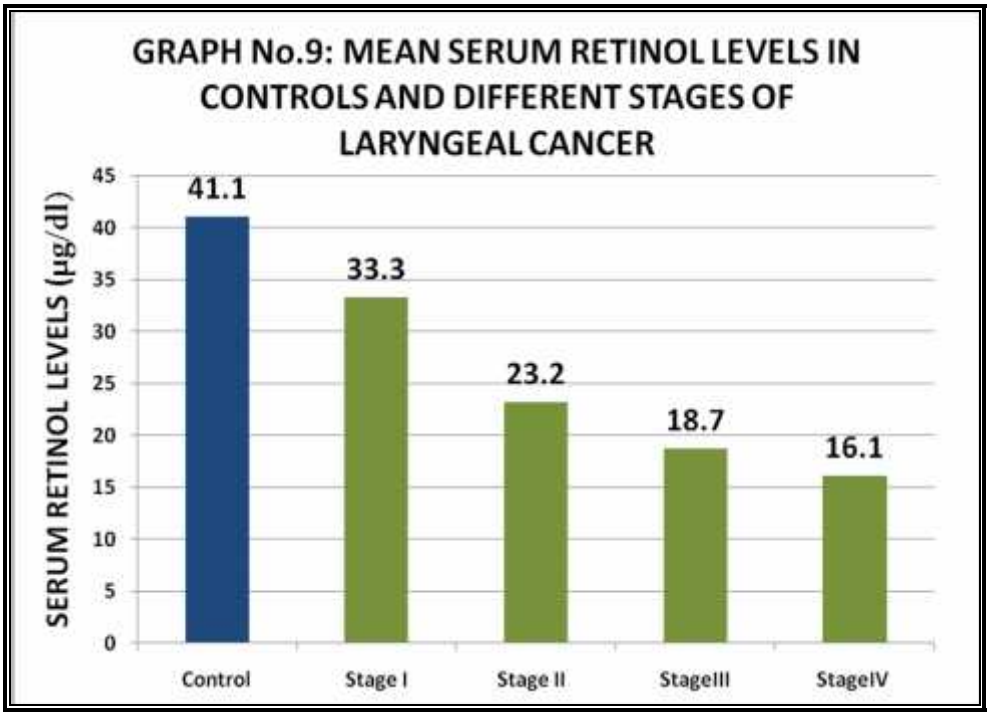
An inter-stage comparison of serum ADA levels showed a non significant increase in levels of ADA when compared between stage I and stage II with p value 0.216. A significant increase was observed between stage I and III with p value of 0.000, between stage I and stage IV with p value of 0.000, between stage II and III with p value of 0.012, between stage II and IV the p value 0.000 and between III and IV the p value 0.002.




Serum 5′-NT levels was also estimated in various stages of laryngeal cancer Stage I (39.0 ± 4.00 U/L), Stage II (46.3 ± 5.51 U/L), Stage III (62.90 ± 5.33 U/L) and Stage IV (77.2 ± 4.16 U/L) and compared with controls (8.7 ± 4.15 U/L). A significant increase in serum 5′-NT was observed in Stage I, II, III and IV when compared to controls with p value < 0.000 (TABLE No.11 and GRAPH No.8).

An inter-stage comparison of serum 5′-NT levels showed a non significant increase in levels of 5′-NT when compared between stage I and stage II with p value 0.478. A significant increase was observed between stage I and III with p value of

0.000, between stage I and stage IV with p value of 0.000, between stage II and III the p value of 0.000, between stage II and IV the p value 0.000 and between III and IV the p value 0.000.




Serum retinol levels at the various stages of laryngeal cancer Stage I (33.3 ± 6.79 µg/dl), Stage II (23.2 ± 3.26 µg/dl), Stage III (18.7 ± 3.77 µg/dl) and Stage IV (16.1 ± 2.95 µg/dl) was also estimated and compared with controls (41.1 ± 9.37 µg/dl). A significant decrease in serum retinol was observed in Stage I, II, III and IV when compared to controls with p value < 0.000 (TABLE No. 11 and GRAPH No.9).

An inter-stage comparison of serum retinol levels showed a non significant decrease in levels of retinol when compared between stage I and stage II with p value 0.026. A significant decrease was observed between stage I and III with p value of 0.000 and between stage I and stage IV with p value of 0.000. A non significant decrease between stage II and III with the p value of 0.537, between stage II and IV with the p value 0.077 and between stage III and IV the p value 0.939.

# DISCUSSION

Non-communicable diseases are fast replacing communicable diseases in India and other developing countries. Cancer is one such disease. Oral and laryngeal cancers remain a serious health problem worldwide. According to the Oral Cancer Foundation, someone dies from oral cancer every hour of the day in the United States alone[18]. This cancer, found in the mouth is often highly curable if diagnosed and treated early. Unfortunately, in its early stages, oral cancer can go unnoticed. For the laryngeal cancer early diagnosis is only possible for the glottic cancers as they present with early hoarseness of voice, whereas supraglottic and subglottic are diagnosed in advanced stages. Therefore despite therapeutic and diagnostic progress in head and neck oncology during the last decade the prognosis of both these cancers remains poor. Cancer that is detected early therefore offers the best chance for cure.

Tumor markers that are used for screening, diagnosis, staging, prognosis and monitoring of treatment of head and neck cancers are expensive require sophisticated technology and well equipped centers. Thus the need of the hour are tests that are simple, less invasive, less time consuming, easy for interpretation, economical and yet quite confirmatory for diagnosis and prognosis. In view of this the present study has been undertaken to assess the clinical utility of some enzyme markers namely, serum adenosine deaminase and 5′-nucleotidase which are inexpensive, rapid and analyzed by easy methods and which may be used as supportive parameters for diagnostic purpose and may add further for prognostic information.

Epidemiological studies worldwide have implicated dietary and nutritional factors in the development of various head and neck cancers. Fruits and vegetables are described to have a protective effect against cancer and precancerous lesions. Non-enzymatic antioxidant “vitamin A” has a stabilizing effect on the mucous membranes.

The deficiency of it causes epithelial atrophy resulting in mucosal irritation. In view of this, serum retinol levels were also evaluated in the present study to assess the degree of oxidative damage in oral and laryngeal cancers which may be helpful in management of the disease in its early stages.

A cross-sectional study was performed. The study sample comprised of 50 cases of oral and laryngeal cancer which were clinically and histopathologically confirmed and were squamous cell carcinoma histopathologically. Site and size of the lesion, tumor stage and nodal status was recorded while doing a thorough clinical examination of the patient. Out of the 50 cases of oral and laryngeal cancer, maximum cases belonged to Stage III (TABLE No. 5 and 8).

In the present study out of the 25 cases of oral cancer, 22 (88%) were males and 3 (12%) were females. Out of 25 cases of laryngeal cancer, 18 (72%) were males and 7 (28%) were females (TABLE No. 2). This is in consistence with findings of Neville and Baback Saedi who state that males have a higher incidence rate than females at all age levels[50,113]. High proportion of cases among males may be due to high prevalence of tobacco and alcohol consumption habits among males. Moreover, tobacco is consumed in both chewing and smoking form in males whereas in our society females indulge lesser in tobacco and alcohol consumption.

The age of oral and laryngeal cancer patients ranged from 45-75 years with oral cancer cases having a mean age of 56.0 ± 8.35 years and laryngeal cancer cases with mean age of 57.2 ± 8.46 years (TABLE No. 1). It has been suggested that the risk of intraoral cancer increases with increasing age especially for males. White men have a higher risk of intraoral cancer after 65 years of age than does any other group[50]. Some authors state that oral carcinoma is a disease of increasing age with 95% cases in people older than 40 years of age[114]. Worldwide, the peak incidence of

laryngeal cancer is highest in men aged between 55 to 65 years. Our data reinforces the data from other studies.

` Variable distribution of cancer at various sites in different populations suggests differences in habits and exposure to the risk factors. In the present study out of 25 cases of oral cancer, 8 cases (32%) had lesions on the buccal mucosa (TABLE No. 4). Most of these patients had combined habits (TABLE No.3). Carcinoma of the buccal mucosa, lateral border of the tongue and vestibule are frequently seen in betel quid chewers because the quid is placed in the buccal or lingual vestibule and compressed against the buccal mucosa or tongue.

Out of the 25 cases of laryngeal cancer, 14 cases (56%) had supraglottic cancer (TABLE No. 7). Most of the patients had history of tobacco smoking along with alcohol consumption (TABLE No. 6). Supraglottic cancer is commonly associated with smoking of black tobacco which is commonly observed in Asian countries. Alcohol plays a synergistic role by acting as a solvent for the carcinogens of tobacco[115]. In India betel quid chewers and tobacco smokers therefore constitute an important risk population for oral and laryngeal cancers.

## ADENOSINE DEAMINASE:

The mean serum ADA level in controls and in patients with oral and laryngeal cancer was 19.8 ± 4.25 U/L, 41.1 ± 8.77 U/L and 48.4 U/L respectively (TABLE No.9). The level of ADA was significantly increased in oral and laryngeal cancer patients as compared to controls ($p < 0.000$). An inter-stage comparison showed a significant increase in levels of ADA from stage I to stage IV. The study results are in accordance with the findings of Borzenko BG[79], Harbans Lal[80], Ashok KJ[81], R. Mishra[82], Orhan Canbolatal[83] and Kalcioğlu MT[84].

They concluded that purine nucleoside adenosine is produced at increased levels in the tissues of solid cancers as a result of local hypoxia. Adenosine inhibits the cell-mediated anti-tumor immune response, promotes tumor cell migration and angiogenesis and stimulates the proliferation of tumor cells. As a result, serum ADA activity is also increased to detoxify high amounts of toxic adenosine and deoxyadenosine substrates produced from accelerated purine metabolism in the cancerous tissues.

## 5å- NUCLEOTIDASE:

The mean serum 5′-NT level in controls and in patients with oral and laryngeal cancer was 8.7 ± 4.15 U/L, 49.0 ± 10.45 U/L and 62.6 ± 13.96 U/L respectively (TABLE No.9). The level of 5′-NT was significantly increased in oral and laryngeal cancer patients as compared to controls ($p < 0.000$). It was also observed that serum 5'-NT increased progressively from stage I to stage IV as compared to controls. The maximum rise was observed in stage IV patients. Similar results were documented by Harbans Lal[93]. Serum 5′-NT activity is also increased to detoxify high amounts of toxic adenosine and deoxyadenosine substrates produced from accelerated purine metabolism in the cancerous tissues.

## RETINOL (VITAMIN A):

The mean serum retinol level in controls and in patients with oral and laryngeal cancer was 41.1 ± 9.37 µg/dl, 22.3 ± 5.81 µg/dl and 20.1 ± 6.53 µg/dl respectively (TABLE No.9). The level of retinol was significantly decreased in oral and laryngeal cancer patients as compared to controls ($p < 0.000$). It was also observed that serum retinol levels decreased from stage I to stage IV as compared to controls. Stage IV oral and laryngeal cancer patients had lowest retinol levels. These

observations were in accordance with findings of Umesh Kapil[39], Kandpal N[101], Begona Olmedilla[102], E Riboli[41], Khanna R[104], Bichler E[109], Drozdz M[110]. They concluded that the decrease in retinol (vitamin A) levels in cancer could be due to the increased utilization by the affected tissues or in combating the excessive oxidative stress in circulation as a result of cancer.

The results of the study conducted by Manoharan S and his co-researchers as well R.C Dwivedi and his co-workers show increased lipid peroxidation in oral and laryngeal cancer patients as a consequence of free radical generation[116,117]. By products of lipid peroxidation cause marked alteration in the structural integrity and function of cell membranes. In accordance with this some of the studies showed significant lipid peroxidation in oral and laryngeal cancer patients[118,119,120] .

Pratibha K and co-investigators studied on the increased activity of ADA in acute infective hepatitis and concluded that lipid per-oxidation is followed by loss of structural integrity of plasma membrane. As a result, there occurs a release of membrane associated enzymes ADA and 5'-NT into the circulation[65]. Enhanced lipid per-oxidation with decline in antioxidants has been reported in the venous blood of oral and laryngeal cancer patients. The extent of oxidative damage caused by ROS can be exacerbated by a decreased efficiency of antioxidant defense mechanisms of the body.

Measurement of malondialdehyde (MDA) in this study therefore would have been useful to state lipid peroxidation as one of the possible causes of oral and laryngeal cancer progression associated with decreased antioxidant levels and to corelate it with the release of membrane associated enzymes ADA and 5'-NT into the circulation.

# CONCLUSION

In conclusion, our present study suggests that serum adenosine deaminase, 5′-nucleotidase and retinol may be used as supportive parameters for diagnostic purpose and may add further for prognostic information. These biochemical parameters are inexpensive, rapid and can easily be analyzed in smaller laboratories which are not exposed to sophisticated technology.

However studies on lipid per-oxidation are required for correlating it with antioxidant status and release of membrane associated enzymes ADA and 5′-NT into the circulation.

Further studies on a larger sample size are needed to substantiate our findings before firm conclusions can be drawn on the utility of these enzymes and retinol for the diagnosis and assessment of progression of oral and laryngeal cancers.

# SUMMARY

Oral and laryngeal cancers remain a serious health problem worldwide with a poor prognosis. Improvement in the early detection is required, because in the initial stages, treatment is more effective resulting in better survival rate and also the morbidity is reduced. In view of this the present study has been undertaken to assess the clinical utility of some promising enzyme markers namely adenosine deaminase, 5′-nucleotidase and retinol which are accurate, easily identifiable and may be of some diagnostic and prognostic significance.

- A total of 50 cases of oral and laryngeal cancers in different stages were included.
- The age of the patients ranged from 45-75 years with majority being males.
- Most of the patients had habits of tobacco, betel quid chewing along with smoking.
- Most common site affected in oral cavity was buccal mucosa and in larynx supraglottic region.

In the present study there was a significant increase in DNA turn over enzymes, serum adenosine deaminase and 5′-nucleotidase in oral and laryngeal cancer patients in comparison to controls. This could be as a result of local hypoxia which inhibits the cell mediated anti tumor response, promotes tumor cell migration and angiogenesis and stimulates the proliferation of tumor cells. As a consequence the activity of serum ADA and 5′-NT is also increased to detoxify the high amounts of toxic adenosine and deoxyadenosine substrates produced from accelerated purine metabolism within the cancerous tissues. Serum retinol level was significantly decreased in oral and laryngeal cancer patients in comparison to controls. Enzymatic and non-enzymatic antioxidants scavenge lipid per-oxidation by products formed under physiological and pathological conditions. The decrease in retinol levels could therefore be due to utilization of it by the affected cancerous tissues or in combating the excessive oxidative stress in circulation.

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# ANNEXURE f - CONSENT FORM

Mr./Mrs/Ms. ……

you are invited to participate in our research study that is a study to know the purine salvage enzyme markers and serum retinol in oral and laryngeal cancers.

Participation in this study is completely voluntary. About 50 patients and equal number of healthy volunteers will be enrolled in this study at J. N. Medical College, Belgaum under the supervision of Head of Department of Biochemistry, and Head of Department of Otorhinolaryngology and Head and Neck Surgery, J. N. Medical College, Belgaum. The study will be carried out by Dr…… ( REG.No. BC0108001), P.G. in Department of Biochemistry, K.L.E. University, Belgaum for M.D. dissertation to be submitted to KLE University, Belgaum.

**PURPOSE OF THE STUDY**

The incidence and mortality cases due to oral and laryngeal cancers are increasing in India. Hence this work is undertaken for early screening and diagnosis of cases by evaluating the enzyme markers and anti oxidants in oral and laryngeal cancers.

**PROCEDURE**

For both oral and laryngeal cancer patients (Cases) and healthy subjects (Controls), 5 ml of venous blood will be collected under aseptic precautionary measures using sterile disposable syringe.

**RISKS**

Since the blood is drawn under aseptic precautionary measures by trained persons there is no scope for any risks. Further only small volume of blood is

collected which will be spontaneously replenished in the body. However there may be minor risks associated with having blood drawn that may include bruising, redness, discomfort or bleeding at the puncture site.

**BENEFITS**

No direct benefit is guaranteed to you from participating in our study. You can make use of blood levels of studied parameters if desired.

**OPTIONS**

If you decide not to participate in this study, the hospital will provide you the usual standard care and treatment.

**NEW INFORMATION**

Does not apply to this research.

**PRIVACY AND CONFIDENTIALITY**

All information collected about you during the course of the study will be kept confidential to the extent permitted by law. You will be identified in this research record by the code numbers. Information which identifies you personally will not be revealed without your written permission. However your records may be revealed to the sponsor of the study. Information from this study may be published but your identity will be confidential in any publication.

**INSTITUTIONAL POLICY**

In the event that you are physically injured as a result of participating in this research emergency care will be available. There is no commitment to provide any compensation for research related injury. The J. N. Medical College will provide, within the limitations of the laws of the state of Karnataka, facilities and medical

attention to subjects who suffered any harm as the result of your participation in this study. In the event you believe that you have suffered any how as a result of your participation in this study you may contact research guide Dr……., Department of Biochemistry or Dr……., Department of Otorhinolaryngology and Head and Neck Surgery.

**COST FOR PARTICIPATION**

You will not be charged for the test to be carried out on your blood sample.

**FINANCIAL INCENTIVE FOR PARTICIPATION**

You will not receive any remuneration for participating in this study.

**VOLUNTARY PARTICIPATION/WITHDRAWAL**

If you decide not to participate in this study, it will not affect the quality of the medical care you receive at this institution.

You may withdraw from the study anytime. The researchers might use the information learned from the study in scientific journal articles or in presentations.

In case you have any questions regarding your rights as a study participant, you may please contact Dr……., J. N. M. C., KLE University, Belgaum and Chairman of J. N. M. C. Institutional Ethics Committee of Human Subjects Research.

**EMERGENCY PROVISION**

If you have questions as a participant in our study, you can contact the study investigator Dr……., (REG. No. BC0108001 or the research guide Dr…….

# CONSENT TO PARTICIPATE IN A RESEARCH TRIAL

I voluntarily agree to take part in this study. If I choose to take part in the study, I may withdraw at anytime. I am not giving any of my legal right by signing this form. My signature below indicates that I have read, or had read to me, this entire consent form including the risks and benefits. I may ask questions at any time.

| | |
|---|---|
| _____ | _____ |
| Signature of participant | Date |
| _____ | _____ |
| Participants Name (Printed): | Date |
| _____ | _____ |
| Name and Signature of witness-1 | Date |
| _____ | _____ |
| Name and Signature of witness-2 | Date |
| _____ | _____ |
| Signature of researchers or Person obtaining consent | Date |

**ANNEXURE ff - PROFORMA**

**"THE DIAGNOSTIC USES OF SERUM ADENOSINE DEAMINASE, 5à-NUCLEOTIDASE AND RETINOL IN ORAL AND LARYNGEAL CANCERS – A CROSS SECTIONAL STUDY"**

**I. Patient Identification**

Name : OPD No. :

Age : Sex :

Occupation: Religion :

**II. Presenting Complaints:**

1. **Mouth ulcer / growth**

| | | | |
|---|---|---|---|
| Onset | : | H/o. trauma | : |
| Duration | : | H/o. bleeding | : |
| Site | : | H/o. difficulty in speech | : |
| Pain | : | H/o excessive salivation | : |
| Frequency | : | H/o alteration of voice | : |

2. **Larynx**

H/o hoarseness of voice H/o vomiting blood

H/o pain in throat H/o neck swelling.

H/o difficulty in breathing

H/o difficulty in swallowing

**Past History**

H/o similar complaints in past

H/o trauma, allergy, jaundice

H/o past surgery

H/o chemotherapy

H/o radiotherapy

**Personal History**

H/o smoking, tobacco chewing, betel nut / quid chewing, alcohol intake, spicy food consumption etc.

**Family History**

H/o of similar complaints in family

H/o of smoking.

**III. General Physical Examination**

| | | | |
|---|---|---|---|
| Weight (Kg) | : | Pulse | : |
| Height (m) | : | Blood pressure | : |
| Pallor | : | Respiratory rate | : |
| Icterus | : | Temperature | : |
| Clubbing | : | Lymphadenopathy | : |
| Cyanosis | : | Oedema | : |

**IV. Local examination**

***Inspection and palpation of oral cavity***

Ulcer/growth: Size, shape, site, mobility, presence of blood, discharge.

***Inspection of larynx:*** By indirect and direct laryngoscopy

Ulcer/growth: Size, shape, site, mobility, presence of blood, discharge.

**Cervical lymph node examination**

**Systemic examination**

CVS :

Respiratory :

Abdominal :

**Investigations**

Hb% : Biopsy report :

ESR : Blood sugar :

Blood group :

HIV :

# ANNEXURE fff - MASTER CHART

## CONTROLS

| Sl. No. | NAME | AGE (YEARS) | SEX | ADA (U/L) | 5'-NT (U/L) | RETINOL (µg/dl) |
|---|---|---|---|---|---|---|
| 1 | B.K. | 45 | Male | 26.00 | 2.0 | 35.70 |
| 2 | G.M. | 52 | Male | 19.00 | 12.0 | 47.40 |
| 3 | S.S. | 48 | Male | 23.66 | 5.0 | 30.20 |
| 4 | N.K. | 46 | Male | 28.00 | 6.0 | 31.00 |
| 5 | D.R. | 63 | Male | 17.56 | 10.0 | 33.30 |
| 6 | H.S.K | 71 | Male | 13.33 | 7.0 | 46.40 |
| 7 | G.H. | 48 | Male | 21.08 | 12.0 | 62.80 |
| 8 | D.S. | 59 | Male | 18.33 | 6.0 | 54.60 |
| 9 | P.K. | 45 | Male | 23.56 | 9.0 | 42.00 |
| 10 | T.K. | 53 | Male | 14.60 | 7.0 | 48.50 |
| 11 | T.L. | 51 | Male | 25.67 | 14.0 | 52.30 |
| 12 | R.K. | 72 | Male | 22.38 | 9.0 | 35.20 |
| 13 | S.M. | 66 | Male | 20.00 | 6.0 | 66.00 |
| 14 | K.K. | 65 | Male | 19.60 | 3.0 | 32.20 |
| 15 | P.P.K | 63 | Male | 23.33 | 8.0 | 49.60 |
| 16 | B.L. | 71 | Male | 17.66 | 11.0 | 29.30 |
| 17 | C.S. | 55 | Male | 24.56 | 12.0 | 37.00 |
| 18 | A.N. | 54 | Male | 20.76 | 15.0 | 46.60 |
| 19 | V.G. | 43 | Male | 12.80 | 5.0 | 38.70 |
| 20 | S.K. | 55 | Male | 16.66 | 10.0 | 52.30 |
| 21 | M.K. | 75 | Male | 17.56 | 14.0 | 45.30 |
| 22 | R.P. | 62 | Male | 21.38 | 18.0 | 37.00 |
| 23 | K.K. | 61 | Male | 24.44 | 12.0 | 28.00 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 24 | S.W. | 65 | Male | 23.33 | 15.0 | 45.80 |
| 25 | A.P. | 53 | Male | 18.77 | 13.0 | 48.50 |
| 26 | K.R. | 48 | Male | 22.15 | 7.0 | 36.20 |
| 27 | R.H. | 51 | Male | 10.33 | 3.0 | 47.20 |
| 28 | G.K. | 46 | Male | 15.33 | 7.0 | 43.00 |
| 29 | H.K. | 56 | Male | 21.08 | 2.0 | 36.30 |
| 30 | K.R. | 63 | Male | 25.00 | 14.0 | 27.20 |
| 31 | P.G. | 58 | Male | 23.00 | 5.0 | 35.50 |
| 32 | T.K. | 47 | Male | 20.00 | 6.0 | 49.30 |
| 33 | R.S. | 46 | Male | 19.00 | 7.0 | 52.60 |
| 34 | M.K. | 39 | Male | 20.00 | 4.0 | 45.30 |
| 35 | S.H. | 40 | Male | 16.66 | 3.0 | 26.70 |
| 36 | B.K. | 51 | Female | 10.00 | 15.0 | 45.30 |
| 37 | J.K. | 48 | Female | 25.00 | 12.0 | 33.70 |
| 38 | D.M. | 62 | Female | 16.60 | 13.0 | 39.80 |
| 39 | S.D. | 53 | Female | 23.33 | 7.0 | 25.80 |
| 40 | H.K. | 51 | Female | 19.00 | 3.0 | 56.70 |
| 41 | V.B. | 50 | Female | 25.00 | 2.0 | 37.30 |
| 42 | S.K. | 57 | Female | 23.30 | 3.0 | 41.00 |
| 43 | R.K. | 46 | Female | 16.60 | 8.0 | 27.30 |
| 44 | A.M. | 54 | Female | 14.00 | 9.0 | 45.30 |
| 45 | K.G. | 38 | Female | 18.33 | 11.0 | 37.00 |
| 46 | B.H. | 45 | Female | 22.00 | 13.0 | 42.30 |
| 47 | P.J. | 52 | Female | 15.33 | 13.0 | 36.40 |
| 48 | N.S. | 50 | Female | 19.60 | 11.0 | 41.20 |
| 49 | G.K. | 58 | Female | 25.00 | 10.0 | 43.30 |
| 50 | R.S. | 56 | Female | 13.33 | 9.0 | 29.70 |

# ORAL CANCER CASES

| S.I No. | NAME | AGE | SEX | SITE | TNM STAGE | ADA (U/L) | 5'-NT (U/L) | RETINOL (µg/dl) |
|---|---|---|---|---|---|---|---|---|
| 1 | K.B. | 65 | MALE | TONGUE | I | 28.66 | 35.00 | 35.40 |
| 2 | H.Y.B | 58 | MALE | TONGUE | II | 30.33 | 37.00 | 22.30 |
| 3 | M.B.N | 70 | MALE | HARD PALATE | II | 38.53 | 42.00 | 25.20 |
| 4 | J.L. | 58 | MALE | LIP | II | 32.00 | 38.00 | 31.30 |
| 5 | J.M.B | 55 | MALE | LIP | II | 35.65 | 41.00 | 23.40 |
| 6 | P.K. | 56 | MALE | FLOOR OF MOUTH | II | 35.00 | 43.00 | 30.00 |
| 7 | N.K. | 54 | MALE | FLOOR OF MOUTH | II | 38.33 | 45.00 | 25.70 |
| 8 | S.P. | 66 | FEMALE | TONGUE | II | 33.66 | 39.00 | 20.20 |
| 9 | D.G. | 45 | MALE | TONGUE | II | 31.08 | 40.00 | 21.30 |
| 10 | B.J. | 46 | MALE | BUCCAL MUCOSA | II | 40.33 | 45.00 | 30.40 |
| 11 | T.A. | 52 | MALE | BUCCAL MUCOSA | II | 36.40 | 48.00 | 27.30 |
| 12 | R.K. | 47 | MALE | HARD PALATE | III | 32.30 | 41.00 | 26.30 |
| 13 | B.G.S | 55 | MALE | BUCCAL MUCOSA | III | 38.20 | 46.00 | 23.40 |
| 14 | G.M.M | 41 | MALE | BUCCAL MUCOSA | III | 45.35 | 56.00 | 21.30 |
| 15 | A.B.B | 64 | MALE | BUCCAL MUCOSA | III | 42.50 | 58.00 | 18.20 |
| 16 | B.G. | 75 | MALE | TONGUE | III | 45.00 | 52.00 | 25.30 |
| 17 | G.N.P | 57 | MALE | TONGUE | III | 39.57 | 48.00 | 15.30 |
| 18 | A.K. | 55 | FEMALE | ALVEOLUS | III | 37.52 | 41.00 | 17.40 |
| 19 | Z.A. | 66 | MALE | TONGUE | III | 42.66 | 56.00 | 20.30 |
| 20 | H.L. | 56 | MALE | HARD PALATE | III | 51.63 | 61.00 | 21.20 |
| 21 | S.S. | 50 | MALE | LIP | IV | 58.66 | 65.00 | 12.30 |
| 22 | D.C.T | 46 | MALE | HARD PALATE | IV | 52.15 | 67.00 | 18.20 |
| 23 | B.M. | 60 | FEMALE | BUCCAL MUCOSA | IV | 48.76 | 59.00 | 14.20 |
| 24 | M.M. | 56 | MALE | BUCCAL MUCOSA | IV | 52.33 | 62.00 | 15.40 |
| 25 | P.M. | 48 | MALE | BUCCAL MUCOSA | IV | 60.39 | 70.00 | 17.00 |

# LARYNGEAL CANCER CASES

| S.I No. | NAME | AGE | SEX | SITE | TNM STAGE | ADA (U/L) | 5'-NT (U/L) | RETINOL (µg/dl) |
|---|---|---|---|---|---|---|---|---|
| 1 | P.K. | 63 | MALE | GLOTTIC | I | 32.84 | 43.00 | 38.20 |
| 2 | S.M. | 79 | MALE | GLOTTIC | I | 28.73 | 39.00 | 25.60 |
| 3 | N.S. | 60 | MALE | GLOTTIC | I | 26.66 | 35.00 | 36.30 |
| 4 | D.K. | 47 | MALE | GLOTTIC | II | 33.18 | 41.00 | 26.30 |
| 5 | S.K. | 60 | MALE | SUPRAGLOTTIC | II | 39.33 | 46.00 | 19.80 |
| 6 | M.R. | 60 | MALE | GLOTTIC | II | 40.82 | 52.00 | 23.60 |
| 7 | B.S. | 57 | MALE | SUPRAGLOTTIC | III | 45.24 | 62.00 | 16.80 |
| 8 | S.R.T | 53 | MALE | SUPRAGLOTTIC | III | 43.67 | 55.00 | 14.30 |
| 9 | C.S. | 52 | MALE | SUPRAGLOTTIC | III | 49.50 | 57.00 | 20.60 |
| 10 | A.A. | 66 | MALE | SUPRAGLOTTIC | III | 44.66 | 60.00 | 23.80 |
| 11 | D.K. | 63 | MALE | SUPRAGLOTTIC | III | 53.33 | 72.00 | 14.30 |
| 12 | S.R.S | 60 | FEMALE | SUPRAGLOTTIC | III | 46.76 | 63.00 | 24.00 |
| 13 | M.R | 68 | MALE | SUPRAGLOTTIC | III | 56.77 | 71.00 | 13.80 |
| 14 | S.F. | 56 | MALE | GLOTTIC | III | 43.41 | 65.00 | 18.70 |
| 15 | T.M. | 54 | MALE | GLOTTIC | III | 54.33 | 62.00 | 19.80 |
| 16 | L.M. | 45 | FEMALE | SUPRAGLOTTIC | III | 48.80 | 59.00 | 22.60 |
| 17 | B.C.S | 62 | MALE | GLOTTIC | III | 52.66 | 66.00 | 17.30 |
| 18 | R.K. | 61 | MALE | GLOTTIC | IV | 51.33 | 72.00 | 12.60 |
| 19 | S.H. | 53 | FEMALE | SUPRAGLOTTIC | IV | 54.41 | 80.00 | 18.30 |
| 20 | D.S. | 45 | FEMALE | SUPRAGLOTTIC | IV | 63.66 | 78.00 | 19.90 |
| 21 | T.A. | 54 | MALE | GLOTTIC | IV | 60.67 | 74.00 | 12.50 |
| 22 | L.S.P | 62 | FEMALE | GLOTTIC | IV | 58.73 | 72.00 | 16.50 |
| 23 | S.V. | 52 | FEMALE | SUPRAGLOTTIC | IV | 52.43 | 78.00 | 18.39 |
| 24 | S.D. | 60 | MALE | SUPRAGLOTTIC | IV | 67.65 | 83.00 | 13.00 |
| 25 | S.S. | 38 | FEMALE | SUPRAGLOTTIC | IV | 61.66 | 81.00 | 17.40 |

# KEY TO MASTER CHART

| | | |
|---|---|---|
| ADA | - | Adenosine deaminase |
| 5′-NT | - | 5′- Nucleotidase |
| SI. No | - | Serial Number |
| U/L | - | Units per liter |
| µg/dl | - | microgram per deciliter |
| T | - | Primary tumor |
| N | - | Lymph nodes |
| M | - | Distant metastasis |