INTRODUCTION

Cancer is a large family of diseases, arises from a series of genetic alterations that promote resistance to apoptosis, self-sufficiency in growth, cellular immortalisation and escape from cell cycle exist. The acquisition of these properties ultimately facilitates angiogenesis, invasion and metastasis. The process of tumorigenesis that is transformation of normal cells to malignant neoplasm involves genetic mutations and epigenetic abnormalities that cooperate with genetic lesions to generate the cancer phenotype. The global scenario of cancer incidence, mortality and prevalence is projected by the International Agency for Research on Cancer (IARC), a specialized cancer agency of the World Health Organization (WHO). According to IARC, an estimated 14.1 million new cancer cases and 8.2 million cancer-related deaths occurred in 2012, compared with 12.7 million and 7.6 million, respectively, in 2008. Prevalence estimates for 2012 show that there were 32.6 million people (over the age of 15 years) alive who had a cancer diagnosed in the previous five years (Ferlay et al, 2012).

Epidemiology of Oral Cancer

The Head and Neck Cancers remain a significant cause of morbidity worldwide, with approximately 650,000 new cases diagnosed each year. Head and Neck Cancer is the most common malignancy developed in the oral cavity, nasal cavity, paranasal sinuses, pharynx, larynx and salivary gland. Further, Lip and oral cavity cancer is the third most common human cancer, representing 7.6% of all types of cancer (Ferlay et al, 2012). The incidence of Oral Cancer has significant local variation; India and other Asian countries have higher rate of Oral Cancer than western countries. The latest findings of National Cancer Registry Programme (NCRP) of the Indian Council of Medical Research (ICMR) show that Oral Cancer is the second leading cancer site among
males across all population based registries. An age-adjusted rate of Oral Cancer in India is 20 per 100,000 population and accounts for over 30% of all cancers in the country. This particularly high prevalence in India is attributed to the habit of tobacco and chewing betel quid (Figure 1).

Figure 1: Incidence and mortality rate of Lip and Oral cavity cancer in India (Modified from Ferlay et al, 2012)

At Gujarat Cancer and Research Institute, Head and Neck Cancer constituted 30.03% of total cancer. Further, among males, Oral Cancer is the most predominant site of cancer that constitutes 17.53% of total cancer. Moreover, tobacco related cancer accounted for 55.95% of all cancer in males and 16.42% in females.
Risk factors
Cancer risk factors can be divided into four major groups; behavioral risk factors, environmental risk factors, hereditary risk factors and biological risk factors. The incidence of Oral Cancer is related to accumulation of genetic changes, duration of exposure to chemical and physical irritants, viruses, hormonal effects and cellular aging. The major risk factors for Oral Squamous Cell Carcinoma (OSCC) are as follows.

Tobacco
The majority of Oral Cancers are associated with tobacco consumption in various forms such as betel quid and areca nut chewing and smoking. Tobacco contains more than 60 known carcinogens including nitrosamines (nicotine), polycyclic aromatic hydrocarbons, nitrosodictanolamine, nitrosoproline and polonium. Nicotine is a powerful and addicting drug. Tobacco smoke contains carbon monoxide, thiocyanate, hydrogen cyanide, nicotine, and metabolites of these constituents; consumption of tobacco is a primary mode of involvement of the carcinogens, use of tobacco cause irritation of mucous membrane of oral cavity. Both tobacco smoking and quid chewing cause oxidative stress to tissues and generate reactive oxygen species, which can damage cellular protein, lipids, carbohydrates and forms DNA adducts. Cellular repair mechanisms such as direct base repair, excision of damaged DNA by excision repair, mismatch repair and double strand repair can remove DNA adducts and return the structure of DNA to normal. If repair system fails, then adducts will persist and cause miscoding during replication which ultimately cause increase probability of developing cancer. Moreover, oxidative stress results in inflammation with increased neutrophils, T cells and pro
inflammatory cytokines (Figure 2). This create immune suppressive environment for tumor progression (IARC, 2004).

**Figure 2**: Smoking induced oxidative stress and inflammation (Modified from Maes et al 2010)

*Alcohol*

Use of alcohol and other toxic liquids is another high-risk factor associated with Oral Cancer. There is strong synergistic effect on Oral Cancer risk when a person is both a heavy smoker and drinker. Alcohol damages the phospholipids of cell membranes and increases permeability. It has also shown to enhance penetration of tobacco specific carcinogens across the oral mucosa. It also impairs the DNA repair mechanism (Odgen et al, 1998).
Genetic Predisposition

There is considerable evidence for an inherited, genetic predisposition in Oral Cancer, related to polymorphisms in carcinogen- metabolizing enzyme like glutathione-S transferase, UDP-glucuronosyl transferase and sulfotransferase. Some rare cancer syndrome like Cowden syndrome caused by mutation in tumor suppressor gene PTEN; involved in Oral Cancer (Handley et al, 2006).

Microorganisms

It is shown that high risk HPV genotypes, particularly HPV 16 and 18, are important causative factor for Oral Cancer. The role of bacteria in the etiology of Oral Cancer is also receiving more attention. Endogenous production of acetaldehyde and reduction of nitrate to nitrites by oral flora is higher in drinkers with poor oral hygiene. (Meurman et al, 2008). Understanding of the role of oral flora is certainly important in the management of the distressing mucositis associated with cancer therapy (Homann et al, 2001).

Air pollution

Part of urban/rural difference in the incidence of Oral Cancer has been also related to atmospheric pollution. One study carried out in Brazil has reported exposure to wood smoke as a risk factor for Oral Cancer (Franco et al, 1989).

Solar Radiation

Prolonged exposure to sunlight represents an important risk for the development of squamous cell carcinoma of lip; usually the lower lip is involved because it receives considerably more direct sunlight than the upper lip (Baker et al, 1980). The evidences have came from many countries including India, in which long hours of sunshine and

**Hematopoietic stem cell transplantation**

Patients after hematopoietic stem cell transplantation (HSCT) are at a higher risk for oral squamous cell carcinoma. Post-HSCT Oral Cancer may have more aggressive behavior with poorer prognosis, when compared to Oral Cancer in non-HSCT patients. This effect is supposed to be owing to the continuous life-long immune suppression and chronic oral graft-versus-host disease (Elad et al, 2010).

**Anatomy of Oral Cavity**

The oral cavity includes the lips, the inside lining of the lips and cheeks (buccal mucosa), the teeth, the gums, the front two-thirds of the tongue, the floor of the mouth below the tongue, and the bony roof of the mouth (hard palate). The area behind the wisdom teeth (called the retromolar trigone) can be included as a part of the oral cavity, although it is often considered part of the oropharynx (Figure 3). The different parts of the oral cavity and oropharynx are made up of several types of cells. Different cancers can develop from each type of cell. The differences are important, because they can influence a person’s treatment options and prognosis (Koch et al, 2009).
Figure 3: Diagram of Oral cavity (Adapted from Koch et al, 2009)

Many types of tumors (abnormal growth of cells) can develop in the oral cavity. They fit into 3 general categories:

1) Benign or non-cancerous
2) Pre-cancerous conditions.
3) Cancerous.

Benign tumors

Many types of benign tumors and tumor-like conditions can occur in the oral cavity such as Eosinophilic granuloma, Fibroma, Granular cell tumor, Keratoacanthoma, Leiomyoma, Osteochondroma, Lipoma, Schwannoma, Neurofibroma, Papilloma, Condyloma acuminatum, Verruciform xanthoma, Pyogenic granuloma, Rhabdomyoma, Odontogenic tumors (tumors that start in tooth-forming tissues). These non-cancerous tumors arise from different types of cells and have a variety of causes. Some of them may cause problems, but they are not likely to be life-threatening. The usual treatment is to surgically remove them since they are unlikely to recur (Greenberg et al, 2003).
Pre cancerous conditions

*Oral Potentially Malignant Disorders*

The term Oral Potentially Malignant Disorders (OPMD) has been recommended by an International working group convened by WHO collaborating Centre for Oral Cancer and Pre Cancer in London in 2005. Leukoplakia, Erythroplakia, Oral submucous fibrosis, Oral Lichen Planus, palatal lesions in reverse smokers, Actinic keratosis are described under the broad definition of OPMD. They conveyed that not all these disorders will transform to invasive cancer (Warnakulasuriya et al, 2007). Further, a meta analysis of 23 primary studies on Oral Leukoplakia, from international data published between 1986 to 2002, revealed that Leukoplakia was significantly more prevalent among males (prevalence ratio 3.22), but no difference between geographical area and between younger and older adults. The mean global prevalence is 2.6% for Leukoplakia (Petti et al, 2003). The prevalence of Leukoplakia in India varies from 0.2% to 5.2% (Mehta et al, 1975).

**Etiology of OPMD**

In western countries majority of premalignant disorders are associated with HPV and Candida infection, while in India majority of the premalignant lesions are associated with tobacco and guthka chewing. A deficiency of micronutrients such as vitamin A, B complex, C, D, E and minerals have been associated with submucous fibrosis.

**Leukoplakia**

The term Leukoplakia was first used by Schwimmer in 1877 to describe a white lesion of the tongue, which probably represented a syphilitic glossitis. As defined by the World
Health Organization, Leukoplakia is a white patch or plaque that cannot be characterized clinically or pathologically as any other disease. As such, Leukoplakia should be used only as a clinical term; it has no specific histopathological connotation and should never be used as a microscopic diagnosis. In the evaluation of the patient, Leukoplakia is a clinical diagnosis of exclusion. There are two main clinical variants of oral Leukoplakia, namely homogenous Leukoplakia and non-homogenous (heterogenous) Leukoplakia. Homogenous Leukoplakia is usually well defined white patch of uniform, flat appearance and texture, although there may be superficial irregularities. Homogenous Leukoplakia is usually slightly elevated compared to surrounding mucosa, and often has a fissured, wrinkled or corrugated surface texture, when homogenous Leukoplakia is palpated, it may feel leathery, dry, or like cracked mud. Non-homogenous Leukoplakia is a lesion of non-uniform appearance. The color may be predominantly white or a mixed white and red. The surface texture is irregular as compared to homogenous Leukoplakia, may be flat, nodular or exophytic. Verrucous Leukoplakia is a descriptive term used for thick, white, papillary lesions. Verrucous Leukoplakia is usually heavily keratinized and often seen in elderly people. Some verrucous Leukoplakia may have an exophytic growth pattern and some may slowly invade surrounding mucosa, when the term proliferative verrucous Leukoplakia may be used (Neville et al, 2002).

At the time of histological identification of Leukoplakia, biopsy reveals Dysplasia in 12 to 25% of patients. Dysplastic lesions have been categorized as mild, moderate, or severe, based on histological criteria. Mild Dysplasia has dysplastic cells that are limited to the basal layer of the epithelium; moderate Dysplasia and severe Dysplasia involve
increasing changes in cellular morphology and increasing thickness of the epithelium. Carcinoma in situ is a lesion in which abnormal cells involve the entire epithelium without invasion through the basement membrane, and carcinoma is diagnosed when there is disruption of the basement membrane and invasion into connective tissue. Dysplasia is present more frequently in Leukoplakia that involves the tongue, lips, and floor of the mouth and less frequently in Leukoplakia that involves the palate and retromolar regions. The presence of Dysplasia within a Leukoplakia lesion implies an increased risk of malignant transformation. Histomorphologic classification has been proposed by two study groups (Pindborg et al, 1996; Schepman et al, 1996).

The classifications include histologic findings and the clinical description, site, and size of the lesion as shown in Table 1 (Greenberg et al, 2003).

**Table 1**: Classification and Staging of Oral Leukoplakia

<table>
<thead>
<tr>
<th>Classification and Staging of Oral Leukoplakia</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>L</strong></td>
</tr>
<tr>
<td><strong>L₀</strong></td>
</tr>
<tr>
<td><strong>L₁</strong></td>
</tr>
<tr>
<td><strong>L₂</strong></td>
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<tr>
<td><strong>L₃</strong></td>
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<tr>
<td><strong>Lₓ</strong></td>
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<tr>
<td><strong>S</strong></td>
</tr>
<tr>
<td><strong>S₁</strong></td>
</tr>
<tr>
<td><strong>S₂</strong></td>
</tr>
<tr>
<td><strong>Sₓ</strong></td>
</tr>
<tr>
<td><strong>C</strong></td>
</tr>
<tr>
<td><strong>C₁</strong></td>
</tr>
<tr>
<td><strong>C₂</strong></td>
</tr>
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</table>
**Histopathological features**

<table>
<thead>
<tr>
<th>P</th>
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</tr>
</thead>
<tbody>
<tr>
<td>P₁</td>
<td>No Dysplasia</td>
</tr>
<tr>
<td>P₂</td>
<td>Mild Dysplasia</td>
</tr>
<tr>
<td>P₃</td>
<td>Moderate Dysplasia</td>
</tr>
<tr>
<td>P₄</td>
<td>Not specified</td>
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</tbody>
</table>

**Staging**

<table>
<thead>
<tr>
<th>Stage</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>any L, S₁, C₁, P₁ or P₂</td>
</tr>
<tr>
<td>2</td>
<td>any L, S₁ or S₂, C₂, P₁ or P₂</td>
</tr>
<tr>
<td>3</td>
<td>any L, S₂, C₂, P₁ or P₂</td>
</tr>
<tr>
<td>4</td>
<td>any L, any S, any C, P₃ or P₄</td>
</tr>
</tbody>
</table>

**Diagnosis**

Early detection of dysplastic lesions is a continuing goal. Thorough head and neck and intraoral examination is a prerequisite. Aids to oral examination include vital tissue staining using toluidine blue and computer-assisted cytology of oral brush biopsy specimens. Toluidine blue can be applied directly to suspicious lesions or used as an oral rinse. A study of the application of toluidine blue to a consecutive series of patients who had prior Head and Neck Cancer and in whom all lesions were examined by biopsy revealed 100% of the carcinomas in situ and SCCs (no false negatives; sensitivity was 100%) whereas clinical findings would not have led to biopsy being performed on 28% of these malignant lesions. Toluidine blue has documented clinical use in choosing biopsy sites, may guide surgical treatment of malignant lesions. Positive retention of toluidine blue (particularly in areas of Leukoplakia, erythroplakia, and uptake in a peripheral pattern of an ulcer) may indicate the need for biopsy. False-positive dye
retention may occur in inflammatory and ulcerative lesions, but false-negative retention is uncommon (Epstein et al, 1997).

**Erythroplakia**

Similar to Leukoplakia, Erythroplakia is a clinical term that refers to a red patch that cannot be defined clinically or pathologically as any other condition. This definition excludes inflammatory conditions that may result in a red clinical appearance. Oral erythroplakia occurs most frequently in older men and appears as a red maculae or plaque with a soft, velvety texture. The floor of mouth, lateral tongue, retromolar pad, and soft palate are the most common sites of involvement. Often the lesion is well demarcated, but some examples may gradually blend into the surrounding mucosa. Some lesions may be intermixed with white areas (erythroleukoplakia). Erythroplakia is often asymptomatic, although some patients may complain of a sore, burning sensation. Similar to Leukoplakia, it is also most likely to show Dysplasia or carcinoma on biopsy (Kramer et al, 1978).

**Sub mucous fibrosis**

Submucous fibrosis is a disease of the oral mucosa, characterized by epithelial atrophy and fibrosis of lamina propria and the submucosa with increasing loss of tissue mobility. Submucous fibrosis is most common among East Indians. While the etiology is unknown, consumption of spices and irritants has been suspected to be the cause. Squamous cell carcinoma has been described in up to one-third of patients with submucous fibrosis (Greenberg et al, 2003).
Oral Lichen planus (OLP)

Oral Lichen Planus is a chronic inflammatory disease of unknown etiology. Oral Lichen planus presents as white striations, white papules, white plaques, erythema, erosions or blisters affecting predominantly the buccal mucosa, tongue and gingivae, although other sites are occasionally involved. It affects 1-2% of general adult population and also affects women more than men. The two main types of OLP are Reticular and Erosive OLPs (Edwards et al, 2002).

Reticular OLP is most common type of OLP. It presents as interlacing white keratotic lines (known as Wickham's striae) with an erythematous border. The striae are typically located bilaterally on the buccal mucosa, mucobuccal fold, gingiva and less commonly, the tongue, palate and lips (Edwards et al, 2002).

Erosive OLP is the second most common type. It presents as a mix of erythematous and ulcerated areas surrounded by finely radiating keratotic striae. Two additional presentations are the atrophic and bullous forms, which are considered variants of the erosive type (Edwards et al, 2002).

The histopathological features of OLP include liquefaction of the basal cell layer accompanied by apoptosis of the keratinocytes, a dense band-like lymphocytic infiltrate at the interface between the epithelium and the connective tissue, focal areas of hyperkeratinized epithelium (Figure 4). Eosinophilic colloid bodies (Civatte bodies), which represent degenerating keratinocytes, are often visible in the lower half of the surface epithelium (Edwards et al, 2002).
Figure 4: Histopathological features of Oral Lichen planus (Adapted from Edwards et al, 2002)

Chronic lichen planus has been shown to present a low but measurable risk of cancer and Oral Cancer has been identified as arising from areas of erythematous atrophic lichen planus. Malignant transformation of lichen planus has been reported in 0.4 to 2.5% of cases in long-term studies, which represents a 50-fold or greater increase in risk (Greenberg et al, 2003).

Palatal lesions in reverse smokers

In some Asian and South American countries, persons with habit of reverse smoking, in which the lit end of the cigarette is placed in the mouth has been associated with a palatal lesion. This habit causes severe heat related alteration of the palatal mucosa. The individuals with palatal lesion have higher risk for malignant transformation (Pindborg et al, 1971; Ortiz et al, 2002).
Actinic Keratosis

Actinic Keratosis is a thick scaly patch of skin, associated with constant sun exposure. Untreated lesion of Actinic Keratosis has 20% risk to progress into squamous cell carcinoma.

Management of pre cancerous lesions

Treatment of Leukoplakia includes laser excision followed by topical therapies. The potential advantage of laser therapy is improved healing of the treated area; however, a potential disadvantage is the inability to carefully assess histologic margins. Topical application of vitamin A acid may achieve remission in cases of mild Dysplasia; however, lesions often recur when therapy is discontinued. Systemic retinoids (analogues of vitamin A) have antiproliferative and differentiating effects on human squamous epithelial cells. In addition, retinoids may play a role in reducing the risk of recurrence or second primary lesions although recurrence of the lesion is common if the retinoid is discontinued. Bleomycin has been applied in a topical solution of dimethyl-sulfoxide and has shown reduction and elimination of oral lesions in short- and long-term follow-ups. Close follow-up is needed for persistent Leukoplakia as some of these lesions will progress to SCC. If changes in behaviour or appearance occur, a repeat biopsy is indicated (Greenberg et al, 2003).

Oral Carcinogenesis

Oral carcinogenesis is a highly complex multifocal process that takes place when squamous epithelium is affected by several genetic alterations.
It is evident that Oral Cancer, develops over many years, and during this period, there are multiple sites of neoplastic transformation occurs throughout the oral cavity such as Hyperplasia, Dysplasia, Carcinoma in situ and finally invasive cancer (Figure 5).

**Malignant transformation of OPMD**

Risk of malignant transformation varies from site to site within mouth, from population to population and from study to study. A classic study conducted in India with follow up for 7 years of more than 30,000 villagers, showed transformation rate from 10-24 per 100,000 per year (Mehta et al, 1975). The mean global transformation rate for Leukoplakia is 1.36% per year (Petti et al, 2003). Malignant transformation rates in Leukoplakia varied from 0.13% to 10% in various Indian populations (Mehta et al,
Non-homogenous Leukoplakia has a greater risk of malignant transformation than homogenous Leukoplakia (Soames et al, 1999; Greenberg et al, 2003).

**Oral Cancer**

**Signs and Symptoms**

Oral Cancer is initially asymptomatic, and unfortunately, patients are most often identified only after the development of symptoms and after progression of disease. Skin lesion or ulcer that does not resolve for fourteen days, dysphagia, odynophagia, otalgia, limited movement are common symptoms, oral bleeding occurs less frequently. Change in oral cavity tissue that may include a red, white, or mixed red-and-white lesion; a change in the surface texture of the lesion, producing a smooth, granular, rough, or crusted lesion; or the presence of a mass or ulceration. The lesion may be flat or elevated and ulcerated or nonulcerated, and it may be minimally palpable or indurated. Loss of function involving the tongue can affect speech, swallowing and diet. Lymphatic spread of oral carcinoma usually involves the submandibular and digastric nodes, the upper cervical nodes, and finally the remaining nodes along the cervical chain. Lymph nodes associated with cancer become enlarged and firm, too hard in texture (Greenberg et al, 2003).

**Diagnosis**

**Imaging**

Imaging including routine radiology, Computed Tomography (CT), Nuclear Scintiscanning, Magnetic Resonance Imaging (MRI) and Ultrasonography can provide evidence of bone involvement and can indicate the extent of some soft tissue lesions.
CT and MRI aid in determining the status of the cervical lymph nodes (Greenberg et al, 2003).

**Histopathology**

A non-invasive brush biopsy can be performed to rule out the presence of cancer on areas of the mouth that exhibit an unexplained color variation or lesion. However, tissue biopsy, of oral tissues and microscopic examination of the lesion confirm the diagnosis of Oral Cancer. Oral Cancers may be of varied histological types such as teratoma, adenocarcinoma derived from a major or minor salivary gland, lymphoma from tonsillar or other lymphoid tissue, or melanoma from the pigment-producing cells of the oral mucosa. Around 90% of Oral Cancers are squamous cell carcinoma originating in the tissues that line the mouth and lips (Figure 6). Moreover, invasion of lymphatic or blood vessels, and perineural spaces is of critical importance (Greenberg et al, 2003).

![Figure 6: Squamous Cell Carcinoma of Oral cavity](image_url)
**Staging of Lip and Oral cavity Cancer**

The American Joint Committee on Cancer (AJCC) has developed the tumor, node, metastasis (TNM) system of cancer classification. The AJCC classification is principally a clinical description of the disease but also includes imaging in the classification. The TNM staging of tumors of the oral cavity is shown in Table 2.

**Table 2**: TNM staging of Lip and Oral cavity Cancer

<table>
<thead>
<tr>
<th>Primary Tumor</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>T&lt;sub&gt;x&lt;/sub&gt;</td>
<td>Primary tumor cannot be assessed</td>
</tr>
<tr>
<td>T&lt;sub&gt;0&lt;/sub&gt;</td>
<td>No evidence of primary tumor</td>
</tr>
<tr>
<td>T&lt;sub&gt;is&lt;/sub&gt;</td>
<td>Carcinoma in situ</td>
</tr>
<tr>
<td>T&lt;sub&gt;1&lt;/sub&gt;</td>
<td>Tumor &lt; 2 cm or less in greatest dimension</td>
</tr>
<tr>
<td>T&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Tumor more than 2 cm but not more than 4 cm in greatest dimension</td>
</tr>
<tr>
<td>T&lt;sub&gt;3&lt;/sub&gt;</td>
<td>Tumor more than 4 cm in greatest dimension</td>
</tr>
<tr>
<td>T&lt;sub&gt;4&lt;/sub&gt;</td>
<td>Tumor invades adjacent structures (e.g., through cortical bone, into maxillary sinus, skin, pterygoid muscle, deep muscle of tongue)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Regional Lymph node</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>N&lt;sub&gt;x&lt;/sub&gt;</td>
<td>Regional lymph node cannot be assessed</td>
</tr>
<tr>
<td>N&lt;sub&gt;0&lt;/sub&gt;</td>
<td>No regional lymph node metastasis</td>
</tr>
<tr>
<td>N&lt;sub&gt;1&lt;/sub&gt;</td>
<td>Metastasis in a single ipsilateral lymph node, 3 cm or less in greatest dimension</td>
</tr>
<tr>
<td>N&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Metastasis in a single ipsilateral lymph node, more than 3 cm but not more than 6 cm in greatest dimension; or in multiple ipsilateral lymph nodes, none more than 6 cm in greatest dimension; or in bilateral or contralateral lymph nodes, none more than 6 cm in greatest dimension</td>
</tr>
<tr>
<td>N&lt;sub&gt;2a&lt;/sub&gt;</td>
<td>Metastasis in a single ipsilateral lymph node, more than 3 cm but not more than 6 cm in greatest dimension</td>
</tr>
<tr>
<td>N&lt;sub&gt;2b&lt;/sub&gt;</td>
<td>Metastasis in multiple ipsilateral lymph nodes, none more than 6 cm in greatest dimension</td>
</tr>
</tbody>
</table>
Table 2 continued

| N<sub>2c</sub> | Metastasis in bilateral or contralateral lymph nodes, none more than 6 cm in greatest dimension |
| N<sub>3</sub>   | Metastasis in a lymph node more than 6 cm in greatest dimension |
| Distant Metastasis |                                           |
| M<sub>x</sub>   | Distant metastasis cannot be assessed       |
| M<sub>0</sub>    | No distant metastasis                        |
| M<sub>1</sub>    | Distant metastasis                           |
| Stage grouping   |                                           |
| Stage<sub>0</sub> | Tis N0 M0                                   |
| Stage<sub>1</sub> | T1 N0 M0                                    |
| Stage<sub>II</sub>| T2 N0 M0                                    |
| Stage<sub>III</sub> | T3 N0 M0; T1 or T2 or T3 N1 M0            |
| Stage<sub>IV</sub> | Any T4 lesion, or Any N2 or N3 lesions, or Any M1 lesion |

Management of Oral Cancer

Surgical excision of the tumor is usually recommended if the tumor is small enough, and if surgery is likely to result in a functionally satisfactory result. Radiation therapy with or without chemotherapy is often used in conjunction with surgery or as the definitive radical treatment, especially if the tumor is inoperable. Surgeries for Oral Cancers include Maxillectomy, Mandibulectomy, Glossectomy and Radical neck dissection. Owing to the vital nature of the structures in the head and neck area, surgery for larger cancers is technically demanding. Reconstructive surgery may be required to give an acceptable cosmetic and functional result. Bone graft and surgical flaps such as the radical forearm flaps are used to help rebuild the structures removed during excision of
the cancer. An oral prosthesis may also be required. Most Oral Cancer patients depend on a feeding tube for their hydration and nutrition. Some will also get a port for the chemo to be delivered. Many Oral Cancer patients are disfigured and suffer from many long terms after effects. The after effects often include fatigue, speech problems, trouble maintaining weight, thyroid issues, swallowing difficulties, inability to swallow, memory loss, weakness, dizziness, high frequency hearing loss and sinus damage (Greenberg et al, 2003).

Chemotherapy is useful in Oral Cancers when used in combination with other treatment modalities such as radiation therapy. It is not used alone as a monotherapy. When cure is unlikely it can also be used to extend life and can be considered palliative but not curative care. Biological agents, such as Cetuximab have recently been shown to be effective in the treatment of squamous cell Head and Neck Cancers, and are likely to have an increasing role in the future management of this condition when used in conjunction with other treatments. Survival rates for Oral Cancer depend on the precise site, and the stage of the cancer at diagnosis. Overall survival is around 50% at five years when all stages of initial diagnosis are considered (Werning et al, 2007).

Following treatment, rehabilitation may be necessary to improve movement, chewing, swallowing and speech. Language and speech pathologists may be involved at this stage. Treatment of Oral Cancer will usually be by a multidisciplinary team, with treatment professionals from the realms of radiation, surgery, chemotherapy, nutrition, dental professionals, and even psychology all possibly involved with diagnosis, treatment, rehabilitation and patient care (Werning et al, 2007).
Immune system in Oral Cancer

The immune system plays a key role in the progression of Oral Cancer. A greater understanding of dysregulation of immune system and its contribution in the development of Oral Cancer should lead to improved therapies and outcome for patients.

Overview of the Immune System

Every organism in nature, from the simplest microbe to the most complex forms of plants and animals, seeks to protect itself against the threats presented by other organisms. The immune system is one of the most important means by which more complex animals protect themselves from being invaded and ingested by microbes and parasites. The immune system is a system of biological structures and processes within an organism that protects against disease.

There are in fact, two types of immune system: the innate and the adaptive. Defense against microbes is mediated by the early reactions of innate immunity and the later responses of adaptive immunity. The characteristics of innate and adaptive system are listed in Table 3 (Abbas et al, 2007).

Table 3: Type of immune system

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Innate</th>
<th>Adaptive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specificity</td>
<td>For molecules shared by groups related to microbes and molecules produced by damaged host cells</td>
<td>For microbial and non microbial antigens</td>
</tr>
<tr>
<td>Diversity</td>
<td>Limited; germ line encoded</td>
<td>Very large; receptors are produced by somatic recombination of gene segments</td>
</tr>
</tbody>
</table>
### Table 3 continued

<table>
<thead>
<tr>
<th>Memory</th>
<th>None</th>
<th>Yes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non reactivity to self</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

The cells and molecules responsible for immunity constitute the immune system, and their collective and coordinated response to the introduction of foreign substances is called the immune response.

**Innate and adaptive immune response**

Defence against threat is mediated by the early reactions of innate immunity and the later responses of adaptive immunity.

**Innate immune response**

Innate immunity (also called natural or native immunity) provides the early line of defence against microbes. It mainly consists of two parts. First is a set of barriers 1. Mechanical barriers (e.g., the skin and mucous membranes) 2. Chemical barriers (e.g., the high acidity of stomach, the salts and secretion of various enzymes) 3. Biological barriers (e.g., commensal microbes that occupy various parts of the body). The second part is based on the activity of a set of cells and molecules that can detect the presence of microbes and act against them once detected. The cellular part is mainly composed of phagocytic cells (neutrophils, macrophages), dendritic cells, and natural killer (NK) cells. Among the non cellular part, blood proteins, including members of the complement system, other mediators of inflammation, cytokines are important. The mechanisms of innate immunity are specific for structures that are common to groups of related microbes and may not distinguish fine differences between microbes. The innate
system generally recognizes the pathogen associated molecular patterns that can present on numerous types of microbes but not on host cells. The receptor and binding sites that recognise pathogen associated molecular patterns called pattern reorganisation receptor (PRR). In human, one of the most prominent types of PRRs is Toll like receptors (TLR). Binding of TLRs and other PRRs expressed on phagocytic cells by pathogen associated molecules triggers the activation of phagocytes. Activated phagocytes become enlarged, increase their production of antimicrobial product, and begin to ingest and degrade microbes (Abbas et al, 2007).

Natural Killer cells

Natural killer cells are part of lymphoid lineage but are distinct from T and B lymphocytes because they do not express the specialized receptors associated with the adaptive immune response. Instead they having two other types of receptors that determine their ability to identify and kill targeted host cells: killer activation receptors and killer inhibitor receptors. NK cells are important element in the innate defences against virally infected and cancerous host cells (Abbas et al, 2007).

Adaptive immune response

In contrast to innate immunity, there are other immune responses that are stimulated by exposure to infectious agents and increase in magnitude and defensive capabilities with each successive exposure to a particular microbe. Because this form of immunity develops as a response to infection and adapts to the infection, it is called adaptive immunity. The defining characteristics of adaptive immunity are exquisite specificity for distinct molecules and an ability to “remember” and respond more vigorously to repeated exposures to the same microbe. The adaptive immune system is able to
recognize and react to a large number of microbial and non-microbial substances. In addition, it has an extraordinary capacity to distinguish between different, even closely related, microbes and molecules, and for this reason it is also called specific immunity. It is also sometimes called acquired immunity, to emphasize that potent protective responses are “acquired” by experience. The main components of adaptive immunity are cells called lymphocytes and their secreted products, such as antibodies (Abbas et al, 2007).

There are many connections between the innate and adaptive immune systems. The innate immune response to microbes stimulates adaptive immune responses and influences the nature of the adaptive responses. Conversely, adaptive immune responses often work by enhancing the protective mechanisms of innate immunity, making them capable of effectively combating pathogenic microbes (Abbas et al, 2007).

Types of adaptive immunity

There are two types of adaptive immune responses, called humoral immunity and cell-mediated immunity, which are mediated by different components of the immune system and function to eliminate different types of threats.

**Humoral immunity**

Humoral immunity is mediated by molecules in the blood and mucosal secretions, called antibodies, which are produced by cells called B lymphocytes. Antibodies recognize microbial antigens, neutralize the infectivity of the microbes, and target microbes for elimination by various effector mechanisms. Humoral immunity is the principal defense mechanism against extracellular microbes and their toxins because secreted antibodies can bind to these microbes and toxins and assist in their elimination. Antibodies
themselves are specialized and may activate different effector mechanisms. For example, different types of antibodies promote the ingestion of microbes by host cells (phagocytosis), bind to and trigger the release of inflammatory mediators from cells, and are actively transported into the lumens of mucosal organs and through the placenta to provide defense against ingested and inhaled microbes and against infections of the newborn, respectively (Abbas et al, 2007).

**Cell-mediated immunity**

Cell-mediated immunity, also called cellular immunity, is mediated by T lymphocytes. Intracellular microbes such as viruses and some bacteria survive and proliferate inside phagocytes and other host cells, where they are inaccessible to circulating antibodies. Defense against such infections is a function of cell-mediated immunity, which promotes the destruction of microbes residing in phagocytes or the killing of infected cells to eliminate reservoirs of infection (Abbas et al, 2007).

Immune responses are regulated by a system of positive feedback loops that amplify the reaction and by control mechanisms that prevent inappropriate or pathologic reactions. When lymphocytes are activated, they trigger mechanisms that further increase the magnitude of the response. This positive feedback is important to enable the small number of lymphocytes that are specific for any microbe to generate the response needed to eradicate that infection. Many control mechanisms become active in immune responses to prevent excessive activation of lymphocytes, which may cause collateral damage to normal tissues, and to avoid responses against self antigens. In fact, a balance between activating and inhibitory signals is characteristic of all immune responses (Abbas et al, 2007).
Cellular components of immune system

The cells that serve specialized roles in innate and adaptive immune responses are phagocytes, dendritic cells, antigen specific lymphocytes, and various other leukocytes that function to eliminate antigens.

Phagocytes

Neutrophils

Phagocytes, including neutrophils and macrophages, are cells whose primary function is to identify, and destroy damaged cells. Neutrophils, also called polymorphonuclear leukocytes, are the most abundant population of circulating white blood cells and mediate the earliest phases of inflammatory reactions. The nucleus of a neutrophil is segmented into three to five connected lobules, hence the synonym polymorphonuclear leukocyte. The cytoplasm contains granules of two types. The majority, called specific granules, are filled with enzymes such as lysozyme, collagenase, and elastase (Abbas et al, 2007).

Monocytes

Monocytes are a type of mononuclear phagocytes present in circulation. Monocytes are heterogeneous and consist of at least two subsets, which are distinguishable by cell surface proteins and kinetics of migration into tissues. One population is called inflammatory because it is the precursors of tissue macrophages, rapidly recruited from the blood into sites of tissue inflammation. The other type may be the source of tissue resident macrophages and some dendritic cells. Once they enter tissues, these monocytes mature and become macrophages. Macrophages in different tissues have been given special names to designate specific locations. The major function of
Macrophages is defence against infection. In addition to killing the microbes, they also recognise and engulf the apoptotic cells. Activated monocytes also secrete cytokines. (Abbas et al, 2007).

*Mast cells, basophils, eosinophils*

Mast cells, basophils, and eosinophils are three additional cells that play roles in innate and adaptive immune response.

*Antigen-Presenting Cells*

Antigen-presenting cells (APCs) are cell populations that are specialized to capture the antigens, display them to lymphocytes, and provide signals that stimulate the proliferation and differentiation of the lymphocytes. The major type of APC that is involved in initiating T cell responses is the dendritic cell. Macrophages and B cells also present antigens to T lymphocytes in different types of immune responses (Abbas et al, 2007).

*Lymphocytes*

Lymphocytes, the unique cells of adaptive immunity, are the only cells in the body that express clonally distributed antigen receptors, each with a fine specificity for a different antigenic determinant. Each clone of lymphocytes consists of the progeny of one cell and expresses antigen receptors with a single specificity. This is why the total population of antigen receptors in the adaptive immune system is said to be clonally distributed. There are millions of lymphocyte clones in the body, enabling the organism to recognize and respond to millions of foreign antigens. The total number of lymphocytes in a healthy adult is about $5 \times 10^{11}$. Of these, ~2% are in the blood, ~10%
in the bone marrow, ~15% in the mucosal lymphoid tissues of the gastrointestinal and respiratory tracts, and ~65% in lymphoid organs (mainly the lymph nodes and spleen). Lymphocytes consist of distinct subsets that are different in their functions and protein products. Morphologically, all lymphocytes are similar, and their appearance does not reflect their heterogeneity or their diverse functions. B lymphocytes, the cells that produce antibodies, were so called because, the early stages of B cell maturation occur in the bone marrow. Thus, “B” lymphocytes refer to bone marrow–derived lymphocytes. T lymphocytes, the mediators of cellular immunity, were named because their precursors, which arise in the bone marrow, migrate to and mature in the thymus; “T” lymphocytes refer to thymus-derived lymphocytes. B and T lymphocytes each consist of subsets with distinct phenotypic and functional characteristics. In addition to B and T cells, other population of lymphocytes on basis of morphology and certain functional and molecular criteria but that are not readily categorized as T or B cells are Natural killer cells (Abbas et al, 2007).

Development of Lymphocytes

Lymphocytes arise from stem cells in the bone marrow. All lymphocytes go through complex maturation stages during which they express antigen receptors and acquire the functional and phenotypic characteristics of mature cells; these mature lymphocytes are called naive lymphocytes. After activation by antigen, lymphocytes go through sequential changes in phenotype and functional capacity (Figure 7) (Abbas et al, 2007).
Naive Lymphocytes

Naive lymphocytes are mature T or B cells that reside in the peripheral lymphoid organs and circulation and have never encountered foreign antigen. Naive lymphocytes typically die after 1 to 3 months if they do not recognize antigens. Naive and memory lymphocytes are both called resting lymphocytes because they are not actively dividing, nor are they performing effector functions. Before antigenic stimulation, naïve lymphocytes are in a state of rest, or in the G0 stage of the cell cycle. In response to stimulation, they undergo proliferation, resulting in increased size of the antigen-specific clones, a process that is called clonal expansion. Concurrently with clonal expansion, antigen-stimulated lymphocytes differentiate into effector cells whose function is to eliminate the antigen. Some of the progeny of lymphocytes differentiate into long-lived memory cells, whose function is to mediate rapid and enhanced (i.e., secondary) responses to subsequent exposures to antigens. Distinct populations of lymphocytes (naive, effector, and memory) are always present in various sites throughout the body, and these populations can be distinguished by several functional and phenotypic criteria (Table 4) (Abbas et al, 2007).
### Table 4: Characteristics of Naive, Effector and Memory Lymphocytes

<table>
<thead>
<tr>
<th>T Lymphocytes</th>
<th>Naive cells</th>
<th>Activated or Effector Lymphocytes</th>
<th>Memory Lymphocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Migration</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Preferentially to peripheral lymph nodes</td>
<td>Preferentially to inflamed tissues</td>
<td>Preferentially to inflamed tissues, mucosal tissues</td>
</tr>
<tr>
<td>Frequency of cells responsive to particular antigen</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Very low</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Effector functions</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>None</td>
<td>Cytokine secretion; cytotoxic activity</td>
<td>None</td>
</tr>
<tr>
<td>Cell cycling</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>Yes</td>
<td>+/-</td>
</tr>
<tr>
<td>Surface protein expression</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-2R (CD25)</td>
<td>Low</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>L- selectin (CD62L)</td>
<td>High</td>
<td>Low</td>
<td>Variable</td>
</tr>
<tr>
<td>Major CD45 isoform</td>
<td>Moderately High</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Chemokine receptor: CCR7</td>
<td>Low</td>
<td>High</td>
<td>High</td>
</tr>
<tr>
<td>Adhesion molecules: Intigrins, CD44</td>
<td>High</td>
<td>Low</td>
<td>Variable</td>
</tr>
<tr>
<td>Morphology</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Small Scant cytoplasm</td>
<td>Large, more cytoplasm</td>
<td>Small</td>
</tr>
</tbody>
</table>
Migration of Leukocytes at site of injury

The movement of immune cells from the blood to the site of injury are accomplished in three main steps (Abbas et al, 2007).

Delivery of phagocytic cell from blood

Neutrophils and Monocytes, after maturing in the bone marrow, enter the blood and circulate throughout the body. Although these cells can perform some phagocytic functions within the blood, their main functions, including phagocytosis of microbes and dead tissue cells, take place in extra vascular sites of infection virtually anywhere in the body.

Lymphocytes are entered from their sites of maturation (bone marrow or thymus) to blood stream, lymphatics and secondary lymphoid organs, where they encounter antigens and differentiate into effector lymphocytes.

Effector lymphocytes from the secondary lymphoid organs migrate to site of injury in any tissue, where they perform their protective functions (Figure 8).
Figure 8: Migration of Leucocytes at the site of injury (Modified from Abbas et al, 2007)

**Phenotypic markers to distinguish distinct populations of T lymphocytes**

Many surface proteins are used to identify and discriminate between different lymphocyte populations. These markers not only delineate the different classes of lymphocytes but also have many functions in the cell types in which they are expressed. The cluster of differentiation (CD) system is a widely adopted uniform method for naming cell surface
molecules that are characteristic of a particular cell lineage or differentiation stage, have a defined structure, and are recognized by a group (“cluster”) of monoclonal antibodies.

**Key surface markers to identify different T cell population**

**T cell receptor**: The T cell receptor or TCR is a molecule found on the surface of T lymphocytes (or T cells) that is responsible for recognizing antigens bound to major histocompatibility complex (MHC) molecules. The TCR is a disulfide-linked membrane-anchored heterodimer normally consisting of the highly variable alpha (α) and beta (β) chains as a part of complex with CD3 chain molecules (Figure 9). T cells expressing this receptor are referred to as α:β (or αβ) T cells, though a minority of T cells express an alternate receptor, formed by variable gamma (γ) and delta (δ) chains, referred as γδ T cells (Table 6) (Abbas et al, 2007).

![Figure 9: Structure of T cell receptor (Adopted from Abbas et al, 2007).](image)

**αβ TCR**: 95% of T cells, consists of an alpha (α) and beta (β) chain of TCR. Each chain is composed of two extracellular domains: Variable (V) region and a Constant (C)
region, both of Immunoglobulin super family domains forming anti parallel β-sheets. The constant region is proximal to the cell membrane, followed by a transmembrane region and a short cytoplasmic tail, while the variable region binds to the peptide/MHC complex. The variable domain of both the TCR α-chain and β-chain have three hyper variable or complementarily (CDRs), whereas the variable region of the β-chain has an additional area of hyper variability (HV4) that does not normally contact antigen and, therefore, is not considered a CDR.

The residues are located in two regions of TCR, at the interface of the α and β chains and in the β chain framework region that is thought to be in proximity to the CD3 signal transduction complex. CDR3 is the main CDR responsible for recognizing processed antigen, although CDR1 of the alpha chain has also been shown to interact with the N-terminal part of the antigenic peptide, whereas CDR1 of the β-chain interacts with the C-terminal part of the peptide. CDR2 is thought to recognize the MHC. CDR4 of the β-chain is not thought to participate in antigen recognition, but has been shown to interact with super antigens (Abbas et al, 2007).

**γδ TCR:** 5% of T cells, consists of an gamma (γ) and delta (δ) chain of TCR. This group of T cells is usually much less common than αβ T cells, but are at their highest abundance in the gut mucosa, within a population of lymphocytes known as intraepithelial lymphocytes. The antigenic molecules that activate γδ T cells are still largely unknown. However, γδ T cells are peculiar in that they do not seem to require antigen processing and major-histocompatibility-complex (MHC) presentation of peptide epitopes, although some recognize MHC class Ib molecules. Furthermore, γδ T cells are believed to have a prominent role in recognition of lipid antigens. They are of an
invariant nature and may be triggered by alarm signals, such as heat shock proteins (HSP) (Holtmeier et al 2005).

**CD3**: The CD3 is a T-cell co-receptor protein complex found on every T cells and is composed of four distinct chains, CD3γ, CD3δ and two CD3ε chains. These chains associate with a molecule known as the T-cell receptor (TCR) and the ζ-chain to generate activation signal in T lymphocytes. The TCR, ζ-chain, and CD3 molecules together comprise the TCR complex (Table 5 and 6, Figure 11) (Abbas et al, 2007).

**CD4**: CD4 is a glycoprotein found on the surface of T helper cells. Helper T cells are an essential part of the human immune system. They are often referred to as CD4 cells, T-helper cells or T4 cells (Table 6, Figure 11). They are called helper cells because one of their main roles is to send signals to other types of immune cells. CD4 is a member of the immunoglobulin super family. It has four immunoglobulin domains (D₁ to D₄) that are exposed on the extracellular surface of the cell; D₁ and D₃ resemble immunoglobulin variable (IgV) domains (Figure 10). CD4 is a co-receptor that assists the T cell receptor (TCR) in communicating with an antigen-presenting cell. Using its intracellular domain, CD4 amplifies the signal generated by the TCR by recruiting an enzyme, the tyrosine kinase Lck, which is essential for activating many molecular components of the signaling cascade of an activated T cell (Table 5) (Abbas et al, 2007).

**CD8**: CD8 is a transmembrane glycoprotein, predominantly expressed on the surface of Cytotoxic T cells (Table 6, Figure 11) and serves as a co-receptor for the T cell receptor (TCR). CD8 forms a dimer, consisting of a pair of CD8 chains. The most common form of CD8 is composed of a CD8-α and CD8-β chain, both members of the immunoglobulin super family with an immunoglobulin variable (IgV)-like extracellular
domain connected to the membrane by a thin stalk, and an intracellular tail (Table 5, Figure 10) (Abbas et al, 2007).

**Table 5:** Function of T cell co receptor CD molecules

<table>
<thead>
<tr>
<th>TCR associated CD molecule</th>
<th>Function</th>
<th>Ligand</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD3</td>
<td>Signal transduction by TCR complex</td>
<td>None</td>
</tr>
<tr>
<td>CD4</td>
<td>Signal transduction</td>
<td>Class II MHC</td>
</tr>
<tr>
<td>CD8</td>
<td>Signal transduction</td>
<td>Class I MHC</td>
</tr>
</tbody>
</table>

**Figure 10:** Structure of CD4 and CD8 co receptors (Adapted from Abbas et al, 2007)

**CD 161:** CD161 is type II membrane protein, also called Killer cell lectin-like receptor subfamily B, member 1, expressed by NK cells (Table 6, Figure 11). It contains an extracellular domain with several motifs characteristic of C-type lectins, a transmembrane domain, and a cytoplasmic domain. A small subset of T cells also express CD161 molecules; they recognized lipid antigens presented by CD1d. Upon
activation NK T cell produced large amount of cytokines and chemokines (Godfrey et al, 2004).

**CD56:** CD56 is a homophilic binding glycoprotein of immunoglobulin super family expressed on the surface of natural killer cells. It is also express on NK T cells (Table 6, Figure 11) (Godfrey et al, 2004).

**CD45RA and CD45RO:** CD45 is a type I transmembrane protein that is in various forms present on all differentiated hematopoietic cells except erythrocytes and plasma cells that assists in the activation of those cells (a form of co-stimulation). CD45 is highly glycocylated and have various isofroms. Naive T cells express large isoform of CD45 that is CD45RA. Activated and memory T cells express short isoform that is CD45RO (Table 6, Figure 11). This shortest isoform facilitates T cell activation (Kaplan et al, 1990).

**CD25:** CD25 is the alpha chain of the IL-2 receptor. It is a type I transmembrane protein present on activated T cells (Table 6, Figure 11) (Stauber et al, 2006).

**FOXP3:** FOXP3 (forkheadbox P3) also known as scurfin is a protein involved in immune system responses. A member of the protein family, FOXP3 appears to function as a master regulator (transcription factor) in the development and function of regulatory T cells (Table 6, Figure 11) (Zhang et al, 2007).
Table 6: CD markers to identify T cell subsets

<table>
<thead>
<tr>
<th>T cell class</th>
<th>Phenotype markers</th>
</tr>
</thead>
<tbody>
<tr>
<td>αβ T lymphocytes</td>
<td>CD3⁺ αβ heterodimers</td>
</tr>
<tr>
<td>helper T lymphocytes</td>
<td>CD4⁺</td>
</tr>
<tr>
<td>cytotoxic T lymphocytes</td>
<td>CD8⁺</td>
</tr>
<tr>
<td>NKT cells</td>
<td>CD161⁺ CD56⁺</td>
</tr>
<tr>
<td>Effector/ Memory T cells</td>
<td>CD45RA/RO⁺</td>
</tr>
<tr>
<td>Regulatory T cells</td>
<td>CD4⁺ CD25⁺ FOXP3⁺</td>
</tr>
<tr>
<td>γδ T lymphocytes</td>
<td>CD3⁺ γδ heterodimers</td>
</tr>
</tbody>
</table>

Figure 11: Different T cell subsets with CD marker expression
Function of different T lymphocyte population

**Helper T lymphocytes:** They play important role in both humoral and cell mediated immune response. They help the activity of other immune cells by releasing the T cell cytokines. They are essential in B cell antibody class switching, in the activation and growth of cytotoxic T cells, and in maximizing bactericidal activity of phagocytes such as macrophages. Helper T cell mainly differentiated in to two major subtypes of effector cells known as Th1 (type 1 helper) and Th2 (Type 2 helper) cells. Th1 cells promote the cellular immune system by maximizing the killing efficacy of the macrophages and the proliferation of cytotoxic CD8$^+$ T cells. It also promotes the production of opsonizing antibodies. Th2 cells promote the humoral immune system by stimulating B-cells into proliferation, to induce B-cell antibody class switching, and to increase neutralizing antibody production (Figure 12) (Abbas et al, 2007).

**Cytotoxic T lymphocytes (CTL):** Cytotoxic T lymphocytes kill the cancer cell, cells that are infected with virus or damaged in other way (Figure 12) (Abbas et al, 2007).

**NK T lymphocytes:** NK T lymphocytes suppress or activate innate and adaptive immune response (Abbas et al, 2007).

**Effector / Memory T lymphocytes:** Memory T cells provides specific, rapid and vigorous immune response against pathogen that encounter second time to body. There are two subsets of memory T cells, first is central memory subsets ($T_{CM}$) and second is effector memory ($T_{EM}$) subsets. Central memory ($T_{CM}$) display a capacity for self-renewal due to high levels of phosphorylation of an important transcription factor known as STAT5. In mice, $T_{CM}$ cells have been shown to confer superior
protection against cancer in several different model systems compared with $T_{EM}$ cells (Abbas et al, 2007).

**Regulatory T lymphocytes:** T regulatory cells are a component of the immune system that suppresses immune responses of other cells. This is an important "self-check" built into the immune system to prevent excessive reactions. These cells are involved in shutting down immune responses after they have successfully eliminated invading organisms, and also in preventing autoimmunity (Figure 12) (Abbas et al, 2007).

![Function of T lymphocytes](image)

**Figure 12:** Function of T lymphocytes (Adapted from Abbas et al, 2007).

**Activation of Lymphocytes**

The activation of lymphocytes follows a series of sequential steps beginning with the synthesis of new proteins, such as cytokine receptors and cytokines, which are required for many of the subsequent changes. The naive cells then undergo proliferation, resulting in increased size of the antigen-specific clones, a process that is called clonal
expansion, Concurrently with clonal expansion; antigen-stimulated lymphocytes differentiate into effector cells whose function is to eliminate the antigen (Abbas et al, 2007).

Activation of T cells

The goal of T cell activation is to generate, from a small pool of naive lymphocytes with predetermined receptors for any antigen, a large number of functional effector cells that can eliminate that antigen and a population of memory cells that remain for long periods to rapidly react against the antigen in case it is reintroduced. The initial activation of naive T lymphocytes occurs mainly in secondary lymphoid organs, where they may encounter antigens presented by mature dendritic cells. Reactivation of previously activated T cells can be done by any of the APCs and can occur in the body tissue and lymphoid aggregations. APCs present antigens to T cells in two different ways (Abbas et al, 2007).

- APCs are capable of ingesting and degrading material derived from infectious organisms and the debris of dead cells. Phagosomes that contain the ingested material fuse with lysosomes to form phagolysosomes. Phagolysosomes then can fuse with other organelles that contain newly synthesised MHC class II molecules and are transported to the surface, where they are exposed to T cells.
- APCs also can degrade and present proteins/peptides that are normal cellular proteins that are worn out, malformed or in excessive amounts and proteins encoded by virus within cytoplasm. These various peptides are then loaded within the endoplasmic reticulum onto newly formed MHC class I molecules. The
MHC class I molecules, bearing peptides, moves to the cell surface, where the peptides are exposed to T cells. T cells recognise the [peptide + MHC molecules] combination on APC surface, and if one of them fits the TCR tightly, a signal is transmitted to the T cell nucleus that provides part of stimulus for the activation. Subsequent binding of B7.1 (CD80) and B7.2 (CD86) molecules on APC with CD28 molecules on T cells, initiate differentiation of T cells into activated state. Part of this differentiation involves synthesis of soluble cytokines Interleukin 2, which serve as proliferation signal and high affinity receptor for cytokines (Abbas et al, 2007).

**T cell mediated cellular immune response**

T cell mediated cellular immune response includes two types of responses: delayed type hypersensitivity and cytotoxic T lymphocytes (CTL). Delayed type hypersensitivity responses are mediated by activated CD4⁺ Th1 cells. These Th1 cells may encounter APCs in the tissue that are associated with MHC II molecules and bind to them. This action triggers the reactivation of Th1 cells, which then secrete cytokines like interferon γ to attract and activate phagocytic macrophages. The activated macrophages then attack the antigenic stimulus, because they have no intrinsic antigenic specificity, tissues often can be damaged. Th1 cells provide specificity to delayed-type hypersensitivity responses but do not themselves act directly against the antigenic stimulus. Instead, they recruit and focus the activity of the nonspecific macrophages against the stimulus (Abbas et al, 2007).

Cytotoxic T lymphocytes responses are mediated by activated CD8⁺ T cells. CTLs act directly against their targets by direct action that involves cell-to-cell contact. Activated
CD8+ cells recirculate through the body looking for the (peptide + MHC I) combinations that fit for their TCRs. Upon encountering and binding to cell that express MHC I, CTLs damage the targeted cells by secreting molecules that damaged the membranes and induced targeted cell to undergo apoptosis. The CTLs are not damaged in the process and seek other target cells (Abbas et al, 2007).

**Regulation of T cell function**

The regulation of T cells occurs at several levels (Abbas et al, 2007).

- Elimination of potentially self reactive T cells in thymus: During the T cell development in thymus, most self reactive T cells undergo apoptosis by negative selection.
- The role of APCs in activating T cells: Activation of naive T cells in lymph nodes requires the intervention of APCs. Naive T cells that encounter appropriate (peptide+MHC) on non APCs that are unable to provide additional necessary signals cause these naive T cells to enter permanent state in inactivation or energy.
- Inactivation signals: After activation, T cells begin to express CD152 (CTLA-4), which competes with CD28 to bind CD80/CD86 and provide internal signal that inactivates T cells.
- Regulatory T cells: CD4 positive T cell population, T regulatory cells, inhibits the activity of other T cells.
- Mutual inhibition of Th1 and Th2 cells: IL4, IL10 and TGFβ produce by Th2, inhibits the activity of Th1 cells, whereas IFN-γ produced by Th1 cells inhibits the activity of Th2 cells.
Immunity to tumors

Among the numerous factors which influence tumor establishment, growth, local invasion and metastasis, the impact of immune system has been debated for a long time. The importance of immune system in surveillance and prevention of malignancy has been hypothesized since the early twentieth century. Macfarlane Burnet proposed the concept of immune surveillance in the 1950s. This concept refers to the continual surveillance and destruction of abnormal or mutated cells by immune system. The existence of immune surveillance has been demonstrated by the increased incidence of some types of tumors in immunocompromised experimental animals and humans. Although the overall importance of immune surveillance has been controversial, it is now clear that the innate and adaptive immune systems do react against many tumors. Further, the cancer immune surveillance system not only eliminates tumor cells early but also edits their immunogenicity to reduce the expression of tumor antigens (cancer immunoediting). Further studies have explained the importance of immunomodulatory factors such as IFN-γ, perforin and cells such as NK and natural killer T (NKT) cells in the immune surveillance system (Smyth et al., 2000; Shankaran et al., 2001). The cancer immunoediting concept proposes three stages of immune surveillance during tumor growth (Figure 13). The three stages include Elimination, Equilibrium and Escape to grow a clinically relevant tumor. The Elimination stage is divided into four steps: (1) Recognition of transformed cells by NK T and NK cells (2) chemokines inducted recruitment of NK cells and DCs to tumor site (3) tumor antigen–specific T cells development in lymph nodes (4) migration of T cells to the tumor site and destroy tumor cells. In the Equilibrium stage, T cells and NK cells keep suppressing cancer cells by
persistent attacks, while tumor cell variants show increased resistance to immune surveillance and gradually survive and proliferate. Finally, in the Escape stage, immunologically edited cancer cells expand. Taken together, the immune surveillance system is closely associated with carcinogenesis as well as with progression and metastasis of cancer cells (Dunn et al, 2002; Schreiber et al, 2011).

\[\text{Figure 13: Cancer immunoediting}\]

\textit{Tumor microenvironment}

Once tumor is formed, it grows in very complex network of epithelial and mesenchymal cells, vascular and lymphatic vessels, inflammatory and immune cells (Figure 14). Further, tumor cells grow and metastasis in immunosuppressive tumor microenvironment. Various mechanisms involved in immunosuppresion like defective antigen expression on the tumor cell surface, loss or reduction of MHC (major histocompatibility complex) class 1 molecules, loss of expression of co-stimulatory
molecules, the production of immunosuppressive molecules such as transforming growth factor (TGF)-β, prostaglandin (PG) E2 and adenosine, production of pro-inflammatory cytokines such as interleukin (IL)-6 and IL-10, the expression of Fas ligand (FasL), which leads to the death of tumor-infiltrating lymphocytes (TILs) (Duray et al, 2010).

![Diagram of immunosuppression in tumor microenvironment](image)

**Figure 14:** Immunosuppression in tumor microenvironment (Adapted from Duray et al, 2010)

The interaction of malignant cells with their microenvironment is complicated. Some cells like Macrophage and Helper cells play dual role in tumor microenvironment. Macrophage recruits at tumor site (tumor associated macrophages) are major inflammatory component of tumor microenvironment. Tumor associated macrophages present two different phenotypes. Macrophages of the M1 phenotype kill pathogens and
promote the activation of cytotoxic CD8^+ T cells and the differentiation of Naive CD4^+ T cells into Th1 effector cells and Th17 cells. M2 macrophages stimulate CD4^+ Th2 cells and Regulatory T cell differentiation and can promote angiogenesis and tissue remodelling. Multiple studies have shown a correlation between a large number of macrophages in the tumor microenvironment and a worse prognosis. However, some studies have shown that tumor associated macrophages may correlated with good prognosis. Similarly, Helper T cells can also contribute to tumor destruction or facilitate its development. Type I (Th1) Helper T cells can facilitate tumor rejection by assisting the function of Cytotoxic cells whereas Type II (Th2) cells promote antibody production by B cells by secreting cytokines. Th17 Helper T cells by producing IL 17, promote production of cytokines, chemokines, promoting inflammation. Moreover, Myeloid-derived suppressor cells (MDSCs) induce Regulatory T cells, secrete IL-10, and inhibit CD4^+ and CD8^+ T cells. (Duray et al, 2010).

Moreover, vasculature in tumor microenvironment plays dual role, tumor cells are taking advantage of energy and nutrients carried by the blood vessels but also favouring immune cell infiltration in to tumor. Similarly increased lymphatic vessels promote metastatic cells to invade draining lymph node but also facilitate migration of Dendritic cells and Effector lymphocytes in to tumor (Fridman et al, 2013).

In addition to altered immune response at tumor site, malignant solid tumors give rise to systemic changes such as leukocytosis, neutrophilia and lymphopenia. These haematological findings are found to be correlated with advance tumor stage, and therefore associated with poor clinical outcome. An elevated Neutrophil to Lymphocyte
ratio is found to be an independent prognosticator in various cancers (Schmidt et al, 2005; Fogar et al, 2006; Donskov et al, 2006).

**Circulating and tumor infiltrating T cells in Head and Neck Cancer**

Among the factors associated with immunosupression, T cells are found to be one of the key factors which coordinate the host immune system to survey and eliminate cells with malignant transformation. There has been considerable investigations performed focusing on the alteration and functional state of circulating and tumor infiltrating T lymphocytes in Head and Neck Carcinoma patients. Decreased absolute T cells counts were noted in peripheral blood of patients with squamous cell carcinoma of Head and Neck (Kuss et al, 2004). Moreover, same study group demonstrated significant decreased number of Helper and Cytotoxic T cells in the peripheral circulation in Head and Neck Cancer and their imbalance in patients with no evidence of disease observed for many years. The reduction of T cells is in peripheral blood mainly due to apoptosis; furthermore, Cytotoxic T cells were more sensitive to apoptosis than Helper cells in patients with Head and Neck Cancer (Hoffmann et al, 2002). Moreover, apoptosis of T cells in Head and Neck Cancer was found to be mediated by Fas ligand expressed on SCCHN cells as co-culture of Fas +/-activated peripheral blood mononuclear cells (PBMCs) with FasL+ tumor cells induced apoptosis of T cells (Gastmann et al, 1999). Further the effect of tobacco on circulating T cells was observed by Manchanda et al (2006); they demonstrated a significant reduction in CD3+ and CD4+ T cells in peripheral blood of OSCC patients. Moreover, the same study group demonstrated that frequency of CD3+IL-4+ and CD8+IL-4+ T cells was significantly higher and the number of CD4+IL-2+ T cells significantly lower in these patients than in
healthy controls and the expression of IL-2 in CD4$^+$ and CD8$^+$ cells was also reduced in patients with advance stage cancer. Alteration in circulating γδ cells in Head and Neck Cancer was also observed by some study groups; Bas et al (2006) observed increased γδ cells where as Noguchi et al (2014) observed decreased γδ cells in Head and Neck Cancer patients as compared to healthy controls. Further, low level of circulating NK T cells were observed in Head and Neck Cancer by two study groups (Molling et al, 2007; Bose et al, 2008). Moreover, increase of CD4$^+$CD25$^+$Foxp3$^+$ regulatory T cells was observed in the peripheral blood with Head and Neck Carcinoma. This increase is linked to an increase in the suppressive activity of these cells on the proliferation of CD4$^+$CD25$^-$ T cells, which suggests the involvement of Regulatory T cells in the decreased antitumor immunity of T cells (Schaefer et al, 2005; Lau et al, 2007).

Apart from alteration in circulating T cells, some study groups also demonstrated variation in tumor infiltrating T cells, Yip et al (2008) observed reduced expression of CD3ζ in tumor infiltrating T cells, suggesting signalling abnormality of tumor infiltrating T cells in nasopharyngeal carcinoma. Further, same study group demonstrated increased frequency of tumor infiltrating CD4$^+$FOXP3$^+$ T cells in nasopharyngeal cancer as compared to non malignant nasopharyngeal tissue. Further, increased frequency of tumor infiltrating CD8$^+$ T cells were observed by Zancope et al (2010) in lip and oral cavity Squamous Cell Carcinoma patients as compared to control. Moreover, one study group (Schwarz et al, 2008) compared CD25$^+$Foxp3$^+$ Regulatory T cells and CD3$^+$Foxp3$^+$ and CD8$^+$Foxp3$^+$ in tumor infiltrating lymphocytes between OSCC and 15 human tumor-free tonsils again revealed an increased number of Regulatory T cells in
carcinomas whereas no significant change was noted in the number of CD3^+Foxp3^+ and CD8^+Foxp3^+ in TILs.

Further, prognostic significance of T cells in Head and Neck Cancer patients is controversial. Badoual et al, 2006 examined prognostic value of tumor infiltrating CD4^+ T-cell populations (CD4^+CD25^+, CD4^+CD69^+, and CD4^+FOXP3^+ T cells) in HNSCC patients and revealed a high level of CD4^+CD69^+ T cells and CD4^+Foxp3^+ T cells were positively correlated with better locoregional control (Badoual et al, 2006). Moreover, a higher density of CD4^+CD25^+ Regulatory T cells was also linked to a good prognosis in HNSCCs (Loose et al, 2008). One study group (Zhang et al, 2010) also observed high density of FOXP3^+ TILs was correlated to better overall survival and progression-free survival in nasopharyngeal carcinomas. In contrast, study of Strauss et al (2007) showed that the presence of Regulatory T cells in TILs was linked to a worse prognosis in HNSCC patients. Indeed, suppression in the tumor microenvironment is mediated by a unique subset of CD4^+CD25^{high}Foxp3^+ Regulatory T cells that produce IL-10 and TGF-β, exerting a more suppressive effect on proliferation.

Further, cancer immunoediting concept also suggests the interaction of anaplastic or dysplastic cells with immune system during elimination step. Thus it is important to identify host immune response in premalignant condition to understand immune escapes during tumorigenesis. More than 90% of premalignant lesions are associated with tobacco and alcohol consumption. The carcinogens in tobacco cause damage in mucosal layer cells, which cause transformation of normal cells to dysplastic cells. These dysplastic cells are detected and removed by the immune system.
Alteration in cellular and humoral immune response was also observed in premalignant condition. Some study groups demonstrated that increased exposure of tobacco was associated with increased number of oral mucosal Dendritic Langerhans cells (Daniels et al, 1992; Girod et al, 1994; Barrett et al, 1996; Boyle et al, 2010), which decrease in invasive carcinomas. Further, Lee et al (2010) observed change in circulating Lymphocytes during oral carcinogenesis and Gannot et al (2002) observed increased infiltration of mature immune cells, especially of B cells, to infiltrate the more malignant epithelial lesions. Moreover, a study by Ohman et al (2012) demonstrated increased LCs and T cells (CD3+, CD4+ and CD8+) in tissue compartments with dysplastic epithelial cells, which further increase in OSCC.

In India, few studies have analysed the alteration of T cells and Cytokine profile in Oral Squamous Cell Carcinoma patients. Agrwal et al (2003) observed altered Th2 cytokine profile in OSCC as compared to healthy controls; they also observed higher IL4 and IL10 gene expression in advance stage tumors. Kulkarni et al (2009) observed down regulation of TCR zeta chain in Oral Cancer. Gaur et al (2012) observed high percentage of Th17 (CD4+IL14A+) and Regulatory (CD4+CD25+FOP3+) cells in OSCC patients as compared to control. These Indian studies have analysed T cell subsets only in OSCC patients. However, these studies have not analysed its dysfunction in premalignant conditions. Only one Indian study (Pilai et al, 1987) have analysed systemic alteration of Lymphocytes in premalignant and malignant oral lesion. However, they did not examine local immune response in their study. Further, no Indian study has evaluated tumor infiltrating Regulatory T cells and their ratios with Cytotoxic and Helper T cells in OSCC and premalignant conditions.
The literature review implies that T cells play major role in systemic and local immune response in Head and Neck Cancer. Moreover, cancers originating from different sites in the Head and Neck may have different tumor biology. Therefore, in present study alteration in systemic and local T cell immune response was analysed in Oral Cancer and at different stages of oral carcinogenesis.
**AIM OF THE STUDY**

The present study focuses on the systemic and local immune response of Oral Squamous Cell Carcinoma (OSCC) and Leukoplakia patients.

**SPECIFIC AIMS**

**PART I**

The first aim was to understand the systemic immune response in OSCC and Leukoplakia patients.

a) To evaluate frequency of leukocyte subsets [Neutrophils, Monocytes, and Lymphocytes] and T cell subsets [αβ and γδ T cells, Cytotoxic T cells (Naive and Effector T cells), Helper T cells (Naive and Memory T cells, Regulatory T cells) and NK T cells] in peripheral blood of healthy control, Leukoplakia and OSCC patients.

b) To compare leukocyte subsets and T cell subsets between healthy control, Leukoplakia and OSCC.

To correlate leukocyte subsets and T cell subsets with clinical parameters (age, gender, habit, anatomic site), pathological parameters (tumor size, nodal status, stage, histological grade, lymphatic permeation, vascular permeation, neural invasion and margin involvement) and disease status of OSCC patients.

**PART II**

The second aim was to understand the local immune response in OSCC and Leukoplakia patients.
a) To evaluate frequency of T cell subsets [Cytotoxic T cells, Helper T cells and Regulatory T cells] in tumor tissue of OSCC patients.
b) To correlate T cell subsets with clinical parameters (age, gender, habit, anatomic site), pathological parameters (tumor size, nodal status, stage, histological grade, lymphatic permeation, vascular permeation, neural invasion and margin involvement) and disease status of OSCC patients.
c) To evaluate frequency of Cytotoxic T cells and Regulatory T cells in Hyperplasia and Dysplasia and compare them with OSCC.
d) To evaluate and compare Micovessel density (MVD) in Hyperplasia and Dysplasia and OSCC.
e) To correlate MVD with clinicopathological parameters, circulating and tumor infiltrating Cytotoxic, Helper and Regulatory T cells.