CORRELATION OF NEUTROPHIL- LYMPHOCYTE RATIO WITH DEPTH OF INVASION AND LYMPH NODE METASTASIS IN ORAL CANCERS

By

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DISSERTATION SUBMITTED TO

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In partial fulfillment of the requirements for the degree of

MASTER IN SURGERY (MS) IN OTORHINOLARYNGOLOGY

Under the Guidance of

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ABSTRACT

BACKGROUND:

Malignancies of the Head & Neck contribute for approximately 30% of cancers in India. Approximately half of them are oral malignancies. Tobacco chewing, areca nut, betel leaf quid, HPV infection, a record of head and neck carcinoma, fungal infection (Candida) are the principal etiological and predisposing factors for OSCC. Predictive biomarkers are useful in planning of treatment as they give critical data regarding the broad outcome of the patient. However, biomarkers (molecular) requiring specimens or tissues for processing remains tough on patients, since sample collection is an invasive procedure.

According to current research, a relationship exists between inflammatory micro-environment of tumor and systemic response by the tumour. Increased neutrophil counts and/or reduced lymphocyte counts may inhibit killer cells (Lymphokine activated). This is plausible mechanism of tumour aggression. Cancer induced lymphocytopenia may signify a generalized state of immunological decline. NLR may reveal the coexistence of two conflicting inflammatory and immunological pathways in cancer patients. The inflammatory response to a tumour promotes angiogenesis, causes DNA damage, and delays apoptosis, facilitating tumour cell proliferation and increasing the risk of spreading. Recent research has found links between systemic inflammation and tumour progress. As a result, NLR is a valid Marker.

The most current AJCC Staging (8th Edition) takes into account tumour depth, which is the third dimension and is more relevant in tumour dissemination.

The depth of invasion influences disease spread and therefore prognosis, and the cutoff limit evaluated for aggressive behavior in tongue carcinoma is 4mm and for buccal mucosa is 5mm, beyond which tumour is aggressive.

In this study, we intend to assess predictive potentiality of NLR to estimate the invasiveness, regional spread to lymph nodes, severity of malignancy, survival rates (disease specific and overall survival) of patients by correlating preoperative NLR with histopathological DOI and lymph node metastasis.

OBJECTIVES:

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Last but not the least, I wholeheartedly thank all my patients and their families who submitted themselves most gracefully for this study. To these stoic people who showed great strength despite their suffering, let me say, I am greatly indebted. Thank you and God bless.

Dr. Diana Ann Jose

CONTENTS

LIST OF ABBREVATIONS

NLR	Neutrophil to Lymphocyte Ratio
OSCC	Oral Squamous Cell Carcinoma
AJCC	American Joint Committee on Cancer
SCC	Squamous Cell Carcinoma
TT	Tumour Thickness
DOI	Depth of Invasion
PMMC	Pectoralis Major Myo-Cutaneous Flap
END	Elective Neck Dissection
ENE	Extra Nodal Extension

ABSTRACT

BACKGROUND:

Malignancies of the Head & Neck contribute for approximately 30% of cancers in India. Approximately half of them are oral malignancies. Areca nut, tobacco chewing, betel leaf quid, HPV infection, reduced dietary adequacy, a record of head and neck carcinoma, Candida infection are the principal predisposing factors for OSCC. Predictive biomarkers are useful in planning of treatment as they give critical data regarding the broad outcome of the patient. However, molecular biomarkers requiring specimens as tissues for processing remains tough on patients, since sample collection is an intrusive procedure.

According to current research, a relationship exists between inflammatory micro-environment of tumor and systemic response by the tumour. Increased neutrophil counts and/or reduced lymphocyte counts may inhibit killer cells (lymphokine activated). Cancer induced lymphocytopenia may signify a generalized state of immunological decline. NLR may reveal the coexistence of two conflicting inflammatory and immunological pathways in cancer patients. The inflammatory response to a tumour promotes angiogenesis, causes DNA damage, and delays apoptosis, facilitating tumour cell proliferation and increasing the risk of spreading. Recent research has found links between systemic inflammation and tumour prognosis. Thus, NLR is a valid marker of systemic inflammation.

The most current AJCC Staging (8^{th} Edition) takes into account tumour depth, which is the third dimension and is more relevant in tumour dissemination.

Depth of invasion influences disease spread and therefore prognosis, and the cutoff limit evaluated for aggressive behavior in tongue carcinoma is 4mm and for buccal mucosa carcinoma is 5mm, beyond which tumour is aggressive and has a high chance of metastasis to lymph nodes.

In this study, we intend to assess predictive potentiality of NLR to estimate the invasiness, regional spread to lymph nodes, severity of malignancy, disease specific and overall survival rates of patients by correlating preoperative NLR with histopathological DOI and lymph node metastasis.

OBJECTIVES:

1)To document Neutrophil: Lymphocyte ratio preoperatively in patients with T2-T4 staged Squamous Cell Carcinoma of Oral Cavity.

2) To document depth of invasion and number of metastatic lymph nodes if any after HPE of the resected specimen of above-mentioned subset of patients.

3)To determine whether the Neutrophil: Lymphocyte ratio has a correlation with above mentioned parameters for prognostication of Oral carcinoma.

MATERIALS & METHODS:

A prospective, cross-sectional study, involving 88 patients with biopsy proven OSCC staged T2-T4, was carried out at Sri Devaraj Urs Medical College, R.L. Jalappa Hospital & Research Centre, Kolar from January 2021 to July 2022.

A complete blood count (CBC) was done and NLR ratio was estimated preoperatively. After surgery (Primary resection of tumour with neck dissection and reconstruction) the resected specimen was examined histopathologically and size, depth of invasion, number of metastatic lymph nodes was documented. A cut-off limit for NLR was taken as 2.93, with depth of invasion as 4mm for tongue cancer and 5mm for other subsites of oral cavity to decide the aggressiveness of the tumour.

RESULTS:

Study included 88 patients; 15 patients with Carcinoma Tongue and 73 patients with Carcinoma involving the Other Subsites of the Oral Cavity. Majority of patients belonged to the 46 to 60 years age group.

Nearly 2/3rd of the patients were females with male preponderance was seen in case of Carcinoma Tongue. Most common subset involved in OSCC in our study was noted to be Buccal Mucosa. A total of 12 patients showed Perineural Invasion on HPE report of which 11 patients belonged to the other subsites group.

On comparing DOI with NLR with cut-off of 2.93, patients belonging to Carcinoma Tongue group, out of the 14 patients who had DOI > 4mm on HPE of the resected specimen, 29% patients had NLR $\geq$ 2.93 cut-off. In patients belonging to Carcinoma of other subsites of oral

cavity group, out of the 58 patients who had DOI > 5mm on HPE of the resected specimen, 36% patients had NLR ≥ 2.93 cut-off.

Patients with Carcinoma Tongue, the mean NLR was noted to be higher for those with pN+ status as compared to those with a pN0 status. Patients with Carcinoma involving other subsites, no correlation was seen between NLR and pN+ status. A higher DOI was noted in patients with Carcinoma of Other Subsites of Oral Cavity with pN+ status – 12.27mm when compared to those with pN0 status – 10.83mm

On comparing the presence of perineural/ Vascular/ Bone invasion with NLR in patients with Carcinoma Tongue, a negative correlation was again noted – patients with no perineural and bone invasion were noted to have a higher mean NLR.

A total of 6 patients were noted to have recurrence of disease out of the total of 88 patients – out of which 67% patients presented with recurrence within 12 months of surgery. It was noted that the chance of recurrence was more in patients with a higher DOI when compared to those lower DOI.

Further, on comparing the relationship between disease free period and NLR it was noted that in patients belonging to Carcinoma Tongue, disease free period was more in those patients who had a lower NLR – however, this correlation was weak. The same finding was however not noted in patients belonging to Carcinoma of Other subsites of oral cavity.

CONCLUSION

NLR was noted to have a weak but positive correlation with DOI in patients belonging to both Carcinoma Tongue and Carcinoma of Other subsites of Oral Cavity. Also, patients with Carcinoma Tongue who had pN+ status were noted to have a higher NLR ratio when compared to those with pN0 status.

KEY WORDS

Oral Squamous Cell Carcinoma (OSCC), Neutrophil Lymphocyte Ratio (NLR), Depth of Invasion (DOI), Positive Cervical Lymph Nodes, Prognosis

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Introduction

1. INTRODUCTION

Malignancies of the Head & Neck account for approximately 30% of cancers in India. Approximately half of them are oral malignancies.[1] Areca nut, tobacco, betel quid, alcohol, HPV infection, low dietary adequacy, a record of head and neck squamous cell carcinoma, and Candida infection are the principal etiological and predisposing factors for OSCC.[2] Head and neck cancers have emerged as a substantial public health issue in India as a consequence of addictions and lifestyle choices. Despite recent breakthroughs in cancer diagnosis and treatment, the 5-year survival rate of patients with oral cancer has stayed at a dismal 50% during the last few decades.

Prognostic biomarkers are useful for plan of treatment because they give critical information regarding the overall outcome of the patient. However, molecular biomarkers requiring tissue specimens for processing place a burden on patients, since sample collection is an intrusive procedure.[3]

Different tumour markers, such as tumour infiltration thickness, degree of differentiation, lymphovascular invasion, tumour budding, perineural invasion, DOI, NLR may be useful in estimating the risk of unknown metastases in primal stage oral tongue squamous cell carcinoma (OTSCC) [4]. Peripheral blood may identify the onset and progression of numerous malignancies, as well as inflammation in body. According to current research, a relationship exists between inflammatory micro-environment of tumor and systemic response by tumour. Increased neutrophil counts and/or reduced lymphocyte counts may inhibit lymphokine-activated killer cells. This might be a plausible mechanism for tumour aggression. Lymphocytopenia in cancer may signify a generalized state of immunological decline.[5]

Decreased immunological function may have an impact on survival. Several animal studies have indicated that a lack of CD4+ Th1 helper cells, CD8+ cytotoxic T cells, or natural killer (NK) cells makes the host more vulnerable to cancer formation and progression. NLR may reveal the coexistence of two conflicting inflammatory and immunological pathways in cancer patients. The inflammatory response to a tumour promotes angiogenesis, causes DNA damage, and delays apoptosis, facilitating tumour cell proliferation and increasing the risk of spreading. Recent research has found links between the degree of systemic inflammatory response and tumour prognosis. Thus, NLR is a valid marker of systemic inflammation.[5]

TNM (tumour, node, metastasis) is a global staging system developed by American Joint Cancer Committee in collaboration with Union for International Cancer Control that aids in the assessment and evaluation of cancer and its subsequent treatment. Until recently, the AJCC

staging of oral malignancies exclusively took into account the two dimensional size of tumor. The most current 8th Edition of the AJCC Staging takes into account tumour depth, which is the third dimension and is more relevant in tumour dissemination. Depth of invasion is the extent of cancer progression into the tissue beneath an epithelial surface. [6]

Depth of invasion influences disease spread and therefore prognosis, and the cutoff limit evaluated for aggressive behavior in tongue carcinoma is 4mm and for buccal mucosa is 5mm, beyond which tumour is aggressive and has a high chance of metastasis to lymph nodes.[7]

In this study, we intend to assess predictive potentiality of NLR to estimate : invasiveness,regional spread to lymph nodes, severity of malignancy, survival rates (disease specific and overall) of patients by correlating preoperative NLR with histopathological DOI and lymph node metastasis.

Aim and Objectives

2. AIM AND OBJECTIVES

2.1 AIM:

To analyze the relationship between preoperative levels of Neutrophil Lymphocyte ratio (NLR) with Histopathological findings for determining prognosis in patients with Oral Squamous Cell Carcinoma (OSCC).

2.2 OBJECTIVES:

1)To document Neutrophil: Lymphocyte ratio preoperatively in patients with Squamous Cell Carcinoma of Oral Cavity (Stage T2-T4).

2)To document Depth of invasion and number of metastatic lymph nodes after histopathological examination of the resected specimen in above mentioned subset of patients.

3)To determine whether the Neutrophil: Lymphocyte ratio has a correlation with above mentioned parameters for prognostication of Squamous cell carcinoma of oral cavity.

Review of Literature

3. REVIEW OF LITERATURE

3.1 HISTORY OF CANCER

About 3000–1500 BC, the earliest descriptions of cancer were made. The term "cancer" is a Latinized version of the Greek word "crab," which is carcinoma. The word "cancer" is used to describe cells that develop abnormally, infiltrate normal tissue, and spread to organs in certain directions.

Roudolf Virchow, the creator of cellular pathology, supplied the pathologic foundation for the study of cancer, which expanded our comprehension of the disease process. In turn, this opened the door for the development of cancer surgery. To get a clear diagnosis, the specimen has to be evaluated. More essential, pathologists need to document how much of the tumour was removed.

The idea that a tumour may be surgically removed if it was mobile and did not affect nearby tissues was initially put out by John Hunter (1728–1793) [(8)]. Consequently, he established the foundation for surgical oncology.

Three surgeons, Billroth, Hadley, and Halsted, later made significant contributions to the field of cancer surgery. Through their efforts, the whole tumour and local lymph nodes were surgically removed. Halsted's basics, which advocated eliminating the tumour and its lymphatic drainage, served as the foundation for most oral cavity cancer surgeries.

A comprehensive excision of head and neck cancers using the mandible and upper neck lymphatics was carried out in 1885 AD by Sir Henry T. Batlin, a surgeon from St. Bartholomew's Hospital in London. He stressed the benefit of removing cervical lymph nodes that had metastatic disease, much like Kocher.

But in 1906 A.D., George Crile published the first description of en-bloc radical neck dissection. The radical neck dissection procedure is still in use today, and his classic paper

In 1932 AD, Grant Ward carried out the first commando operation. This radical neck dissection and en bloc removal of the primary from the oral cavity, which includes a piece of the mandible, have been done together since 1942.

The term "composite resection" is given to Hayes Martin (originally known as the COMMANDO operation). A primary tumour in the oropharynx or oral cavity is removed with this surgical procedure, along with a portion of the mandible and the neck nodes.[9]

PMMC flap, which is based on the pectoral branch of thoracoacromial artery, was first reported by Stephan Ariyan in 1979. Especially in underdeveloped countries, this is sometimes referred to as the "workhorse" of head and neck reconstruction.

In the era of antibacterial chemotherapy, improved wound care, diagnostic tools, advancements in pathology, improved surgical techniques, micro vascular free tissue transfer for reconstruction, advancements in anaesthesia, and transfusion methods, prognosis of cancer surgery significantly improved. [10]

3.2 ORAL CAVITY - ANATOMY

According to the American Joint Committee on Cancer Staging [11] anatomical sites within oral cavity are:

LIPS

TONGUE (ANTERIOR 2/3rd)

FLOOR OF MOUTH

GINGIVOBUCCAL SULCUS (UPPER AND LOWER)

BUCAL MUCOSA

RETROMOLAR TRIGONE

HARD PALATE

AJCC DEFINITIONS:

“The oral cavity extends from the skin-vermilion junction of the lips to the junction of the hard palate and soft palate above, to the line of circumvallate papillae below, and to the anterior tonsillar pillars laterally.”

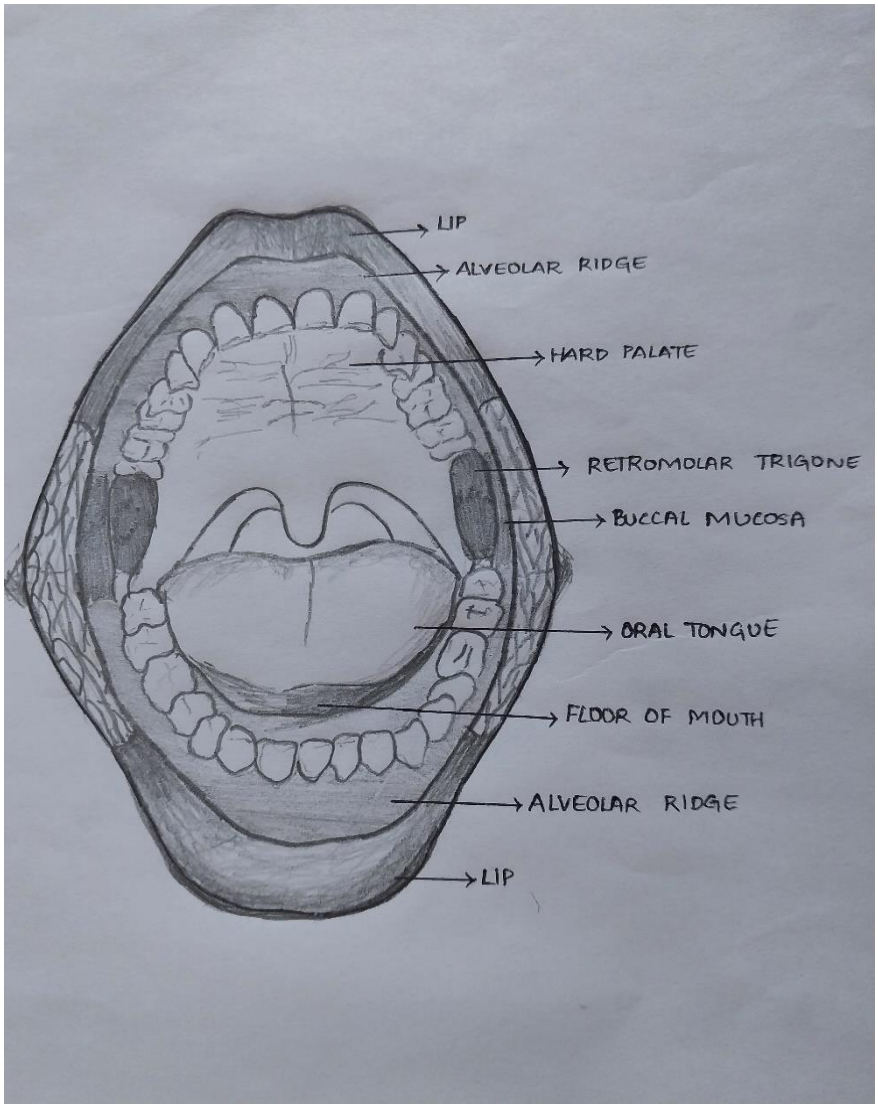

FIGURE 1: ORAL CAVITY SUBSITES

LIP: "The lip begins at the junction of the vermilion border with the skin and includes only the vermilion surface or that portion of the lip that comes into contact with the opposed lip. It is subdivided into an upper and lower lip, joined at the commisures of the mouth."**(11)**

BUCCAL MUCOSA: "The buccal mucosa includes all the mucous membrane lining of the inner surface of cheeks and lips from the line of contact of the opposing lips to the line of attachment of mucosa of the upper alveolar ridge (upper and lower) and pterygomandibular raphe."**(11)**

LOWER ALVEOLAR RIDGE: "The lower alveolar ridge refers to the mucosa overlying the alveolar process of the mandible, which extends from the line of attachment of mucosa in the lower gingivobuccal sulcus to the line of attachment of free mucosa of the floor of mouth."**(11)**

UPPER ALVEOLAR RIDGE: "The upper alveolar ridge refers to the mucosa overlying the alveolar process of maxilla, which extends from the line of attachment of mucosa in the upper gingivobuccal sulcus to the junction of the hard palate."**(11)**

RETROMOLAR TRIGONE: "The retromolar trigone is the attached mucosa overlying the ascending ramus of the mandible from the level of the posterior surface of the last lower molar tooth to the apex superiorly, adjacent to the tuberosity of the maxilla."**(11)**

FLOOR OF MOUTH: "The floor of the mouth is a crescentic surface overlying the mylohyoid and hyoglossus muscle, extending from the inner surface of the lower alveolar ridge to the undersurface of the tongue. Its posterior boundary is the base of the anterior pillar of tonsil. It is divided into two sides by the frenulum of the tongue and harbors the ostia of the submandibular and sublingual salivary glands."**(11)**

HARD PALATE: "The hard palate is the semilunar area between the upper alveolar ridge and the mucous membrane covering the palatine process of the maxillary palatine bones.It extends from the inner surface of the superior alveolar ridge to the posterior edge of palatine bone."**(11)**

ANTERIOR 2/3rd OF TONGUE: "The anterior 2/3rd of the tongue is the freely mobile portion of the tongue that extends anteriorly from the line of circumvallate papillae to the undersurface of the tongue at the junction with the floor of the mouth. It is composed of four areas: the tip, the lateral borders, the dorsum, and the undersurface (nonvillous ventral surface of the tongue)."**(11)**

3.3 ARTERIAL SUPPLY OF ORAL CAVITY

Branches of external carotid artery give arterial supply to oral cavity. Tongue receives supply from lingual artery. Greater palatine and superior alveolar arteries supply hard palate. **(12)** Alveolar arteries arise from maxillary artery's terminal branches and deliver blood to gingiva and upper teeth. Labial arteries provide vascular support to lips. Inferior alveolar artery supplies lower dentition and mandible. **(13)**

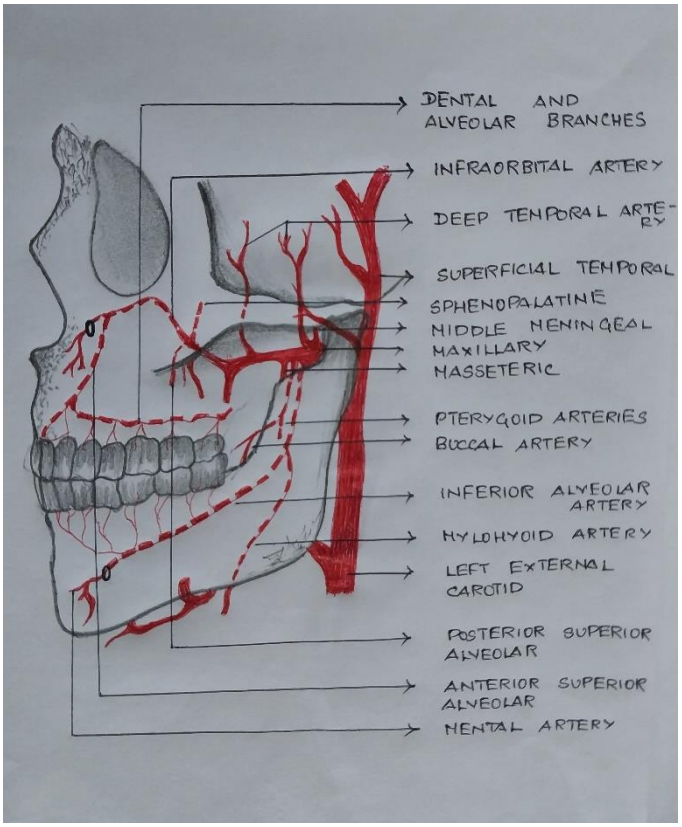

FIGURE 2: ARTERIAL SUPPLY - ORAL CAVITY

3.4 NERVE SUPPLY OF ORAL CAVITY

The innervation of the oral cavity is distinct and is predominantly supplied by the trigeminal nerve's maxillary and mandibular divisions.

Oral mucosa, teeth, and supporting tissues are all supplied by trigeminal nerve's maxillary and mandibular divisions. Soft palate is innervated by lesser palatine branch of maxillary nerve, whereas larger palatine and nasopalatine nerves innervate hard palate.

The tongue is intricately innervated. The hypoglossal nerve innervates all intrinsic and extrinsic muscles, with exception of palatoglossus. Vagus nerve innervates palatoglossus. Anterior and posterior regions of tongue have varied taste and sensory innervations because of their unique embryological base.

Anterior two-thirds of tongue receive general sensation from lingual nerve whereas chorda tympani nerve relay taste sensation. Glossopharyngeal nerve carries sensory and gustatory information to posterior part of tongue. Mandibular division of trigeminal nerve's buccal nerve innervates cheek.

Medial and lateral pterygoid muscles are controlled by second and third divisions of trigeminal nerve's motor components. [14]

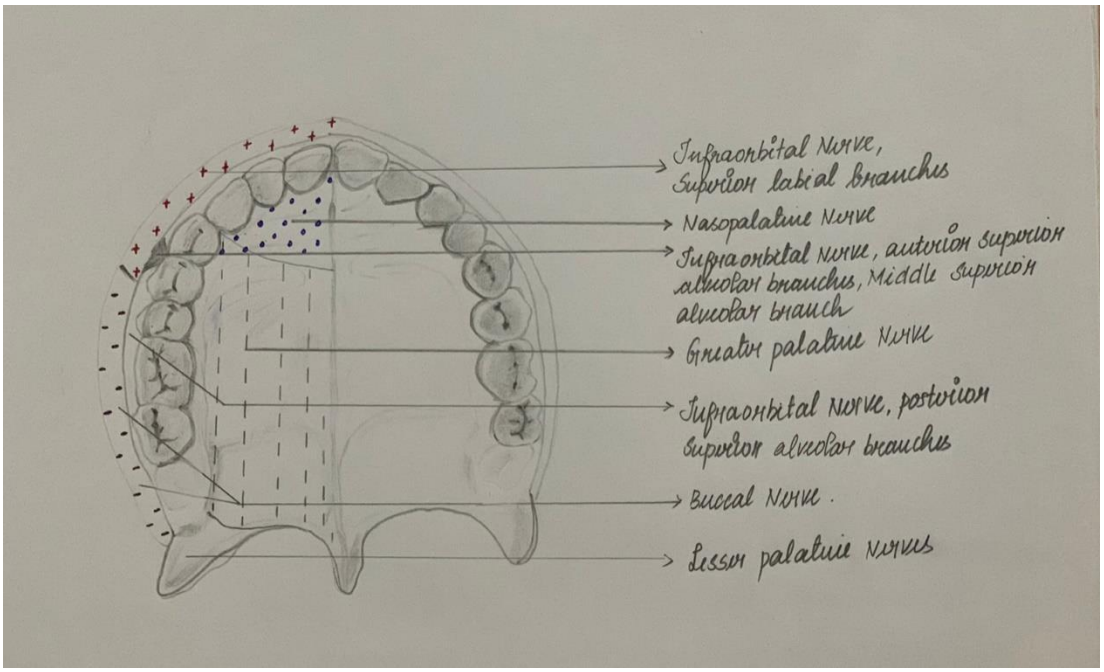

FIGURE 3: INNERVATION OF THE ORAL CAVITY

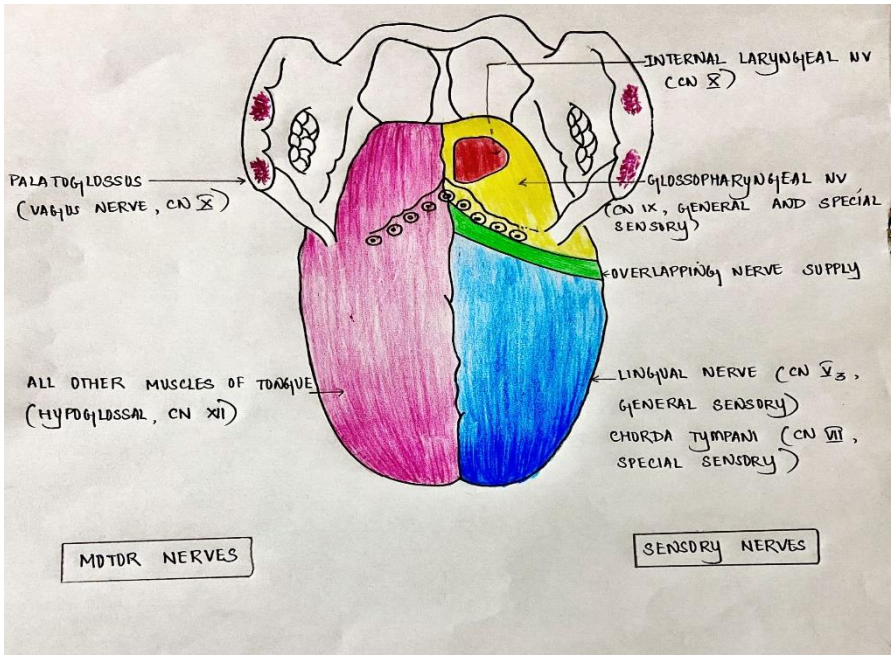

FIGURE 4: INNERVATION OF THE TONGUE

3.5.1 HISTORY OF LYMPHATIC SYSTEM

The earliest account of lymphatic systems was written by Italian anatomy and surgery professor Gaspero Aselli in 1662. An accurate account of the anatomy and physiology of lymphatics was published in a book in London in 1786 by W. Hunter, W. Cruickshank, and W. Hewson.

Additionally, Sappey had a thorough anatomical insight of the lymphatic system, and his representations of lymphatic flow are still in use today. At the time, Virchow and other experts contended that lymph nodes prevented development of cancer and that disease went from a primary tumour to local lymph nodes before spreading to systemic regions. Extreme surgical procedures like Crile's radical neck dissection were developed in reaction to this supposition. **(15)**

3.5.2 DEVELOPMENT OF LYMPHATIC SYSTEM

The emergence of structures known as lymph sacs, which are closely associated to veins, is the first indication of a lymphatic system in intrauterine life. The first to develop are : two jugular lymph sacs. One cisterna chyli, two posterior lymph sacs, and one retroperitoneal lymph sac are also present.

According to Sabin (1916), lymph sacs form as an extension of vein endothelium, and lymph vessels sprout in a radial pattern, with initial connections to veins are lost. **(16)**

According to Huntington (1911) and McClure (1915), all lymph vessels begin as clefts in the mesenchyme, much like blood vessels. Lymph nodes form from the aggregation of cells in mesenchymal strands surrounded by a lymph vascular plexus. Around each nodule vessels are transformed to lymph sinus.

3.5.3 LEVELS OF LYMPH NODES

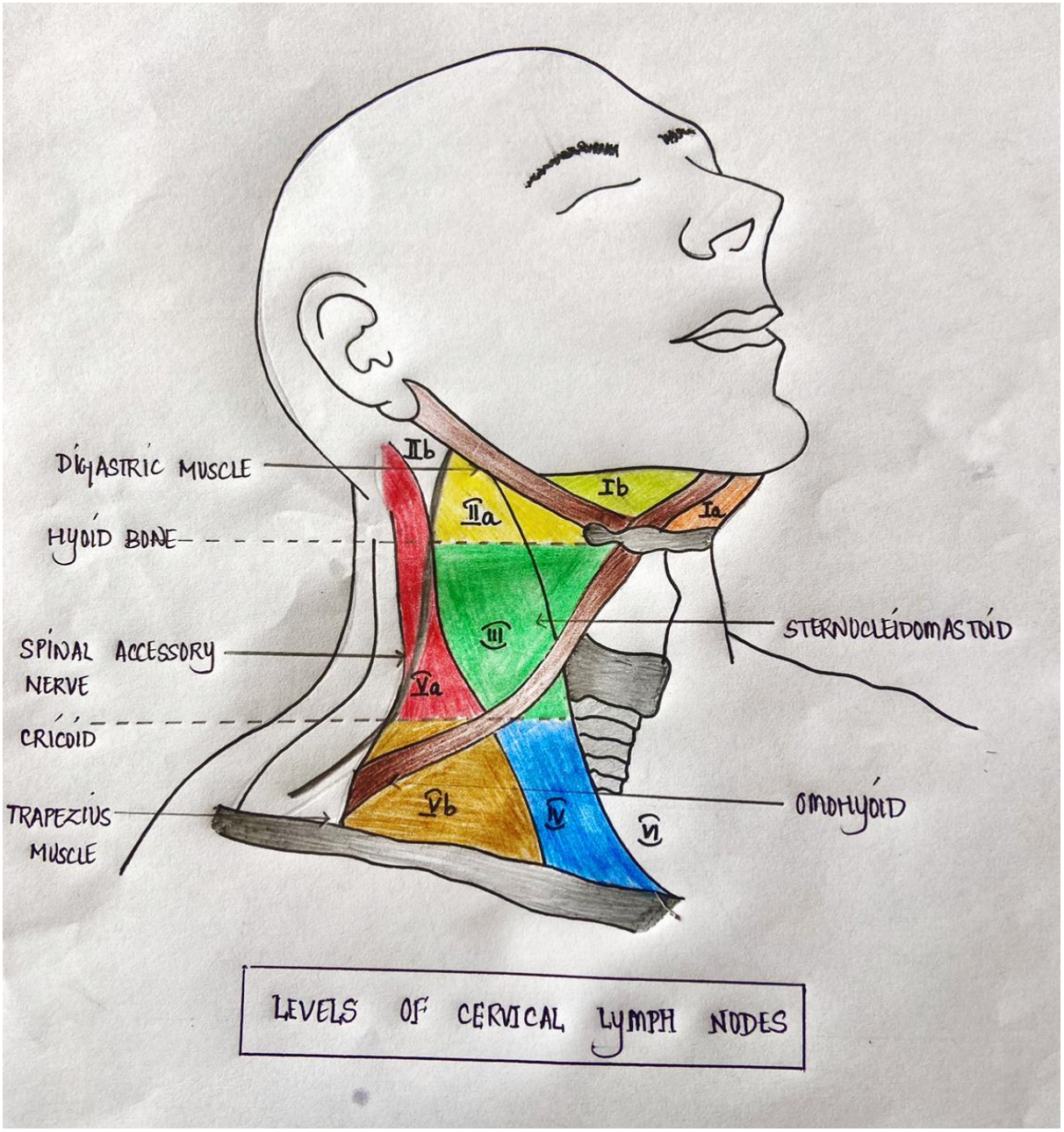

FIGURE 5: REPRESENTATION OF LEVELS OF NECK NODES

LEVEL OF CERVICAL LYMPH NODE	
LEVEL IA	Submental
LEVEL IB	Submandibular
LEVEL II	Upper Internal Jugular/ Deep Cervical Chain
LEVEL III	Middle Internal Jugular/ Deep Cervical Chain
LEVEL IV	Lower Internal Jugular/ Deep Cervical Chain
LEVEL V	Posterior Triangle Group
LEVEL VI	Anterior/ Central Compartment Group
LEVEL VII	Upper Mediastinal Group

TABLE 1: LEVELS OF NECK NODES

3.6 ORAL CAVITY CANCER

3.6.1 EPIDEMIOLOGY

As history shows, man has long attempted to combat malignant conditions. However, it is still a dominant contributor of death and morbidity. Every year, around nine million new cancer cases are diagnosed worldwide.

India, Sri Lanka, Pakistan, Bangladesh, Hungary, and France have the highest rates, with India accounting for 30% of newly discovered cases. **(17)**

Every year, over seven lakh new malignancies are diagnosed in India, and approximately 3.5 lakh people die as a result of cancer. **(18)**

According to the Kidwai Memorial Institute of Oncology's cancer registry in Bangalore, Karnataka, around 5000 new malignancies are recorded each year. One of the top three malignancies in India is oral cancer. In India, oral cancers make up more than 30% of all cancer cases, with age-adjusted incidence rates of 20 per 100,000 individuals.**(19)** In Western culture,

the oral cavity is more in tongue and floor of mouth. In contrast, the most often reported primary sites in India are buccal mucosa and lower alveolus. [16]

Buccal mucosal carcinoma accounts for 40% of oral malignancies in South East Asia. [14] Except in underdeveloped nations, where start might be early owing to tobacco and pan chewing practices, 85% of cases occur after the age of 50. Floor of mouth cancer, which makes up 18-33% of oral cancers and is more prevalent in males in their sixth and seventh decades,22-39% of oral carcinomas develop on the tongue, most typically in the middle third and lateral side. [14]

Retromolar trigone incidence in oral malignancies ranges from 6 to 7% and is more in men. The incidence of carcinoma in the upper alveolus is 3.5 - 6.5%, and that in the hard palate is 1 - 3%. Oral cancers are more common in men, with the exception of hard palate carcinomas, which have a higher female prevalence due to reverse smoking in some areas. Lower alveolar tumour account for 7.5 to 17.5% of all oral malignancies. [14]

However, in the Kolar region, the most common malignancy is carcinoma of the buccal mucosa. [20] It is more common in women due to the addiction to tobacco quid chewing. In India, patients appear in advanced stages, involving the lower gingivobuccal complex, making it difficult to determine epi-center or origin site of tumour. Such tumour involving buccal mucosa and lower alveolar complex have been termed as "Indian oral cancer" and are high volume disease.

3.6.2 ETIOLOGY

The cause of oral cancer is not fully understood. A range of risk factors have been identified.

- **SMOKING:**

Since tobacco smoking causes millions of cancer deaths each year, it continues to pose the largest substantial cancer risk. C onnection between smoking and mouth cancer has been conclusively demonstrated by epidemiological research. The most significant carcinogens in tobacco smoke are the aromatic hydrocarbon benz-pyrene and the tobacco-specific nitrosamines (TSNs), 4-(nitrosomethylamino)-1-(3-pyridyl)-1-butanone (NNK) and N'-nitrosonornicotine (NNN). By forming a covalent bond deoxyribonucleic acid (DNA) of keratinocyte stem cells, NNK, NNN, and their metabolites produce DNA adducts. Important

mutations in DNA replication are brought on by these adducts. These carcinogens are processed by glutathione-S-transferase (GST) and oxygenation by P450 enzymes in cytochromes . The genetic predisposition to tobacco-induced cancer is likely to be significantly influenced by hereditary differences in the genes encoding these enzymes. **(23)**

Tobacco is more widely smoked in the form of cigarette and bidi. Some people smoke chuttas (cigars) with the burning tip inside their mouths. Chemical carcinogens in cigarette smoke or repetitive thermal injury are risk factors for oral cancer. The risk rises with the amount smoked and the total lifetime smoking years. Tobacco is widely smoked in the form of bidi, a type of low-cost cigarette formed by rolling a rectangle dried tendu leaf piece (Diospyros melanoxylon). The length ranges from 4 to 7.5 centimeters. Bidi smoke contains more hazardous toxins than cigarette smoke, including carbon monoxide, ammonia, hydrogen cyanide, phenol, and carcinogenic compounds.

Other tobacco smoking methods include clove-flavored cigarettes, diverse forms of pipes (wooden, clay, and metal), hookahs (the Hubble bubble or water pipe), cheroots (or chuttas), and dhumtis. Tobacco can be used raw, in processed blends, or as pyrolized forms. The raw forms are combined with lime and areca nut (Mawa-smokeless tobacco).

A quid of Khaini is a blend of freshly powdered tobacco and slaked lime. It is swallowed and held for hours in the lower gingivolabial sulcus, which remains risk for khaini cancer (squamous cell carcinoma -lower lip). Tobacco in its processed forms, such as zarda, gutkha, and Manipuri tobacco, is an industrial product. Tobacco that has been pyrolized (roasted) (mishri, bajjar, etc.) is used as a dentifrice. Snuff is also consumed orally in some regions, causes histone hyperacetylation and hypomethylation, which disarms tumour suppressor genes. **(21)**

FIGURE 6: TOBACCO AND ITS DIFFERENT FORMS

- **SPIRITS:**

Intake of calvados, a pot-distilled spirit.

- **SEPSIS:**

Infected and decaying teeth.

- **SHARP TEETH:**

Result of poor oral hygiene, imperfect restorations, and ill-fitting dentures.

- **SPICES**

- **SYPHILIS**

- **HABIT OF CHEWING BETEL QUID:**

The quid is made with a betel leaf wrapped around an areca nut that is strong in tannin, quick lime, and tobacco. Oral cancer develops in the area where quid is commonly maintained. Smoking and chewing betel quid increase risk of mouth cancer by 20- 30 times. In our area, this is the most prevalent risk factor for oral cancer.

FIGURE 7: BETEL LEAVES COATED WITH SLAKED LIME AND ARECA NUT

- **ALCOHOL**

Alcohol use has been linked to the emergence of oral cancer, and this association is dose-dependent. The risk of acquiring mouth cancer has been shown to increase when alcohol and cigarettes are combined. Drinking alcohol suppresses the immune system on a systemic level while also having a cumulative local effect that disperses the carcinogen in the mouth's basement region. It has been shown that alcohol increases the permeability of the oral mucosa, leading to morphological alterations such epithelial atrophy, which makes the oral mucosa more permeable to carcinogens. **(23)** Alcoholics typically lack in some micronutrients. It's also known that alcohol makes histones hypermethylated. **(14)**

It has been established that alcoholic beverages include elements that may cause human cancer. Examples include N-nitroso compounds, mycotoxins, urethane, inorganic arsenic, and others. Most significant alcohol metabolite is acetaldehyde, which is mostly converted by enzyme alcohol dehydrogenase (ADH). Aldehyde dehydrogenase(ALDH) then transforms acetaldehyde into acetate . DNA damage is brought on by acetaldehyde in cultured mammalian cells. It interferes with DNA synthesis and repair. Additionally, it alters certain genes and switches sister chromatids. The enzyme 6-methylguanitransferase, which is control of fixing damage brought on by alkylating agents, is inhibited by acetaldehyde.**(23)**

ADH enzyme activity that is elevated in the oral flora and mucosa might lead to acetaldehyde accumulation. Oral cancer is more common in those with ADH type-3 genotypes, which facilitate alcohol's quick acetaldehyde oxidation. A reduction in ALDH enzyme may also

contribute to acetaldehyde accumulation. Alcohol-related cancers are more likely to develop when there are genetic differences in these two enzymes, ADH and ALDH.. [23]

- **VIRUSES:**

The human papilloma virus (subtype 16) and the herpes simplex virus have both been linked to an increase in the incidence of oral cavity squamous cell carcinoma. These serve as risk factors for those who have several oral sex partners and are often between the ages of 30 and 40.

- **IMMUNE STATUS:**

Low cell-mediated immunity as a result of immunological weakness - Those with weaker immune systems, especially those who have HIV or have undergone organ transplants, are more prone to acquire oral cancer.

- **GENETIC:**

A multi-step process of accumulating genetic alterations results in majority of spontaneous cancers. A activity of epithelial cells is impacted by the loss of chromosomal heterozygosity. This starts a chain of occurrences that ultimately results in severe squamous cell carcinoma. Clinical and microscopic pathology of tumour exhibits related genetic alterations, which range from hyperplasia to invasiveness. Overexpression of mutant p53, p16, and other tumour suppressor genes may make cancer more likely to spread and reccur post treatment. C-erbB-2 overexpression has been associated with nodal disease, metastasis, and reduced survival. Oral cavity caners have been connected to mutagenic sensitivity diseases as Xeroderma pigmentosum, Fanconi's anaemia, and Ataxia telangiectasia. [22]

- **SOCIAL STATUS:**

Due to the increasing exposure to tobacco and its forms, OSCC has been linked to low socioeconomic societal strata.

- **DIET AND NUTRITION:**

Oral cancer risk has been associated with processed food. There is strong evidence that some micronutrients lower risk of oral cancer. Among these are : vitamins A (retinol), C (AA), and E (-tocopherol), as well as potassium, selenium, and carotenoids (-carotene). Antioxidants are essential for reducing free radical reactions that can lead to DNA mutations, changes in enzyme

function, and lipid peroxidation of cellular membranes. These antioxidants include -carotene, retinol, retinoids, vitamin C (AA), and vitamin E (-tocopherol). [23]

- **OCCUPATION:**

It is known that occupational risks, notably prolonged exposure to ultraviolet (UV) light and solar radiation, induce lip cancer. Additionally, UV rays can result in actinic cheilitis, which can develop into OSCCs. The use of fossil fuels, asbestos, sulphur dioxide, pesticides, and mists from strong inorganic acids have all been connected to cancers of the posterior mouth, pharynx, and larynx. [23]

- **DENTAL FACTORS:**

Poor oral hygiene, poor dental health (sharp/fractured teeth due to caries/trauma), and chronic ulceration from an ill-fitting denture have all been linked to promoting neoplasia in the context of other risk factors. This is due to existence of coexisting risk factors including alcoholism and smoking. However, a hamster experiment showed that persistent stress might increase tumour growth in addition to carcinogen exposure. [23]

- **SYPHILIS:**

Tertiary syphilis, along with other risk factors such as tobacco and alcohol, has been linked to development of oral cancer.

3.6.3 PREMALIGNANT CONDITIONS:

Premalignant Condition – a tissue with morphological changes where the risk of cancer is higher than in its more normal counterparts.

A) LEUKOPLAKIA:

"It is defined as a clinical white patch in the oral mucosa that cannot be clinically or pathologically identified as any other disease and cannot be scraped out. Malignant transformation rates range from less than 1% to 17.5%."[24]

ORAL LEUKOPLAKIA TYPES:

"As per Sugar L and Banoczy J, following types of Oral Leukoplakia exists:

* **Leukoplakia Simplex:** A white, homogenous keratinized lesion that exhibits lowest incidence of cancer.

* **Leukoplakia Verrucosa:** A white, verrucous lesion with a wrinkled surface exhibiting highest rate of incidence with cancer.

* **Leukoplakia Erosiva:** A white, erythematous lesion with erosions and fissures exhibiting highest rate of correlation with cancer."

"Lindberg states the following Clinical Types of Oral Leukoplakia:

* **Homogeneous:** A white patch with a varied look that might be smooth or wrinkled; smooth areas with small fissures. It has the lowest rate of malignancy.

* **Speckled or Nodular:** White patches with erythematous base or nodular excrescences. It shows highest rate of association with carcinoma."

"As per Burkhardt, based on microscopic appearance, Oral Leukoplakia can be of following types:

* **Plain Form:** Clinically comparable to leukoplakia simplex

* **Papillary Endophytic:** Erosive leukoplakia.

* **Exophytic Papillomatous Papilloma:** Clinically comparable to verrucous leukoplakia.

* **Proliferative Verrucous Leukoplakia:** It is an aggressive type of leukoplakia. It has a proclivity to be widespread or multifocal. This type of leukoplakia develops into verrucous cancer. They are linked to an increased risk of malignant transformation and dysplasia."**(24)**

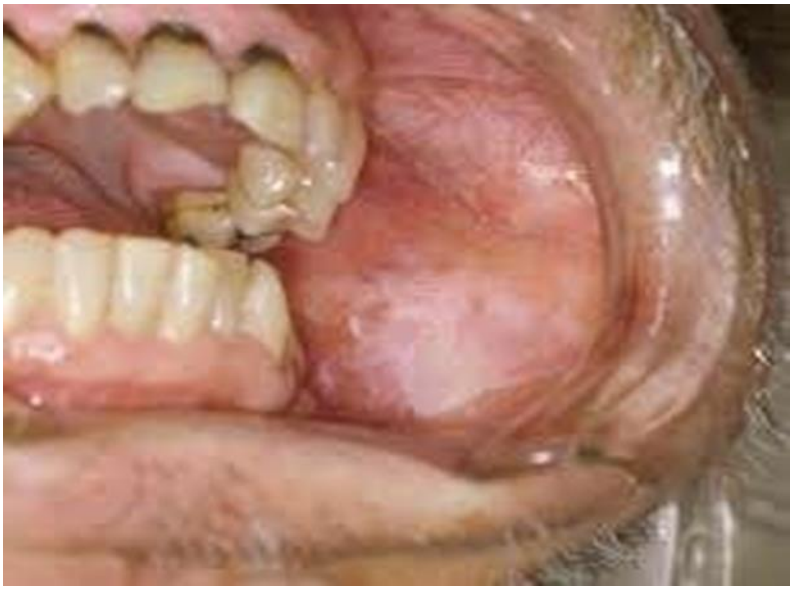

FIGURE 8: LEUKOPLAKIA OVER LEFT BUCCAL MUCOSA

B) ERYTHROPLAKIA:

These are red, velvety plaques on the oral mucosa that cannot be attributed to any other pre-existing disorder clinically or pathologically. Approximately 30-40% of erythroplakia has malignancy or severe epithelial dysplasia.

FIGURE 9: ERYTHROPLAKIA OVER LEFT BUCCAL MUCOSA

C) MELANOPLAKIA

D) ORAL SUBMUCOUS FIBROSIS:

Oral submucous fibrosis is a chronic, complex, premalignant (1% transformation risk) condition of the oral cavity, characterized by juxta-epithelial inflammatory reaction and progressive fibrosis of the submucosal tissues (the lamina propria and deeper connective tissues). As the disease progresses, the oral mucosa becomes fibrotic to the point that the person is unable to open the mouth.

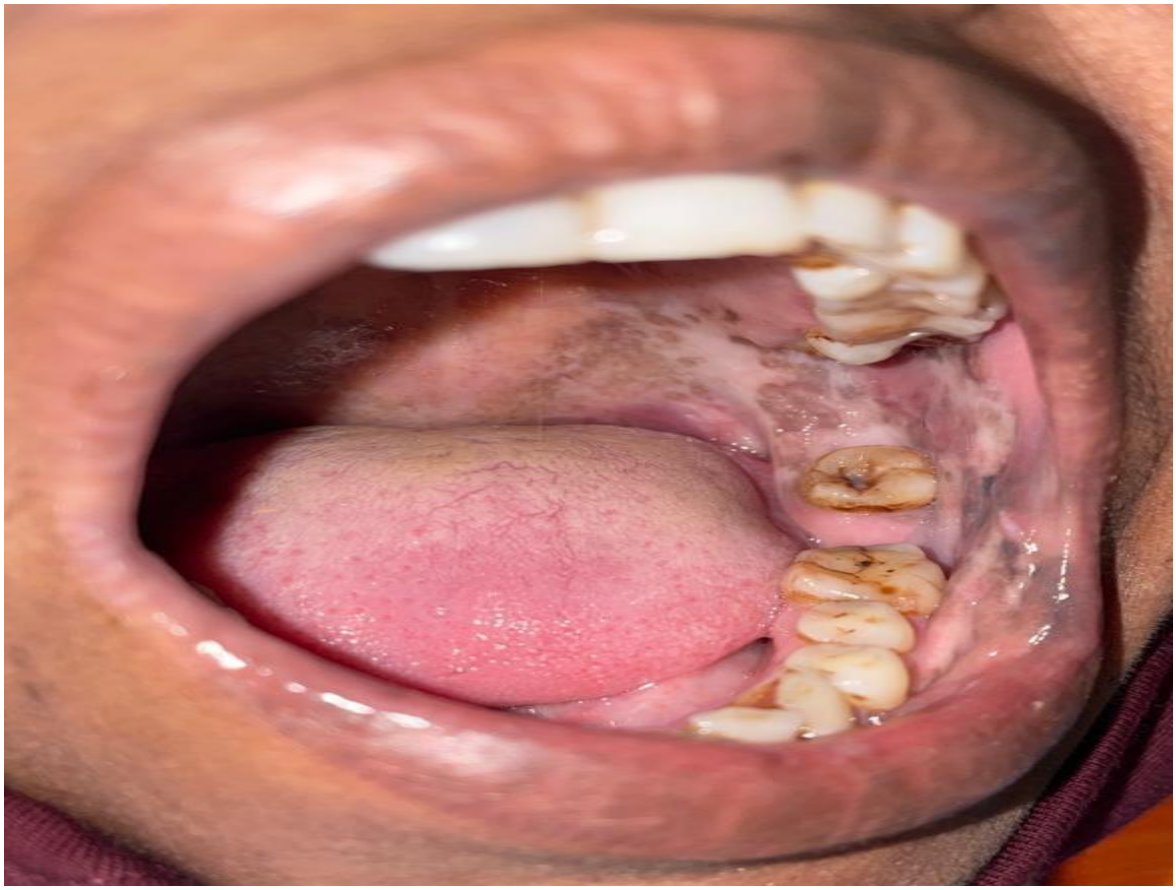

FIGURE 10: ORAL SUBMUCOUS FIBROSIS

E) SIDEROPENIC DYSPHAGIA

F) ORAL LICHEN PLANUS:

It is a chronic T-cell mediated inflammatory disease that affects the oral mucosa. It is characterized by periods of symptomatic exacerbation and remission. Rate of malignant transformation is about 4%. [25]

G) DISCOID LUPUS ERYTHEMATOSUS

H) HYPERKERATOSIS

I) DYSKERATOSIS CONGENITAL

J) SYPHILIS

3.6.4 TUMOR DEVELOPMENT

- TUMOUR BIOLOGY [21]

Tumour development involves three phases:

A) Initiation

b) Promotion

c) Progression

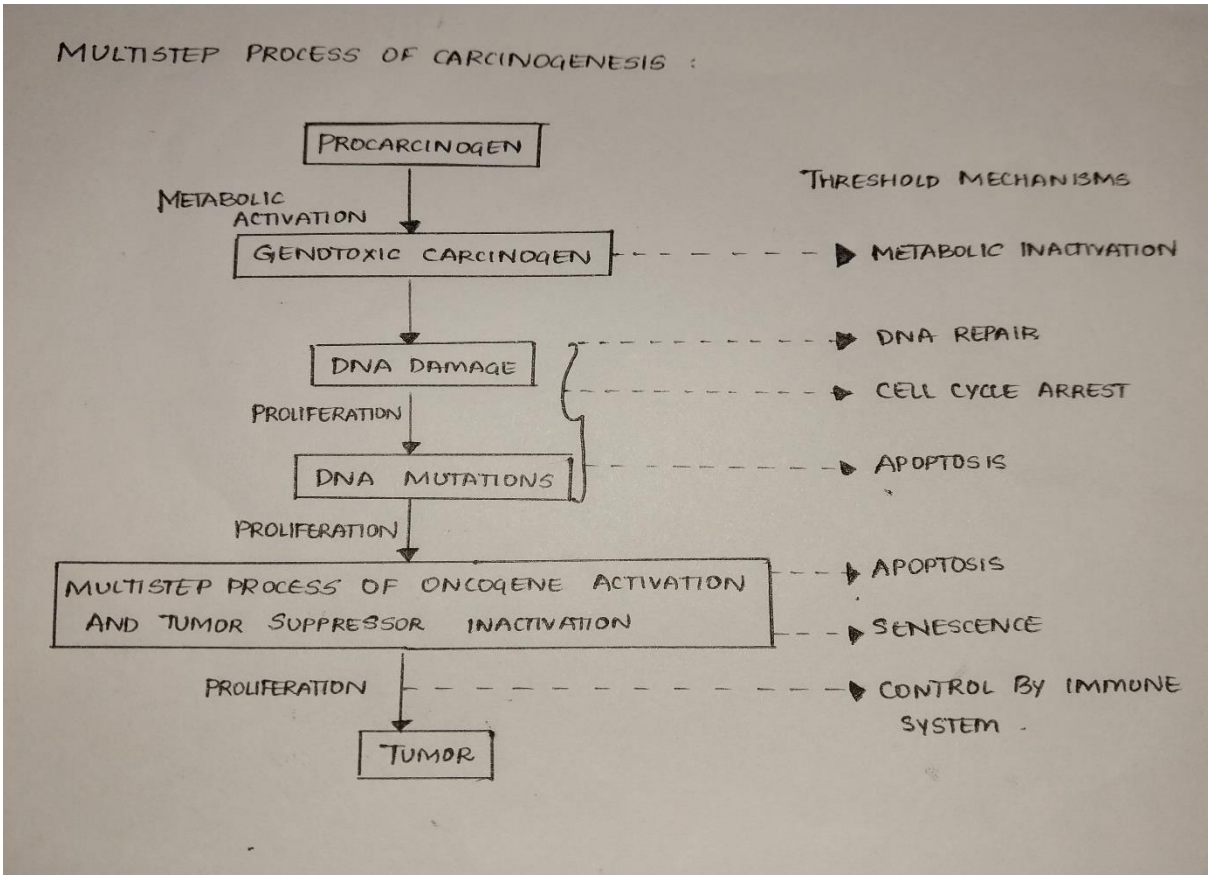

FIGURE 11: FLOWCHART DEMONSTRATING MULTISTEP PROCESS OF CARCINOGENESIS

- **TUMOUR ESCAPE MECHANISMS:** [21]

- Tumour related:
- Tumour is not immunosensitive
- Tumour is not immunogenic

- Host related:
- The tumour grows too quickly for the immune system.
- Immunodeficiency - inherited or acquired
- Immune suppression caused by carcinogens
- Deficiency in antigen presentation by antigen-presenting cells
- Inability of effector cells to reach the tumour
- Expression of immunodominant antigens on the parental tumour that precludes stimulation with additional tumour antigens.

- **CARCINOGENESIS** [21]

Tumor growth is triggered by the absence of normal signaling pathways involved in regulated cell proliferation. The inability of cancer cells to perform apoptosis (programmed cell death) allows for the aggregation and clonal proliferation of cells that would have died if their cell death machinery had been preserved and functional. Tumor growth is the result of cell multiplication minus cell death. Carcinogenesis involves DNA damage and the initiation and propagation of mutant cells through the cell cycle. Overexpression of mitogenic sensors, loss of tumour suppressor proteins, expression of oncogene-derived proteins that impede apoptosis, and overexpression of proteins that derive the cell cycle all contribute to uncontrolled cell division. [26] Tumor growth symbolizes the loss of normal genetic mutations caused by DNA damage, particularly in the 9p, 3p, 11q, 8p, and 17p regions. Smokers have a increased rate of p53 and p16 mutation, which contributes to oral cancer and has a high recurrence rate following treatment.

- **TUMOR THICKNESS AND DEPTH OF INVASION**

"Tumor thickness is defined as the vertical extension of the tumour from the point of maximum projection to the point of maximum infiltration in a perpendicular manner." Breslow was first to discover a strong connection between tumour thickness (TT) and both tumor-free survival and metastasis in individuals with cutaneous melanoma. Following Breslow's concept, other scientists found a link between lymph-node involvement and tumour thickness and oral cavity malignancy. **(27)** Many research has since been conducted to test this association. These studies have revealed that tumour thickness is a major predictor of lymph-node involvement in OSCC. Many writers have also discovered that the thickness of the tumour correlates with survival and lymph node involvement better than its surface diameter. **(27)**

However, further studies showed that the tumor's exophytic development should not be taken into account because it does not denote the removal of tissue resistance. The ulcerated tumor's space is crucial because it represents tissue that the tumor's downward progression has damaged. Tumor depth was consequently proposed as a more accurate predictive predictor for lymph node metastasis. "Tumor depth is defined as the portion of the tumour that extends below the mucosal basement membrane."**(28)**

Breslow created a technique for evaluating the depth of invasion, which he measured vertically from the tumour surface to the base of the ulcer. A line is drawn from this plane to the deepest point of tumour invasion for histological evaluation. **(29)**

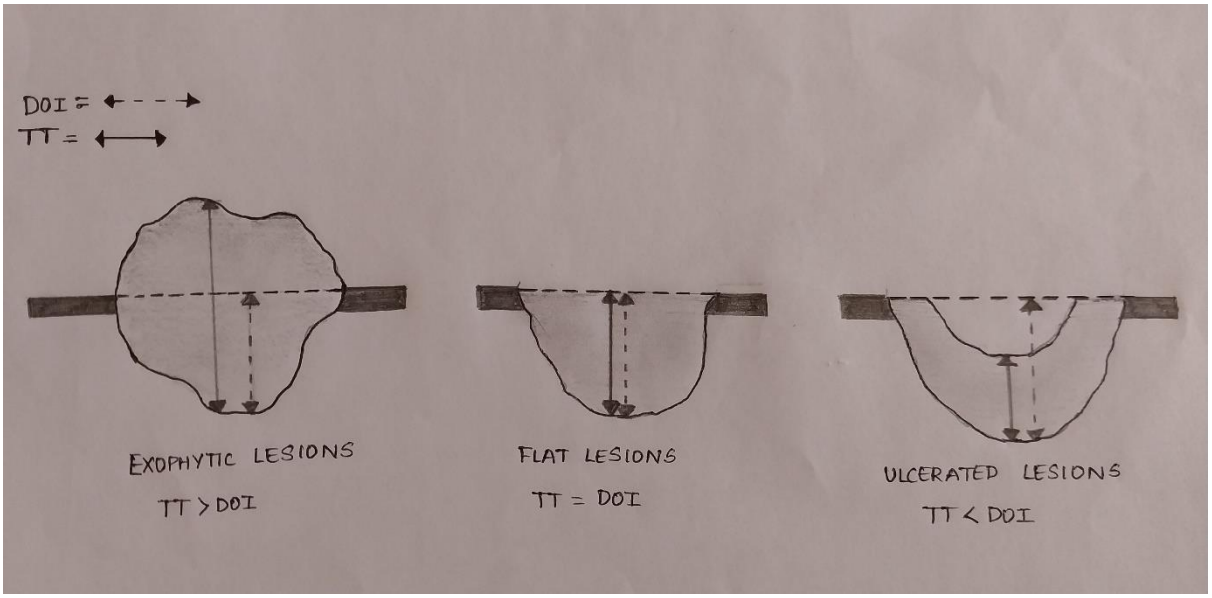

FIGURE 12: EVALUATION OF DOI AS PER BRESLOW

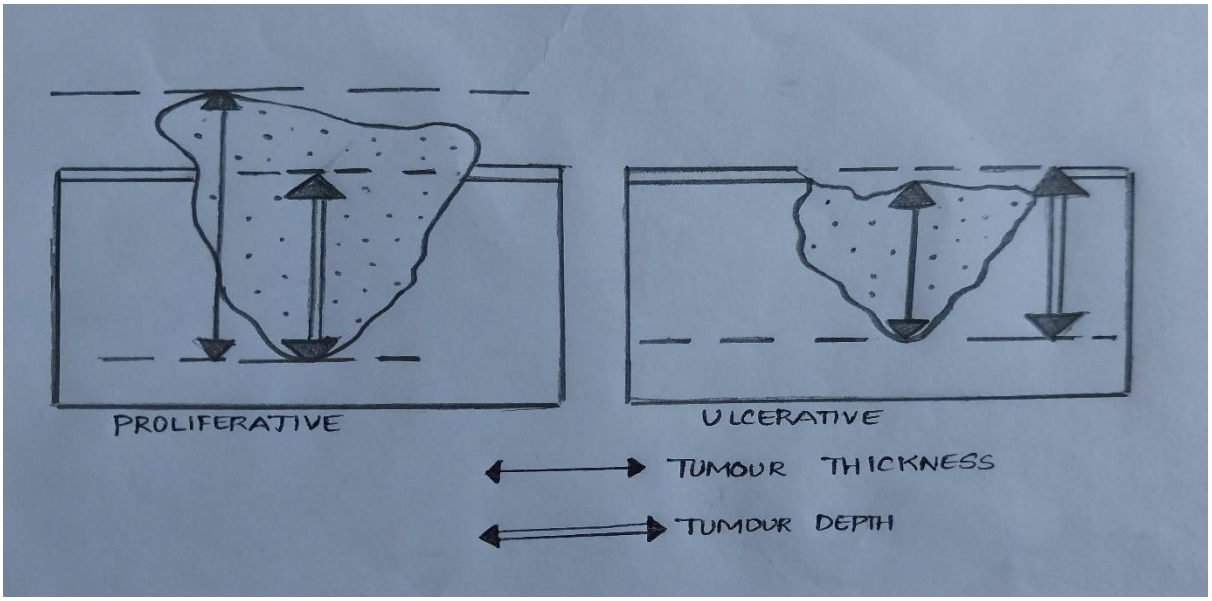

FIGURE 13: DETERMINING THE DEPTH OF INVASION IN RESECTED SPECIMEN

In oral tongue cancer, primary tumour thickness and depth of invasion have been utilized to predict lymph node metastasis. The depth of tumour invasion is thought to be an independent predictor of cervical lymph node metastasis. "**The depth of infiltration** was defined as the maximal depth of tumour infiltration (in millimeters) below the mucosal Basement Membrane. The restored mucosal surface was used in the case of ulcerated or exophytic tumors."[30]

In a meta-analysis of over 50 studies, Pentenero et al. found that depth of invasion was a better predictor of cervical metastases and overall prognosis than tumour thickness. [31] **Depth of invasion is a stronger predictor of nodal status**. Furthermore, investigations have revealed that tumors positioned closer to the midline (i.e., lower alveolus, floor of mouth, and tongue) have a higher proclivity to show cervical metastasis. [32]

According to the literature, **cervical lymph node metastasis is the single most important prognostic factor in the therapy of patients with oral cavity squamous cell carcinoma**, and parameters such as initial site and depth of invasion of tumour are the strongest predictors of nodal metastasis.

These patients are at a higher risk of locoregional recurrences necessitating adjuvant therapy. Because adjuvant therapy might have significant toxic effects, it is critical to develop a reliable strategy for identifying such high-risk individuals promptly after surgery. It takes 1011 cells to create a perceptible mass. Because of the possibility of concealed nodal metastasis,

preventative or elective surgical neck dissection is performed in oral cancer patients, particularly those containing areas that tend to metastasize early.

Patients with head and neck carcinomas have a much worse prognosis when they have cervical lymph node metastases. Metastasis to lymph nodes lowers survival by about 50%. Squamous cell carcinoma frequently spreads lymphatically, and concealed metastases can develop in people who have no palpable lymph nodes. Early-stage cancers have been shown to have occult lymph-node metastasis between 27% and 40% of the time (primary site T-categorization T1 or T2). Lymph nodes will be periodically sectioned step-by-step to help find micro-metastases. **(33- 34)**

Level I (100%) is the most common location for nodal metastases from oral cancers, followed by levels II (32%), III (16%), and IV (8%). (16) The prognosis is often poor despite availability of multimodal treatment intervention. More frequently than any other oral cavity malignancy is buccal carcinoma, followed by hidden lymph node metastases of the oral tongue. **(35)** Despite the fact that the distribution of tumour stage remained similar from the prior 10-year period, the literature shows a general 5-year survival rate of 65%. **(36)** Survival was linked to more vigorous neck treatment, even in the earliest stages of the tumour, and adjuvant radiation in later stages of the tumour. Extra capsular distribution diminishes the likelihood of cure by 50%. As previously stated, the location, size, tumour differentiation, perineural invasion, perivascular invasion, inflammatory response, and DNA content all predict the aggressiveness of cervical lymph node metastasis. **(32)**

The most current AJCC staging recommendations use N3b staging because to extra capsular dissemination from lymph nodes. There are several retrospective studies that link the cervical lymph node's initial location and degree of invasion of oral cavity squamous cell carcinoma to prognosis . There is, however, a dearth of information on the associations between oral cancer tumour volume and lymph node metastasis and recurrence.

- **REGIONAL LYMPH NODES**

Lymph node involvement by metastatic deposits is invariably associated with a less favorable prognosis, typically 50% worse than for patients who have similar tumors without lymph node involvement.

- **PATTERN OF CERVICAL NODE METASTASIS**

The ability to spread metastatically is the single most significant property of a malignant tumour. Initial step in the metastatic process is breakthrough of basement membrane at primary tumour location. This is accomplished by hydrolytic enzymes released by tumors, such as urokinase type plasminogen activator, collagenase, and stereomelysins. [16] The enzymes destroy basement membrane proteins like collagen IV, laminin, and proteoglycans, allowing tumour cells to spread. [38]

Presence of extracapsular spread reduces chance of cure by 50%.[37]The primary mechanism of dissemination beyond the primary site of genesis for squamous cell carcinoma of head and neck region is lymphatic spread. Tumor cells traverse the lymphatic system as emboli. Tumor emboli are transported to first level lymph nodes through afferent lymphatic arteries. The tumour cells then lodge in the subcapsular sinus prior migrating to the cortex and medulla. The tumour eventually invades the node capsule, signaling the onset of extracapsular dissemination. [38] Extracapsular spread can occur in much smaller lymph nodes if tumour emboli first lodge in the capsular lymphatic sinuses and there is focal degradation of capsular collagen by type I collagenase.

As metastatic tumour replaces the first level of lymph nodes, afferent lymph flow is diverted, delivering tumour cells to the second and third level of nodes. Increased blockage in the lymphatics and intranodal sinuses may eventually result in lymphatic flow reversal and retrograde dissemination of tumour cells to unknown nodal groups.

Tumor cells infiltrating blood vessels within the lymph node or penetrating minor lymphatic-venous communication can result in lympho-hematogenous dissemination. When tumour cells reach the draining lymph node, they can multiply, die, stay dormant, or enter the bloodstream via blood arteries in the node. The lymphatic distribution pattern is consistent. In general, well-localized tumors disseminate to the ipsilateral first or second echelon lymph nodes. Patients who have clinically positive lymph nodes in ipsilateral neck are at risk of having metastases to contralateral lymph nodes. This shunting takes place mostly via anastomotic channels that decussate in the midline at submental and submandibular triangles.

The Lindberg research highlighted the nodal groups most at risk for each primary, and the pattern of subclinical microscopic metastasis is comparable· [39] Carcinoma situated in the anterior oral cavity most usually spreads to submental and submandibular lymph nodes, followed by the upper jugular nodes. The oral cancer situated posteriorly is more likely to

extend to the upper jugular nodes and less commonly to the submandibular nodes. Shah presented a detailed histological analysis that confirmed Lindberg's clinical findings. [40] The levels I, II, and III had highest risk of oral cavity cancer metastasis. Thus, first tier of lymph nodes for the oral cavity is located at level I, specifically level Ib (sub-mandibular) for buccal mucosa and the lower alveolar complex.

The proportional risk of nodal metastasis is determined by the primary tumor's position, size, thickness, histological features, and immunological and biological components. [38] The lesser the differentiation, the more probable the tumour would metastasize rapidly. Tumors with infiltrative margins are more likely to metastasize than those with pushing margins.

The table below describes the lymph node levels as well as the nodes that are most likely to harbor metastases from various primary locations. [41]

- **SKIP METASTASIS**

Oral cavity cancer consistently metastasizes to the cervical LNs, depending on the original sites or subsites. Cancers of the oral cavity and tongue are likely to progress to stages I and ll, respectively. However, the term "Skip metastasis" (SM) or "Nodal skip metastasis" is used if the metastasis skips any regional LN and exhibits dysplastic features in the peripheral LN (NSM). NSM is defined as the presence of infiltrating LNs far from the primary tumour but without involvement of nodes close to the primary site. Due to the unrestricted communication between the two sides, some studies have shown that the SM is a more prevalent feature in oral cancers, notably SCC of the tongue and floor of mouth. In the course of normal chewing and swallowing, tongue is stimulated, which can encourage both preliminary and broad lymphatic diffusion.

- **DISTANT METASTASIS**

Distant metastasis is an uncommon clinical manifestation that affects less than 10% of individuals. Lungs are most commonly affected by distant metastases, with skeletal and liver metastases occurring less frequently. Metastases to mediastinal lymph nodes are classified as distant metastases.

TABLE 2: LYMPH NODE LEVELS AT RISK OF HARBORING METASTASIS FROM DIFFERENT PRIMARY

LYMPH NODE GROUP	PRIMARY SITE FOR METASTASIS
LEVEL IA	Floor of mouth, Anterior 2/3 tongue, Anterior part of mandibular ridge, Lower lip.
LEVEL IB	Oral cavity, Anterior nasal cavity, Soft tissue of the mid face, SubmandibularGland.
LEVEL II	Oral cavity, Anterior Nasal cavity, Nasopharynx, Oropharynx, Hypo pharynx, Supra-glottic larynx, Parotid.
LEVEL III	Oral cavity especially tongue, Nasopharynx, Oropharynx, Hypo pharynx,Supra- glottic Larynx, Thyroid
LEVEL IV	Hypopharynx, Thyroid, Larynx, Cervical Esophagus.
LEVEL V	Nasopharynx, Oropharynx, Cutaneous structures of the posterior scalp and Neck
LEVEL VI	Thyroid gland, Glottic and subglottic Larynx, Apex of Pyriform fossa,Cervical Esophagus.

3.6.5 TNM CLASSIFICATION

TNM (tumor, node, metastasis) is a universal staging system developed by American Joint Committee on Cancer in collaboration with Union International Contre de Cancer that aids in the assessment and evaluation of cancer and its subsequent treatment globally. This staging system assesses the degree of disease and outlines a conventional strategy for cancer management, as well as the disease's prognosis. The revised eighth edition of AJCC staging went into effect on January 1, 2018. [6]

TABLE 3: TNM STAGING OF ORAL SQUAMOUS CELL CARCINOMA - AJCC 8th EDITION

Primary tumour (T)

- **TX**: primary tumour cannot be assessed
- **Tis**: carcinoma in situ
- **T1**: tumour ≤2 cm in greatest dimension with depth of invasion (DOI) ≤5 mm
- **T2**
  - tumour ≤2 cm with DOI >5 mm and ≤10 mm, or
  - tumour >2 cm and ≤4 cm with DOI ≤10 mm
- **T3**
  - any tumour with DOI >10 mm, or
  - tumour >4 cm with DOI ≤10 mm
- **T4**: moderately or very advanced
  - **T4a**: moderately advanced local disease:
    - tumour >4 cm with DOI >10 mm, or
    - tumour invades adjacent structures (e.g. through cortical bone of mandible or maxilla, into the maxillary sinus, into the skin of face)*
  - **T4b**: very advanced local disease:
    - tumour invades masticator space, pterygoid plates, or skull base, and/or
    - tumour encases the internal carotid artery

*Superficial erosion alone of bone/tooth socket by gingival primary is not sufficient to classify as T4

Pathologic nodal status (pN)

Pathologic criteria apply for patients treated surgically, with cervical lymph node dissection, for whom multiple whole lymph nodes are available for microscopic evaluation.

- **NX**: nodes cannot be assessed
- **N0**: no regional node metastases
- **N1**: metastasis in single ipsilateral node, ≤3 cm, and no extranodal extension (ENE(−))
- **N2**
  - **N2a**: metastasis in single ipsilateral node, >3 cm and ≤6 cm, and ENE(−); or metastasis in single ipsilateral node, ≤3 cm, and ENE(+)
  - **N2b**: metastasis in multiple ipsilateral nodes, all ≤6 cm, and ENE(−)
  - **N2c**: metastasis in bilateral or contralateral nodes, all ≤6 cm, and ENE(−)
- **N3**
  - **N3a**: metastasis in a node, >6 cm, and ENE(−)
  - **N3b**: metastasis in single ipsilateral node, >3 cm, and ENE(+); or multiple ipsilateral, contralateral, or bilateral nodes any with ENE(+); or single contralateral node of any size and ENE(+)

Distant metastases (M)

The terms pM0 and MX are not valid TNM categories. The following categories may be used:

- **cM0**: no evidence of metastases
- **cM1**: distant metastasis
- **pM1**: distant metastasis, microscopically confirmed

TABLE 4: AJCC STAGING OF ORAL CAVITY SQUAMOUS CELL CARCINOMA

Prognostic Stage Groups			
Stage	T	N	M
Stage 0	Tis	N0	M0
Stage I	T1	N0	M0
Stage II	T2	N0	M0
Stage III	T3	N0	M0
	T1,2,3	N1	M0
Stage IVA	T4a	N0, 1	M0
	T1,2,3,4a	N2	M0
Stage IVB	T4b	Any N	M0
	Any T	N3	M0
	Any T	Any N	M1

The new Edition of AJCC has integrated two important changes in its staging system. These are **: addition of Depth of invasion in T staging and Extranodal expansion in N staging**. Addition of Depth of invasion has advanced the disease's staging, causing changes in the management regimen as well. However, this causes a disparity in clinical and pathological staging. This revised edition has ramifications as well, as it represents the difficulties in determining Depth of invasion in subsites that are directly on bone, potentially leading to a conflict in Clinical (and Radiological) and Histopathological staging.

3.6.6 ORAL CANCER THERAPEUTIC MODALITIES [16]

The elements that determine the primary treatment option are those associated with:

a) Primary tumor features (tumour factors)

b) Related to the patients (patient factors)

c) Related to the treatment delivery system (physician factors).

a) TUMOR FACTORS:

- Site
- Dimension (T stage)
- Location (anterior versus posterior)
- Bone proximity (mandible)
- Lymph node metastasis
- Previous treatment
- Histology (type, grade, depth of invasion)
-

b) PATIENT FACTORS:

- Age
- General medical condition
- Tolerance
- Occupation
- Treatment acceptance and compliance
- Lifestyle (smoking, drinking, tobacco chewing)
- Socioeconomic aspects

c) PHYSICIAN FACTORS:

- Surgery
- Radiotherapy
- Chemotherapy
- Combined modalities treatment
- Services for Dental Rehabilitation
- Services for Prosthetics Support
- Photodynamic treatment
- Immunotherapy
- Gene Therapy

Other than surgery, most therapies are not known to be successful against massive tumors. As a result, treatment of non-metastatic tumors with surgical removal of the primary tumour followed by adjuvant radiotherapy or radiotherapy and chemotherapy may yield the most promising results.

3.6.7 RECONSTRUCTION

Oromandibular reconstruction remains one of the most difficult sections of head and neck reconstruction.

The following strategies can be used to reconstruct the resulting defect: "Split thickness skin grafts/ Full thickness skin grafts; Buccal;Palatal; Periosteal Mucous membrane flaps;Tongue flaps (Posteriorly based lateral tongue flap, Posteriorly based bilateral tongue flap, Anteriorly based ventral tongue flap); Osteo-myocutaneous flap- fifth rib with pectoralis major myo-cutaneous flap; Spine of scapula with trapezius; Free osteo-cutaneous groin flap; Free osteo-cutaneous fibula flap;Masseter flap; Nasolabial flap;Medial based deltopectoral flaps; Forehead flap; Sternocleidomastoid myo-cutaneous flap; Trapezius flap; Platysma myo-cutaneous flap; Pectoralis major myo-cutaneous flap; Latissimus dorsi myo-cutaneous flap ; Costochondral grafts; Scapular Osseo-cutaneous flap; Radial forearm flap; Radial forearm free osteo-cutaneous flap; Free fibula and osseo-integrated implants."

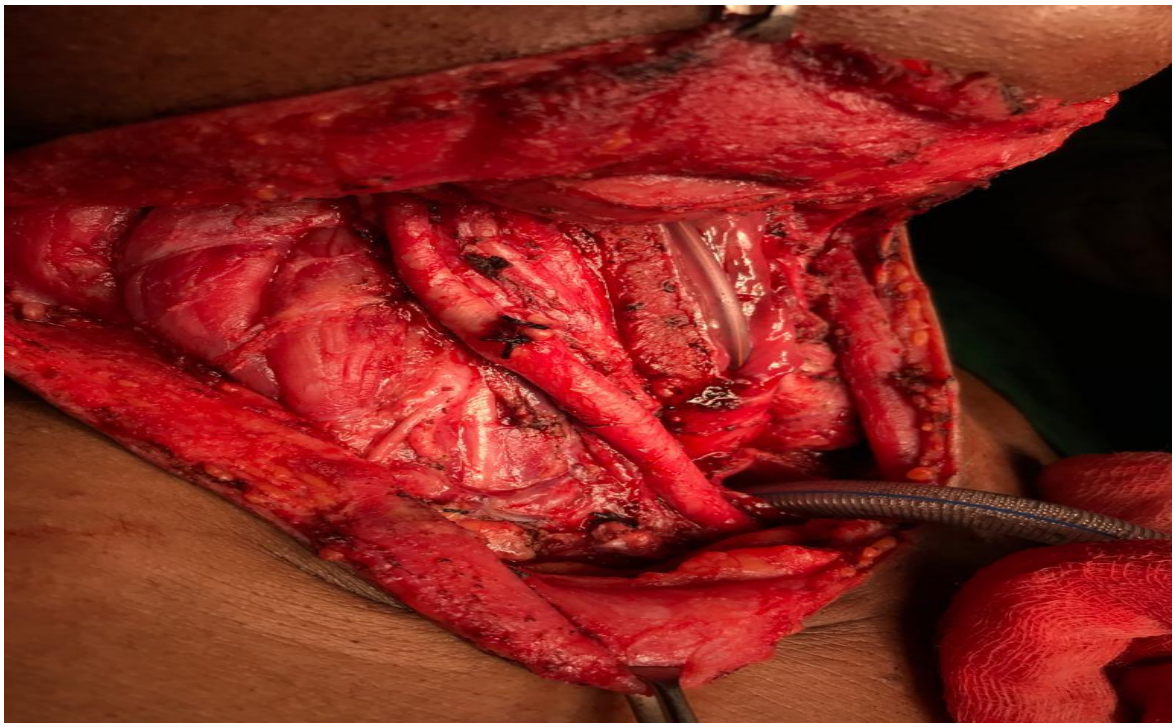

FIGURE 14: IMAGE SHOWING CERVICAL LYMPH NODE DISSECTION

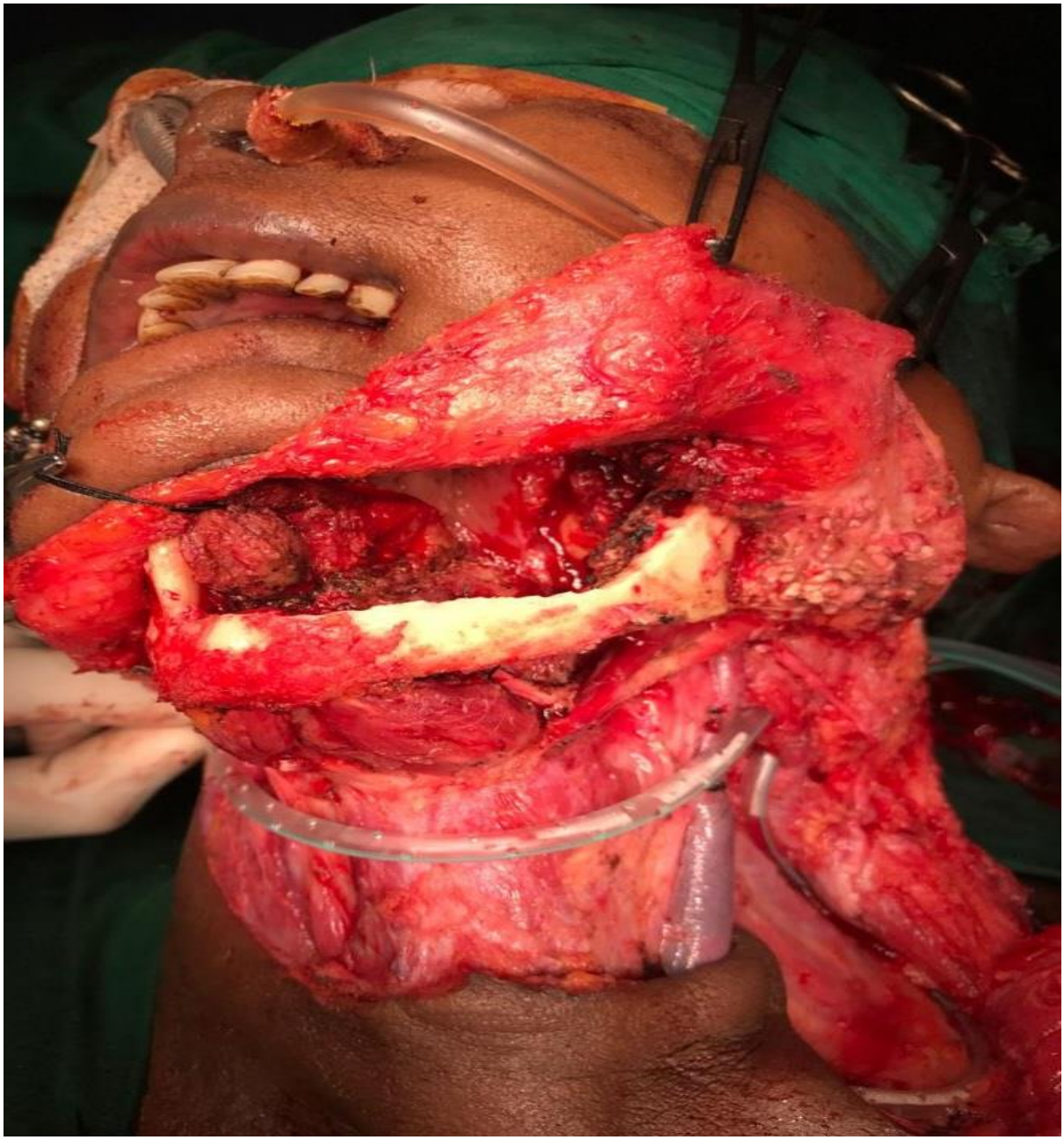

FIGURE 15: IMAGE SHOWING MARGINAL MANDIBULECTOMY

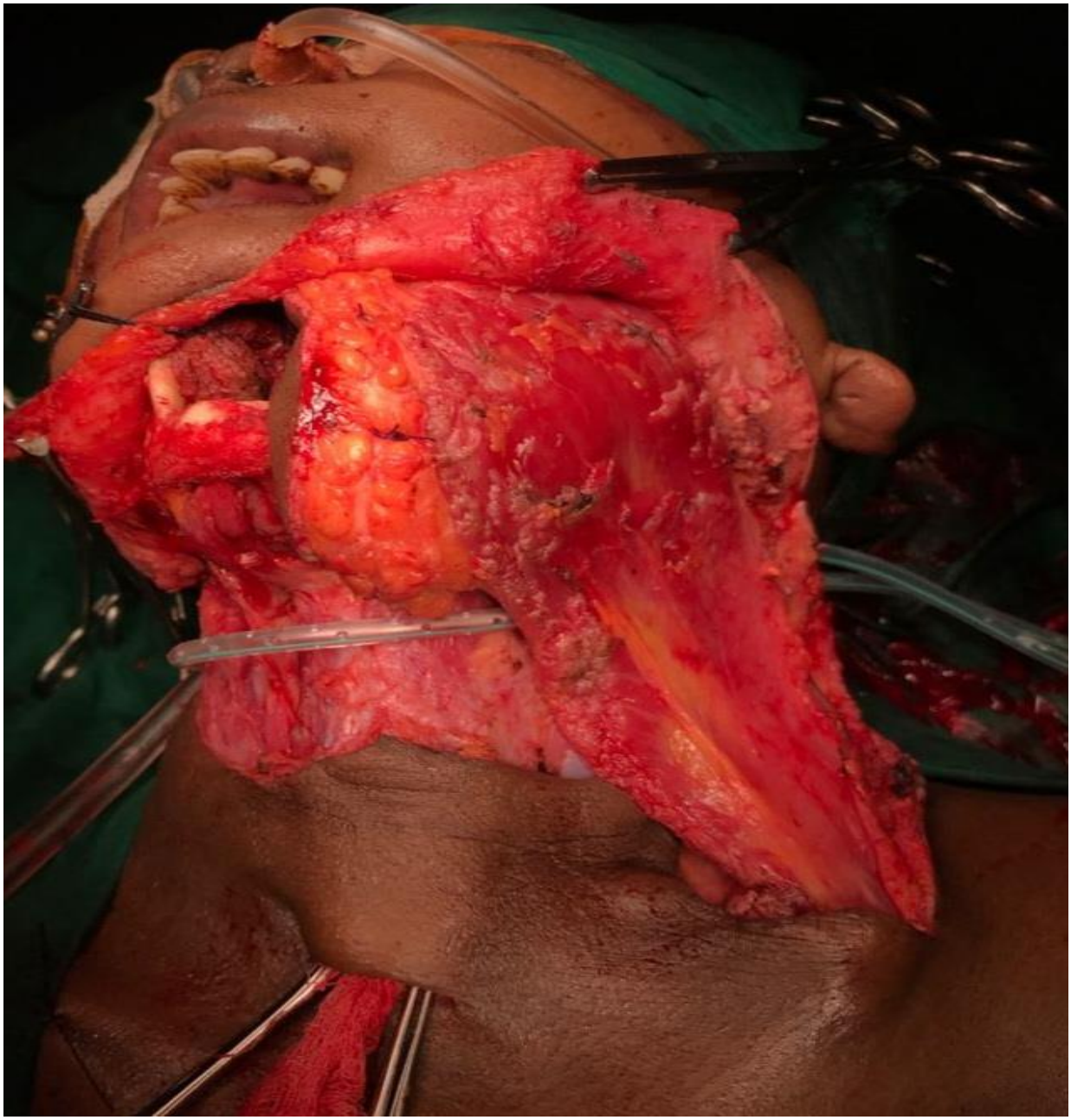

FIGURE 16: IMAGE SHOWING PECTORALIS MAJOR MYO-CUTANEOUS (PMMC) FLAP BEING USED TO COVER THE DEFECT

3.7 QUALITY OF LIFE

The surgical removal of an oral cavity tumour has been linked to severe changes in normal anatomy, functional impairments, and unsatisfactory reconstruction. Outcome markers like as disease-free survival, overall survival, and tumour response rates have been used to assess treatment outcomes. Although these conventional results have proved beneficial to physicians, they have an impact on some of life's most basic functions. Despite the most rigorous treatment regimens, overall survival rates for people with head and neck cancer have remained stable. Greater understanding of the functional impact of surgical resection on patients' function has resulted in improved reconstruction and rehabilitation approaches, as well as the preservation of organs whenever possible.

The term "quality of life" refers to unconventional outcome measurements of functional status and psychosocial well-being.

Different dimensions of quality of life:

- Functional status
- Physical condition
- Psychological issues
- Social Interactions

The distinct characteristics of head and neck surgery, as well as its involvement in speech, swallowing, and deglutition, as well as its cosmetic appeal, enable for socialization. Some of the functional impairments have always been related with mandibular excision. To assess functional status in cancer patients, many quality-of-life assessments are used.

These include:

Karnofsky Performance Scale ECOG 11 scale

Sickness Impact Profile

University of Washington Quality of Life scale

Head & Neck Cancer Specific Quality of Life Instrument [42]

3.8 INFLAMMATORY MARKERS IN TUMOR GENESIS

3.8.1 NEUTROPHILS:

The most prevalent type of granulocyte in humans are neutrophils, also known as neutrocytes, heterophils, or polymorphonuclear leukocytes, which make up 40% to 70% of all white blood cells. They are a crucial component of innate immune system and serve a variety of purposes in various species. [43]

They originate from stem cells in bone marrow and differentiate into neutrophil-killer and neutrophil-cager subpopulations. They have a brief lifespan and are extremely mobile because they may enter tissues that other cells or molecules cannot. Segmented neutrophils and banded neutrophils are two types of neutrophils . Along with basophils and eosinophils, they are part of polymorphonuclear cells (PMNs). [44]

In the circulation, neutrophils, which are phagocytes, are frequently seen. Neutrophils are among the first inflammatory cells to enter site of inflammation during acute phase, mostly as a result of bacterial infection, exposure to the environment, and some types of cancer. Chemotaxis allows them to move across blood arteries and into interstitial space by following chemical signals such interleukin-8 (IL8), C5a, fMLP, leukotriene B4, and H2O2. [45]

They fall within the category of polymorphonuclear cells, so termed because of multilobular form of the nucleus (as compared to lymphocytes and monocytes, the other types of white cells). N ucleus has a distinctly lobbed appearance, with chromatin connecting lobes. In a few other types of nucleated cells, nucleolus disappears as neutrophil develops. Rough endoplasmic reticulum is absent, mitochondria and ribosomes are few, and the Golgi apparatus is small in the cytoplasm. [46]

ANC= Absolute mature neutrophils + absolute immature neutrophils

(OR)

$$\text{ANC} = \frac{\%\ \text{mature neutrophils} + \%\ \text{of immature neutrophila} \times \text{WBC}}{1000}$$

(ANC: Absolute Neutrophil Count)

Normal range : 2.5-7.5 X 10^9/L (40-80%) [46]

LOW NEUTROPHIL COUNT CAUSES (Neutropenia):

Specific causes include: Chemotherapy; Chronic idiopathic neutropenia in adults; Kostmann's syndrome ; Leukaemia; Myelodysplastic syndromes; Myelofibrosis; Myelokathexis; Radiation therapy; Vitamin deficiencies; Aplastic Anaemia; Certain infections: Hepatitis A, Hepatitis B, Hepatitis C, HIV/AIDS, Lyme disease, Malaria, Salmonella infection; Sepsis; Hypersplenism.

HIGH NEUTROPHIL COUNT (Neutrophilia):

Causes include: Acute bacterial infections;Inflammation (e.g., inflammatory bowel disease, rheumatoid arthritis); Tissue necrosis; Physiological (stress, rigorous exercise); Smoking; Pregnancy; Acute lymphocytic leukaemia; Acute myelogenous leukaemia ; Allergy; Chronic lymphocytic leukaemia; Chronic myelogenous leukaemia; Drugs, such as corticosteroids and epinephrine; Polycythaemia vera; Tuberculosis.

3.8.2 NEUTROPHIL ROLES IN TUMOURGENESIS:

TUMOR PROLIFERATION:

It has been established that neutrophil elastase (NE) promotes the development of tumours. NE is taken up by tumour cells and kills the insulin receptor substrate-1, according to research by Houghton et al (IRS-1). A surge in the association between phosphatidylinositol 3-kinase (PI3K) and the active mitogen platelet-derived growth factor receptor (PDGFR) was associated with lower levels of IRS-1, and this relationship led PI3K axis to promote tumour formation. [47]

Neutrophils, on the other hand, can lyse tumour cells by creating hypochlorous acid from reactive oxygen species (ROS). The MET proto-oncogene, which is expressed in neutrophils, is crucial for neutrophil chemoattraction and cytotoxicity against tumour cells in response to its ligand, hepatocyte growth factor. This is vital to keep in mind. Neutrophils can kill tumour cells when TNF- is expressed. Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), which is produced by interferon-stimulated neutrophils, also results in the death of tumour cells. [47]

ANGIOGENESIS AND INVASION:

It is believed that neutrophils contribute significantly to MMP-9 production, which releases VEGF from the extracellular matrix and leads to angiogenesis in cancer (ECM). In addition to its functions in angiogenesis, MMP-9 is hypothesised to aid direct invasion of tumour cells by destroying ECM/basement membrane. [48]

It should be mentioned that neutrophil extracellular traps (NETs), which are produced during neutrophil death and comprise chromatin, NE, and myeloperoxidase, have been shown to play a part in angiogenesis by inducing the production of proangiogenic cytokines by vascular endothelial cells. [49]

IMMUNOMODULATION:

Neutrophils are known to express arginine. An enzyme called arginase breaks down arginine, an essential amino acid necessary for many physiological functions, including proliferation of T cells. High amounts of arginase in tumour microenvironment inhibit development of T cell receptors and antigen-specific responses, aiding tumour evasion. [50] Neutrophils have also been shown to enhance CD8 T cell apoptosis via nitric oxide (NO) and TNF-dependent mechanisms.[51] Additionally, by use of programmed death ligand 1, neutrophils can suppress T cells (PD-L1). Finally, the production of CCL17 by neutrophils causes regulatory T cells to enter tumours, which may inhibit antitumor immunity. [52]

EXTRAVASATION AND METASTATIC SEEDLING:

It has been demonstrated that NETs may capture circulating tumour cells and cause metastases to distant sites. By enhancing anchoring to vascular endothelium, interactions between 2 integrin on neutrophils and ICAM-1 on tumour cells enable extravasation into tissues. By directing bone marrow neutrophils to congregate at the metastatic site before cancer cells arrive, tumor-derived G-CSF can induce a pre-metastatic habitat in distant organs. [53] Contrarily, Granot et al. showed that tumor-entrained neutrophils (tumor-stimulated neutrophils) can reduce metastatic seeding by killing disseminated tumour cells with hydrogen peroxide (H2O2). Transient receptor potential cation channel subfamily M member 2 (TRPM2), a calcium channel, has recently been found to be the mechanism by which neutrophils' H2O2 kills tumour cells. [54]

3.8.3 LYMPHOCYTES:

The majority of immune systems of vertebrates contain lymphocytes, a kind of white blood cell (leukocyte). Lymphocyte types include B cells, T cells, and natural killer cells (for cell-mediated, cytotoxic innate immunity), as well as natural killer cells (for cell-mediated, cytotoxic adaptive immunity) (for humoral, antibody-driven adaptive immunity). Lymphocytes are the most prevalent type of cell found in lymph, thus the term. 18% to 42% of all circulating white blood cells are lymphocytes. [55]

Normal range :1.0–3.0×10^9/l (20–40%).

T-CELLS AND B-CELLS:

T cells (thymus cells) and B cells (bone marrow- or bursa-derived cells) are two main biological components of the adaptive immune response. B cells are involved in humoral immunity, whereas T cells are involved in cell-mediated immunity. Certain "non-self" antigens are detected by T and B cells as a result of a process called antigen presentation. When an invasion is identified, specialised responses are produced by cells that are designed to destroy certain pathogens or pathogen-infected cells. High levels of antibodies are produced by B cells in response to pathogens, which are then neutralised by alien things like bacteria and viruses.

NATURAL KILLER CELLS:

NK cells are a part of innate immune system and help to protect host against cancerous cells and cells that have been infected by viruses. By spotting changes in a surface protein called MHC (major histocompatibility complex) class I, NK cells distinguish between infected and tumour cells from normal and uninfected cells and regulate activity of other cells like macrophages and T cells. C ytokines belonging to the interferon family cause NK cells to activate. Altered cells are eliminated by cytotoxic (cell-killing) granules produced by activated NK cells. They are referred to as "natural killer cells" because they can destroy cells without MHC class I without having to be activated. [55]

LYMPHOCYTOPENIA:

Causes: Undernutrition; HIV and AIDS; Influenza; Autoimmune conditions, such as lupus; Malignancy; Steroid use; Radiation therapy; Certain drugs, including chemotherapy drugs; Inherited disorders, such as Wiskott-Aldrich syndrome and DiGeorge syndrome

LYMPHOCYTOSIS:

Causes: Viral infections:measles, mumps, mononucleosis, adenovirus, hepatitis and influenza; Tuberculosis; Toxoplasmosis; Cytomegalovirus; Brucellosis; Vasculitis; Acute lymphocytic leukaemia; Chronic lymphocytic leukaemia; HIV and AIDS

3.8.4 NEUTROPHIL: LYMPHOCYTE RATIO

The neutrophil to lymphocyte ratio (NLR) is a subclinical inflammatory measure. It is calculated by dividing number of neutrophils by number of lymphocytes, which is obtained from a peripheral blood sample.

$$NLR = \frac{\text{Absolute Neutrophil Count (ANC) (cells/uL)}}{\text{Absolute Lymphocyte Count (ALC) (cells/uL)}}$$

NLR is a novel indicator of cellular immune activation, a reliable measure of stress and systemic inflammation, and it opens up a new area of clinical medicine. It enables a deeper comprehension of biology of inflammation, interaction and competition between innate and adaptive immunity, and clinical implications for health and disease.

Over years, several studies have been conducted to link blood inflammatory indicators and predict survival in OSCC patients. An increase in number of circulating neutrophils in first line of defence indicates presence of systemic inflammation, which promotes cancer cell adhesion. Lymphocytes, especially cytotoxic lymphocytes, have an impact on the development and management of cancer by eliminating tumour cells. Development of cancer is significantly influenced by chronic inflammation. Chronic irritation, autoimmune disorders, and other illnesses that result in infiltration of inflammatory cells at certain places in the body are some of the causes of inflammation. It is thought to encourage development and progression of

various cancers, and inflammation in the body can be detected in peripheral blood. Recent research suggests a connection between a tumor's inflammatory microenvironment and systemic reactions by tumour. A higher neutrophil count or a lower lymphocyte count will reduce lymphokine-activated killer cells. This might be the mechanism underlying tumor's potentially aggressive nature. Lymphocytopenia in cancer patients may be a sign of systemic immunological decline. W Thweakened immune system may have an impact on survival. Insufficient CD4+ Th1 helper cells, CD8+ cytotoxic T cells, or natural killer (NK) cells render the host more vulnerable to onset and spread of cancer, according to several animal studies. NLR may demonstrate coexistence of two antagonistic inflammatory and immunological pathways in cancer patients. **Therefore, NLR is a validated marker of systemic inflammation.**

3.9 REVIEW OF WORLD LITERATURE:

Following studies are available in literature regarding Correlation of NLR with Depth of Invasion and Lymph Node metastasis.

a) **Rashmi G S Phulari et al. in 2017** - "Neutrophil: Lymphocyte ratio and oral squamous cell carcinoma: A preliminary study.The study included both patients with OSCC and healthy participants. The differential leukocyte count was used to calculate NLR. NLR was compared between two groups. According to the findings, NLR and other haematological parameters may serve as surrogate markers for probable aggressive behaviour of OSCC and may aid in the prognosis of these individuals." **(5)**

b) **Salzano G et al. in 2020** –"The prognostic role of the pre-treatment neutrophil to lymphocyte ratio (NLR) and tumour depth of invasion (DOI) in early-stage squamous cell carcinomas of the oral tongue.
A retrospective evaluation of patients with earlystage (cT1T2 cN0) OTSCC who unde rwent elective neck dissection (END) was carried out.Tumors were retrospectively cla ssified using the 8th TNM classification, the DOI wasdetermined using preoperative magnetic resonance imaging, and the NLR was computed for each patient. They came to a conclusion that DOI and NLR can be utilized to effectively anticipate the emergence of occult neck metastases and, as a result, schedule an END in early-stage OTSCC. **(4)**

c) **Charles K A et al. in 2016** - "Systemic inflammation is an independent predictive marker of clinical outcomes in mucosal squamous cell carcinoma of the head and neck in oropharyngeal and non-oropharyngeal patients. A retrospective study was conducted to determine the correlations between NLR and clinicopathological characteristics and recurrence free survival (RFS) and overall survival (OS). Patients with NLR > 5 had a worse overall survival rate, according to the findings."**(56)**

d) **Petrescu et al. in 2020** - "Neutrophil-to-lymphocyte ratio, lymphocyte-to-monocyte ratio and platelet-to-lymphocyte ratio correlates with histopathological grading of oral squamous cell carcinoma. The study looked into the relationship between three inflammatory markers in the peripheral blood of patients with oral squamous cell carcinoma (OSCC): neutrophil to lymphocyte ratio (NLR), platelet to lymphocyte ratio (PLR), and lymphocyte to monocyte ratio (LMR) and cancer histopathological differentiation. Patients' medical records diagnosed with oral maxillofacial cancer between January 2017 and June 2019 were reviewed retrospectively. As SCC differentiation decreased, the NLR, PLR, and LMR for each group indicated a more inflammatory response. They discovered that weaker histological differentiation and lymph node positivity in SCC were linked to a greater inflammatory response in pre-treatment NLR, PLR, and LMR."**(57)**

e) **Agarwal A et al. in 2020** - "Haematological inflammatory parameters: Can they play a role as cancer biomarkers. This was a prospective case control. The haematological parameters (neutrophil, lymphocyte, monocyte count, and platelet count) and indices (NLR, PLR, and LMR) were assessed, and statistical analysis was performed to determine a link between the indices and cancer among cancer patients and control group. NLR and PLR, as well as absolute neutrophil count and platelet count, indicated a significant difference between cancer patients and controls in our investigation."**(58)**

f) **Tsai YT et al.in 2022** - "Prognostic Role of High-Sensitivity Modified Glasgow Prognostic Score for Patients with Operated Oral Cavity Cancer. A retrospective Study was conducted. The HS-mGPS (high sensitivity modified Glasgow prognostic score) has found to be a potential predictive biomarker for individuals with OSCC who had received curative surgery. HS-mGPS along with NLR, proved the clinical usefulness of the combined model with accurate customised survival predictions."**(59)**

g) **Bhola N et al. in 2021**- "Neutrophil-lymphocyte Ratio and Platelet-lymphocyte Ratio for Cervical Metastasis in Patients of Primary Oral Squamous Cell Carcinoma. A

prospective observational study was conducted to evaluate and correlate preoperative serum CRP, NLR, and PLR for cervical metastasis in cases with primary oral squamous cell carcinoma."[2]

h) **Hasegawa T et al. in 2020** - "Neutrophil-lymphocyte ratio associated with poor prognosis in oral cancer. A non-randomized retrospective cohort study was used in this investigation. Numerous risk variables for poor prognosis, such as neutrophil-to-lymphocyte ratio, lymphocyte-to-monocyte ratio, and platelet-to-lymphocyte ratio. Patients' disease-specific survival and overall survival rates were compared across variables and biomarkers. According to the findings, elevated NLR, moderately or poorly differentiated histology, and ENE were all independent predictors of DSS and OS. High NLR, in particular, was linked to a bad prognosis. In Japanese OSCC patients, the NLR could be a significant independent prognostic factor."[3]

i) **Meena M et al. in 2020** - "Diagnostic Role of Neutrophil-Lymphocyte Ratio in Oral Cavity Cancers: A Prospective Study. Individuals were divided into three groups: malignant, premalignant, and control. The lymphocyte count in oral cavity cancers (OCCs) was found to be considerably lower when compared to premalignant oral cavity lesions and the control group. In comparison to premalignant oral cavity lesions and the control group, NLR was considerably higher in OCCs and oral SCC. It was so determined that NLR may be useful in identifying high-risk oral cavity lesions harbouring cancer. As a result, it has great potential as a biomarker for risk classification in oral cavity SCC."[60]

j) **Wu M et al. in 2022** - "Prognostic role of an inflammation scoring system in radical resection of oral squamous cell carcinoma.The major goal of this study was to detect preoperative blood fibrinogen and neutrophil-lymphocyte ratio (NLR) in OSCC patients, as well as to assess the prognostic usefulness of F-NLR (combined fibrinogen and NLR score). Patients with oral cancer were divided into three groups: those with an F-NLR of 2, those with hyperfibrinogenaemia (>250 mg/dL) and a high NLR (>3.2), those with an F-NLR of 1, and those with no higher indices. Preoperative F-NLR was found to be a better predictor of oral cancer prognosis than fibrinogen and NLR alone. According to our findings, blood F-NLR may serve as an independent prognostic factor in OSCC patients."[61]

Similar studies have been conducted that show the significance of pretreatment NLR ratio in determining Tumor Staging in Colorectal Cancer, prognosis in malignant parotid tumors, colorectal carcinoma, stroke patients, HER2 positive breast cancer, osteoporosis, gestational trophoblastic disease, multiple myeloma, and so forth.

Materials and Methods

4. MATERIALS AND METHODS

4.1 STUDY AREA

A prospective study conducted in the Department of Otorhinolaryngology, Sri Devaraj Urs Medical College, R.L. Jalappa Hospital & Research Centre, Tamaka, Kolar.

4.2 STUDY POPULATION

Patients who have been clinically, radiologically and biopsy-proven diagnosed with Squamous Cell Carcinoma of Oral Cavity (cT2 to cT4) and planned for surgery have been included in the study.

4.3 STUDY DESIGN

A prospective, observational and cross-sectional study.

4.4 SAMPLE SIZE

The sample size was calculated based on the number of patients who underwent surgery for oral cancer at our institution in the previous two years.
With 95% confidence interval and with absolute error of 10% - the required sample size was 88 as calculated by the formula.

$$n = \frac{Z\,\alpha 2(p)\,(1-p)}{d2}$$

$$= (1.96)^2\,(36)\,(64)\,/(10)^2$$

n = 88

4.5 STUDY PERIOD

From January 2021 to July 2022

4.6 INCLUSION CRITERIA

- Patients between the ages of 20 - 75 years with biopsy proven oral squamous cell carcinoma.
- Patients belonging to pT2 to pT4 tumour stage.
- Patients who have been planned for surgical management of oral squamous cell carcinoma.

4.7 EXCLUSION CRITERIA

- Patients who have received neoadjuvant treatment prior to surgery.
- Patients with other histopathological types of oral carcinoma other than squamous cell carcinoma.
- Patients with recurrent disease.
- Presence of any active infection during the perioperative period.
- Patients with pre-existing haematological abnormalities/ autoimmune diseases/ history of steroid usage/ chronic renal insufficiency.

4.8 METHODOLOGY

The research was conducted as a prospective, cross-sectional study. The Institutional Ethical Committee approval for study protocol was obtained. With informed written consent, patients with clinical diagnosis of OSCC underwent a complete blood count and incisional biopsy from the ulcerative growth. Biopsies were performed on all individuals with probable malignancy of oral cavity. A total of 88 patients with histologically proven OSCC, fulfilling the above-mentioned inclusion and exclusion criteria were included in study.

In each case, NLR was determined by examining a peripheral blood smear. All patients underwent a thorough pre-surgical evaluation that included a medical history and complete physical examination, as well as regular blood biochemistry and a complete blood count.

The socio-demographic history of each participant was compiled and entered in the study proforma. Each patient's clinical case history was meticulously documented. A local clinical

examination was performed to determine the location, size, and type of growth. Manual palpation was used to assess any expansion neck lymphadenopathy along its with its size, shape, consistency, tenderness, and fixity to overlying and underlying structures. Clinical TNM staging was estimated based on clinical observations as per 8th edition of the AJCC.

4.8.1 CALCULATION OF NLR:

The blood was obtained aseptically from median cubital vein. Differential leukocyte count was determined by examining peripheral blood smears stained with Field's stain. From differential counts, absolute counts of neutrophils and lymphocytes were collected, and NLR was computed for each sample.

NLR = ABSOLUTE NEUTROPHIL COUNT / ABSOLUTE LYMPHOCYTE COUNT

4.8.2 CALCULATION OF DEPTH OF INVASION:

All patients underwent curative surgery with concurrent unilateral or bilateral neck dissection Surgeons employed pedicle, free, or local flaps to repair surgical defects. Surgeries included removal of primary tumor included Wide excision, Composite resection, Total/Partial/ Infrastructure Maxillectomy, Total/Sub-total glossectomy along with neck dissection and reconstruction.

Before formalin fixation, a wedge of tumour margin was extracted shortly after resection of the specimen. This tumour wedge and the primary specimen were histopathologically examined for extent, depth of invasion, and bone involvement. **DOI is obtained by drawing a line perpendicular to that passing through the basal membrane of the normal squamous**

epithelium, contiguous to the neoplasm, up to the deepest tumor invasion point [(4)]

Metastasis of lymph nodes was also observed in the neck dissection specimen. A thorough histopathology report was obtained. The pathological staging of tumor was documented based on AJCC 8th Edition. Histopathologically, oral cavity malignancies were distributed on the basis of their degree of differentiation:

(a) Well differentiated SCC

(b) Moderately differentiated SCC

(c) Poorly differentiated SCC

4.8.3 CUT OFF VALUES FOR DEPTH OF INVASION AND N:L RATIO IN OUR STUDY

In this study, we have taken **cut off value for depth of invasion (DOI)** as > 4 mm for tongue cancer and > 5mm for carcinoma involving rest of the oral cavity. The depth of invasion greatly influences the spread of the disease and thereby the prognosis of the disease. The cutoff point evaluated for tongue carcinoma is 4mm and for the buccal mucosa is 5mm beyond which tumor was hypothesized to be aggressive and will have a higher chance of metastasis to lymph nodes. [(62)(63)]

Cut-**off value for NLR** was taken from the study conducted by **Salzano G et al.-** "The prognostic role of the pre-treatment neutrophil to lymphocyte ratio (NLR) and tumor depth of invasion (DOI) in early-stage squamous cell carcinomas of the oral tongue." The study showed a NLR > 2.93 to associated with an increased risk of aggressive nature of the tumor and presence of occult cervical metastases. [(4)] According to the research, an NLR score of 2.93 was linked to a 49.91% chance of having occult neck metastases. Existence of perineural invasion and tumoral infiltration thickness were two additional neck lymph node metastasis predictors that showed a positive connection with an NLR > 2.93.

4.8.4 ADJUVANT TREATMENT MODALITY

The consensus of our hospital's multidisciplinary tumour board established postoperative adjuvant therapy plans.

Adjuvant chemoradiotherapy (CRT) was administered to patients with multiple metastatic LNs, ENE, and surgical margins (positive), while adjuvant radiotherapy was administered to patients with a single metastatic neck LN with pathological T4 disease (RT).

The total doses for adjuvant RT and adjuvant CRT were 60 Gy and 66 Gy, respectively, and the intensity modulated radiation dosage each fraction was 2 Gy for 5 days per week. The adjuvant chemotherapy treatment was 100mg/ m2 IV Cisplatin once every 3 weeks or 40mg/ m2 IV Cisplatin once a week, in line with the patient choices and medical assessments.

4.8.5 FOLLOW UP

Follow-up was done on a regular basis (second monthly) for first six months, then every six months through phone or clinical follow-up on out-patient basis.

Every follow-up session included a physical examination as well as a flexible fiberoptic examination. In case of recurrence, a repeat CT scan (skull base to T4) was performed, and a biopsy was performed if necessary. We defined OS (overall survival) as the time span between the date of surgery and the date of death from any cause. In case of death due to recurrence, the duration of cancer-specific survival (CSS) was measured from time of surgery until death. We also defined disease-free survival (DFS) as time between the date of curative surgery and recurrence (based on clinical evidence such as locoregional recurrence or distant metastases).Recurrence cases were categorized into local, regional, locoregional and distant metastasis.

4.9 STATISTICAL METHODS

- Data was entered into Microsoft excel data sheet and was analysed using SPSS 22 version software. Categorical data was represented in the form of Frequencies and proportions. **Chi-square test or Fischer's exact test** (for 2x2 tables only) was used as test of significance for qualitative data.
- Continuous data was represented as mean and standard deviation. **Independent t test** was used as test of significance to identify the mean difference between two quantitative variables.
- **ANOVA** was used as test of significance to identify the mean difference between more than two quantitative variables.
- Correlations were performed with **Pearson Correlation Coefficient.**
- **Graphical representation of data:** MS Excel and MS word was used to obtain various types of graphs.
- **P value** (Probability that the result is true) of <0.05 was considered as statistically significant after assuming all the rules of statistical tests.
- **Statistical software:** MS Excel**,** SPSS version 22 **(**IBM SPSS Statistics, Somers NY, USA) was used to analyse data.

Observations and Results

5. OBSERVATIONS AND RESULT ANALYSIS

A total of 88 patients satisfied the study criteria in this prospective study. The contents of each table are explained during discussion. Graphs are made to portray the relation existing among data wherever necessary.

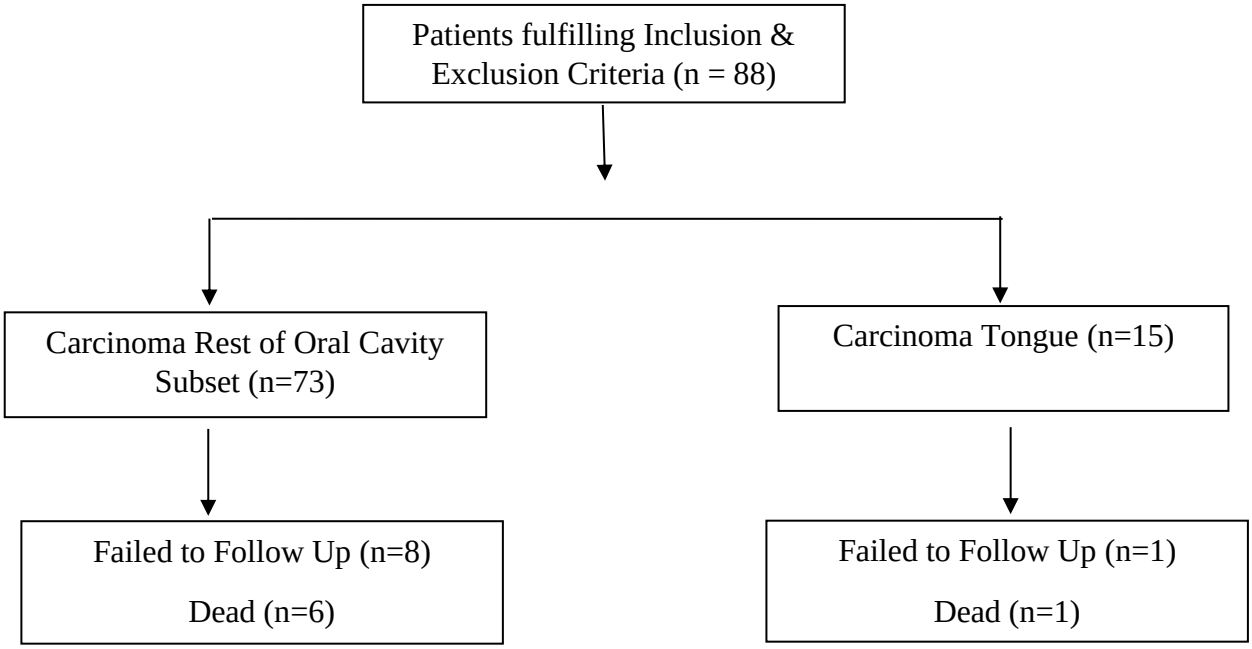

TABLE 5: STUDY FLOW DIAGRAM

5.1 AGE DISTRIBUTION

TABLE 6: AGE DISTRIBUTION OF SUBJECTS

Age in years	Carcinoma Tongue	Rest of Oral Cavity Subset Carcinoma	Total	Percentage (%)
≤ 30	2	2	4	4.5
31 – 45	2	16	18	20.5
46– 60	10	26	36	40.9
≥ 60	1	29	30	34.1
TOTAL	15	73	88	100.0

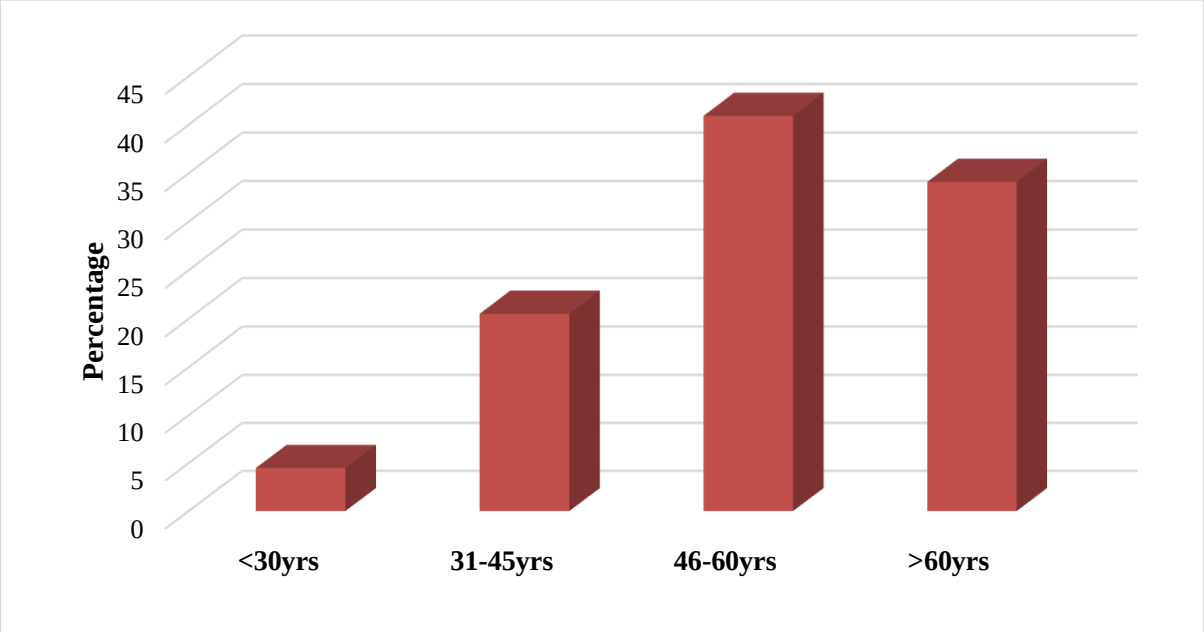

CHART 1: GRAPH SHOWING DISTRIBUTION OF SUBJECTS ACCORDING TO AGE GROUP

A majority of the patients belonged to the age group 46-60 years, with the mean age of the patients in the study being 55.15 ± 12.63 years.

Mean ± SD in each group:

* For Carcinoma Tongue – 49.13 ± 12.03 years

* For Rest of Oral Cavity Subset Carcinoma – 56.39 ± 12.47 year

5.2 GENDER DISTRIBUTION

TABLE 7: DISTRIBUTION OF SUBJECTS ACCORDING TO SEX

Gender	Carcinoma Tongue	Rest of Oral Cavity Subset Carcinoma	Total	Percentage (%)
Male	11	23	34	38.6
Female	04	50	54	61.4
TOTAL	15	73	88	100.0

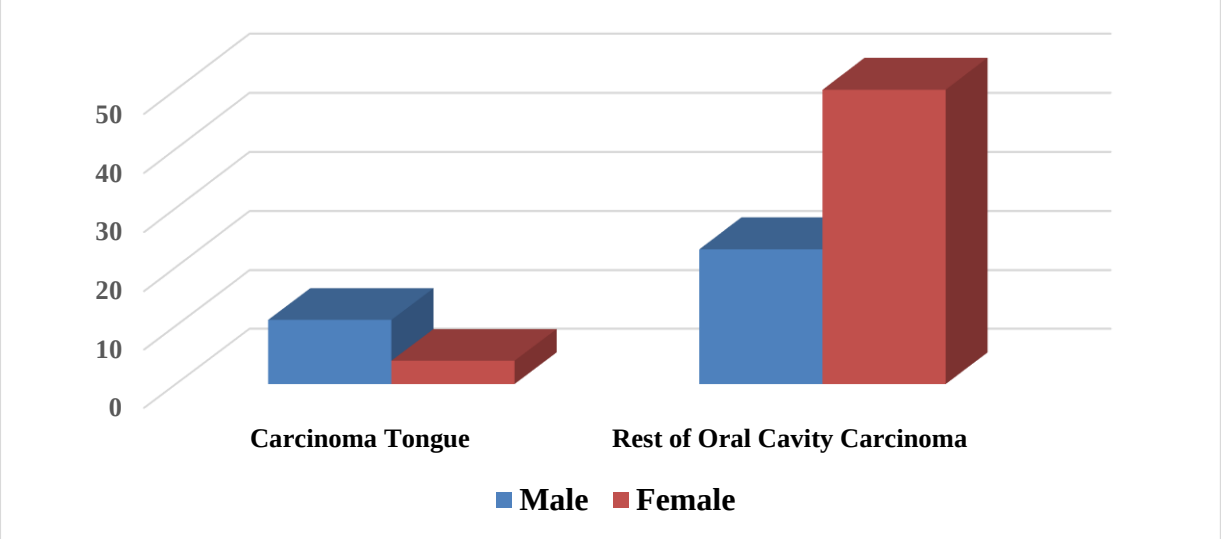

CHART 2: GRAPH SHOWING DISTRIBUTION OF SUBJECTS ACCORDING TO SEX

Of the total 88 patients enrolled in this study, 38.6% (34) were males while 61.4% (54) were females.

Incidence of Carcinoma Tongue was noted to be higher among males when compared to Carcinoma of the rest of the oral cavity wherein females were noted to have a higher incidence of malignancy.

5.3 SUBSET OF ORAL CAVITY INVOLVED WITH MALIGNANCY

TABLE 8: DISTRIBUTION OF SUBJECTS ACCORDING TO SUBSET OF ORAL CAVITY INVOLVED

Subset of Oral Cavity Involved	Total	Percentage (%)
Tongue	15	17.2
Rest of Oral Cavity	73	82.8
TOTAL	88	100.0

Rest of Oral Cavity	Total	Percentage (%)
Gingiva Buccal Sulcus	19	26
Buccal Mucosa	35	47.9
Alveolus	11	15.1
Lip	2	2.7 .
Maxilla	1	1.4
Retro Molar Trigone	4	5.5
Mandible	1	1.4
TOTAL	73	100

Most common subset of oral cavity involved in malignancy was noted to be the Buccal Mucosa – contributing to nearly 40% of all the cases.

Gingiva buccal Sulcus (21.5%) and Tongue (17.2%) were noted to be the next most common subsets to be involved with malignancy.

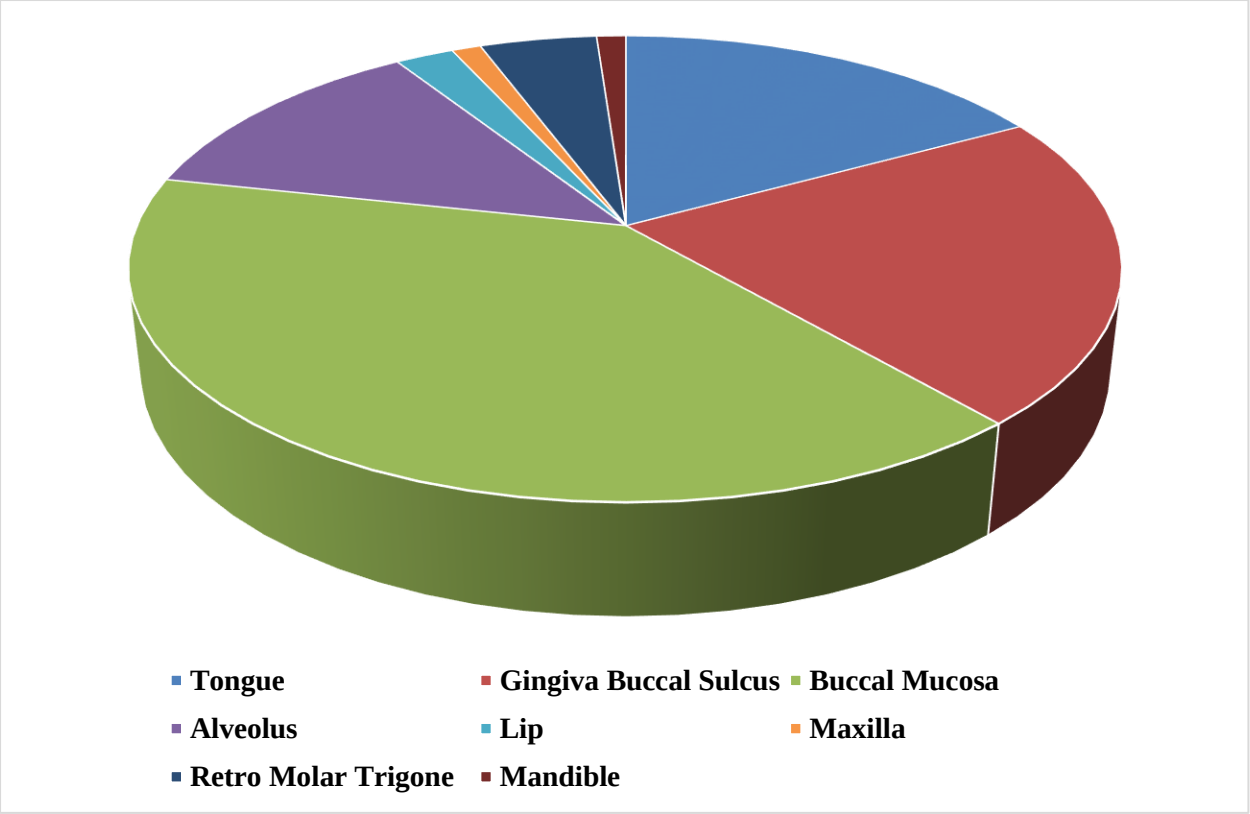

CHART 3: GRAPH SHOWING DISTRIBUTION OF SUBJECTS ACCORDING TO SITE

5.4 DURATION OF SYMPTOMS

Average duration of symptoms in patients presenting with Carcinoma Tongue was noted to be 5.2 ± 2.04 months.

Average duration of symptoms in patients presenting with Carcinoma involving the Other Subsets of the Oral Cavity was seen to be 6.35 ± 3.19 months.

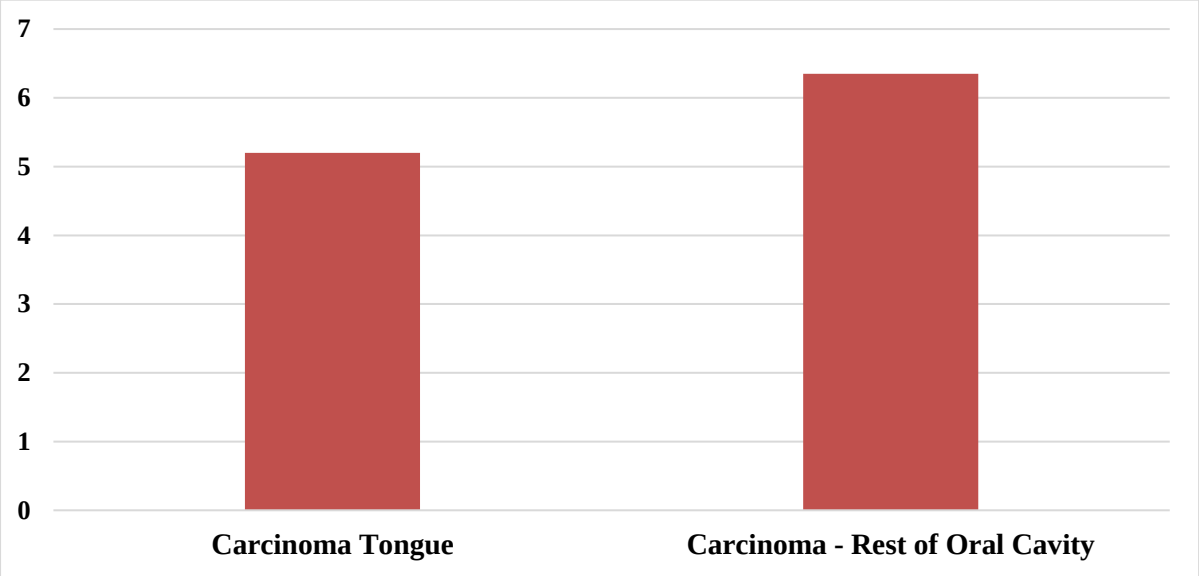

CHART 4: GRAPH SHOWING AVERAGE DURATION OF PRESENTATION OF SYMPTOMS

5.5 GRADE OF TRISMUS

TABLE 9: DISTRIBUTION OF PATIENTS BASED OF GRADE OF TRISMUS

TRISMUS	Carcinoma Tongue	Carcinoma - Rest of Oral Cavity Subset	Percentage (%)
Grade 0	2	2	4.5
Grade 1	5	28	37.5
Grade 2	4	26	34.1
Grade 3	2	13	17.1
Grade 4	2	4	6.8
TOTAL	15	73	100

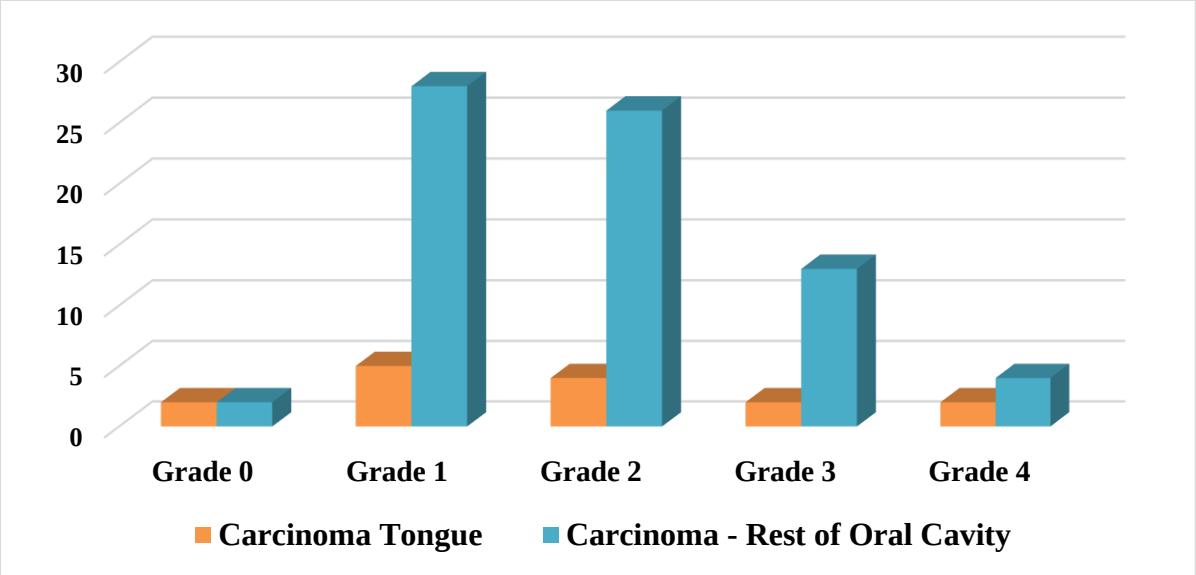

CHART 5: GRAPH SHOWING DISTRIBUTION OF GRADE OF TRISMUS AMONG SUBJECTS

37.5% of the patients enrolled in this study presented with Grade I Trismus, which was followed by Grade II Trismus.

5.6 CLINICAL TUMOUR (T) STAGE

TABLE 10: DISTRIBUTION OF PATIENTS WITH RESPECT TO CLINICAL TUMOUR (T) STAGE (cT)

Tumour (T) Status	Carcinoma Tongue	Carcinoma – Rest of Oral Cavity Subset	Total	Percentage (%)
T1	0	0	0	0
T2	5	33	38	43.2
T3	8	22	30	34.1
T4	2	18	20	22.7
TOTAL	15	73	88	100.0

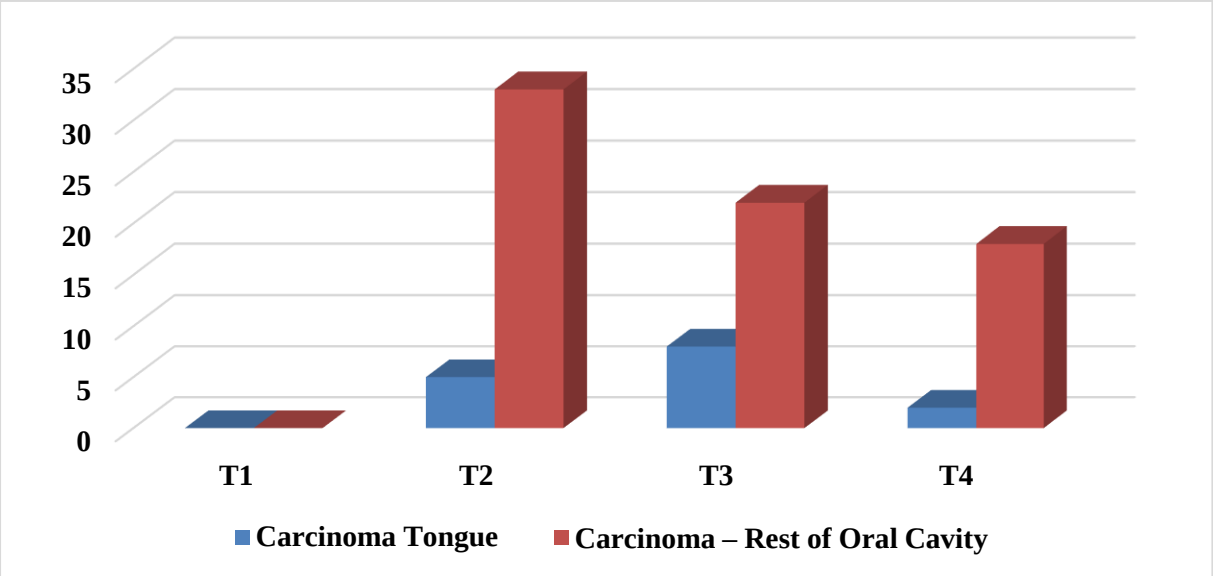

CHART 6: DISTRIBUTION OF SUBJECTS BASED ON CLINICAL TUMOUR (T) STAGE

Majority of the patients presented with T2 stage of tumour (accounting for 43.2% of all cases) – Tumour ≥ 2cm but < 4cm in greatest dimension.

Nearly 23% of patients presented with tumour involving the adjacent structure (T4) on clinical examination.

5.7 PATHOLOGICAL TUMOUR (T) STAGE

TABLE 11: DISTRIBUTION OF PATIENTS WITH RESPECT TO PATHOLOGICAL TUMOUR (T) STAGE (pT)

Tumour (T) Status	Carcinoma Tongue	Carcinoma – Rest of Oral Cavity Subset	Total	Percentage (%)
T1	0	0	0	0
T2	5	32	37	42.1
T3	7	16	23	26.1
T4	3	25	28	31.8
TOTAL	15	73	88	100.0

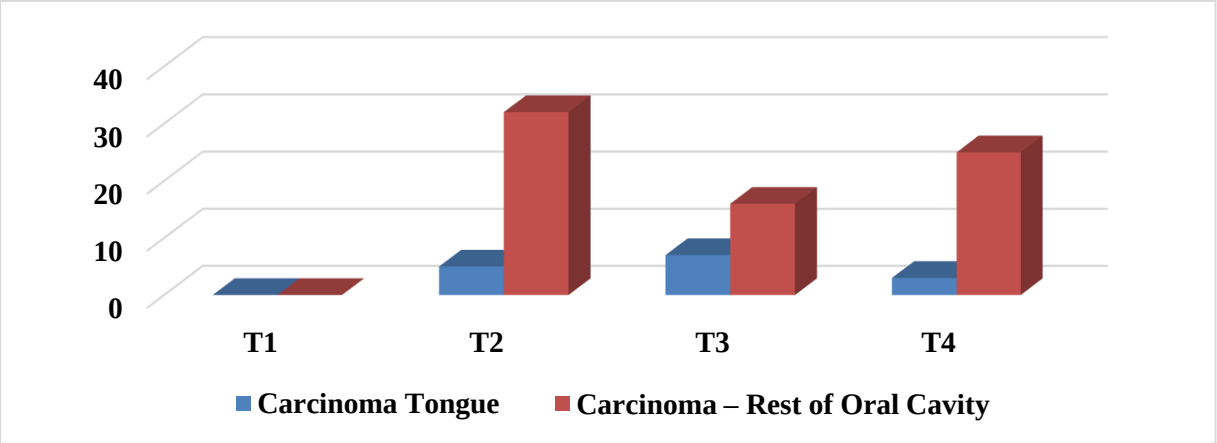

CHART 7: DISTRIBUTION OF SUBJECTS BASED ON PATHOLOGICAL TUMOUR (T) STAGE

Based on HPE findings, 42% patients were noted to have T2 disease (tumour ≥ 2 cm but < 4cm in dimension) which was similar to number of patients with clinical T2 disease. However, nearly 32% patients had T4 pathological disease when compared to clinical T4 disease which was seen in 23% patients.

5.8 CLINICAL LYMPH NODE (N) STAGING

TABLE 12: DISTRIBUTION OF PATIENTS ACCORDING TO CLINICAL LYMPH NODE STAGE (cN)

Clinical Nodal (T) Status	Carcinoma Tongue	Carcinoma – Rest of Oral Cavity Subset	Total	Percentage (%)
N0	7	38	45	51.2
N1	5	22	27	30.7
N2	2	10	12	13.6
N3	1	3	4	4.5
TOTAL	15	73	88	100.0

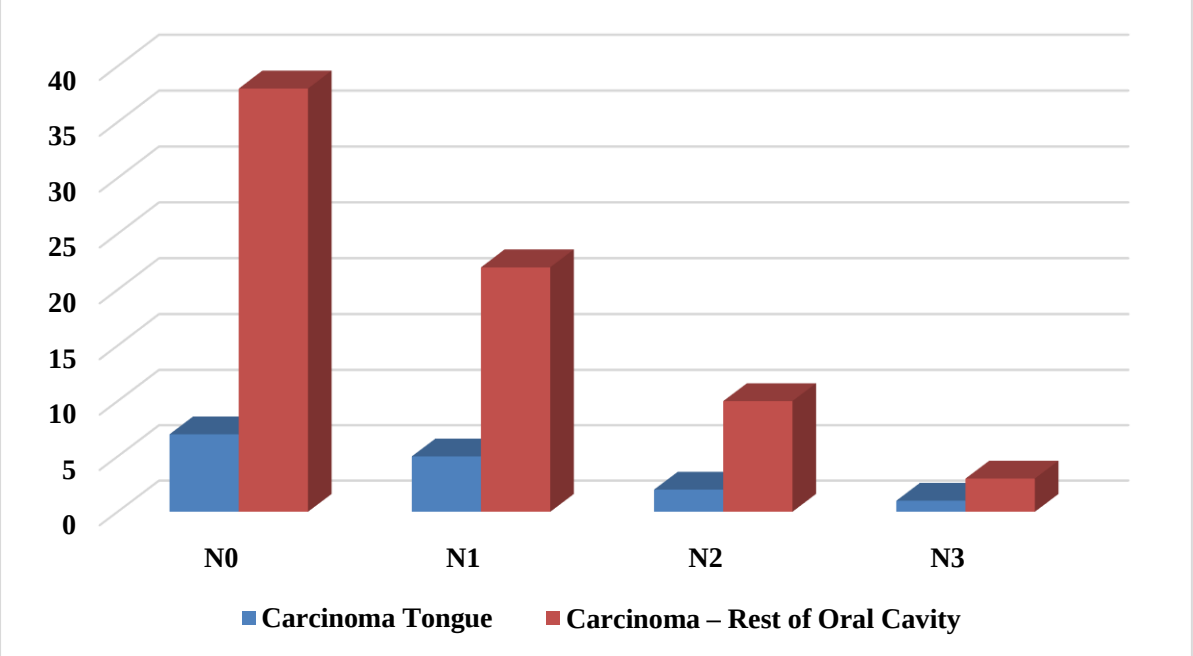

CHART 8: DISTRIBUTION OF SUBJECTS BASED ON CLINICAL LYMPH NODE (N) STAGE

50% of the patients included in this study were noted to clinical lymph node status N1 and above, with majority of patients having N1 status (31%)

5.9 PATHOLOGICAL LYMPH NODE (N) STAGING

TABLE 13: DISTRIBUTION OF PATIENTS ACCORDING TO PATHOLOGICAL LYMPH NODE STAGE (pN)

Pathological Nodal (T) Status	Carcinoma Tongue	Carcinoma – Rest of Oral Cavity Subset	Total	Percentage (%)
N0	8	36	44	50
N1	3	16	19	21.6
N2	2	13	15	17.1
N3	2	8	10	11.3
TOTAL	15	73	88	100.0

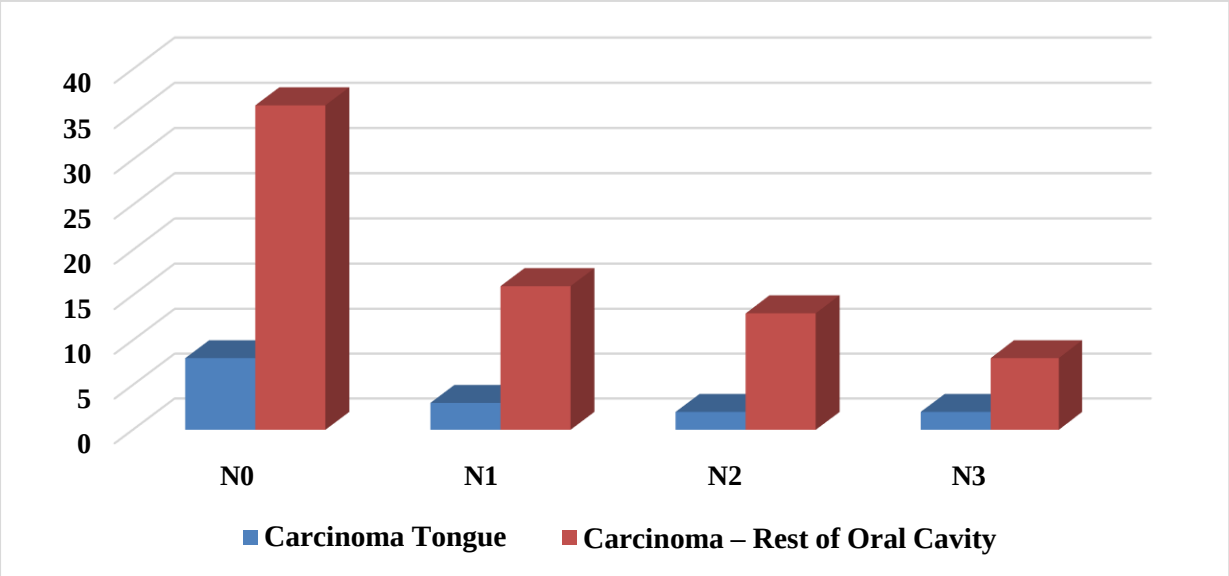

CHART 9: DISTRIBUTION OF SUBJECTS BASED ON PATHOLOGICAL LYMPH NODE (N) STAGE

5.10 CLINICAL NODAL STATUS

TABLE 14: DISTRIBUTION OF PATIENTS WITH RESPECT TO INVOLVEMENT OF LYMPH NODES CLINICALLY (cN)

Nodal Status	Carcinoma Tongue	Carcinoma – Rest of Oral Cavity Subset	Total	Percentage (%)
Present	8	35	43	48.9
Absent	7	38	45	51.1
TOTAL	15	73	88	100.0

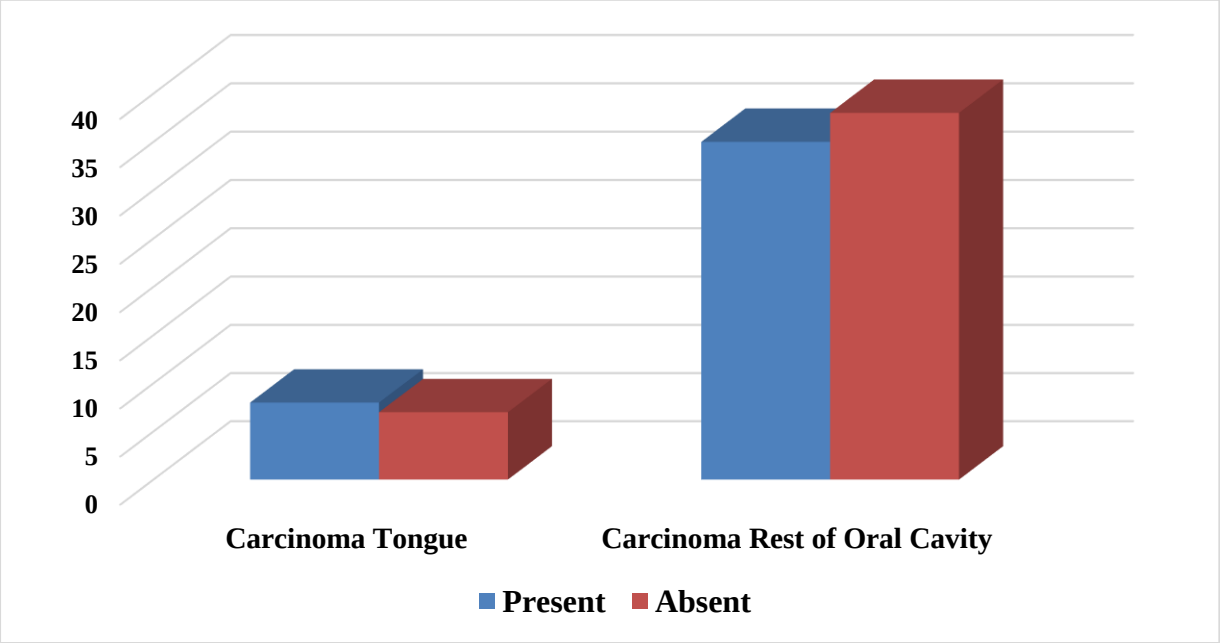

CHART 10: DISTRIBUTION OF SUBJECTS BASED ON CLINICAL NODAL INVOLVEMENT

Nearly 50% of the patients enrolled in this study presented with regional lymph node involvement on clinical examination.

5.11 PATHOLOGICAL NODAL STATUS

TABLE 15: DISTRIBUTION OF PATIENTS WITH RESPECT TO INVOLVEMENT OF LYMPH NODES PATHOLOGICALLY (pN)

Pathological Nodal Status	Carcinoma Tongue	Carcinoma – Rest of Oral Cavity Subset	Total	Percentage (%)
Present	7	37	44	50
Absent	8	36	44	50
TOTAL	15	73	88	100.0

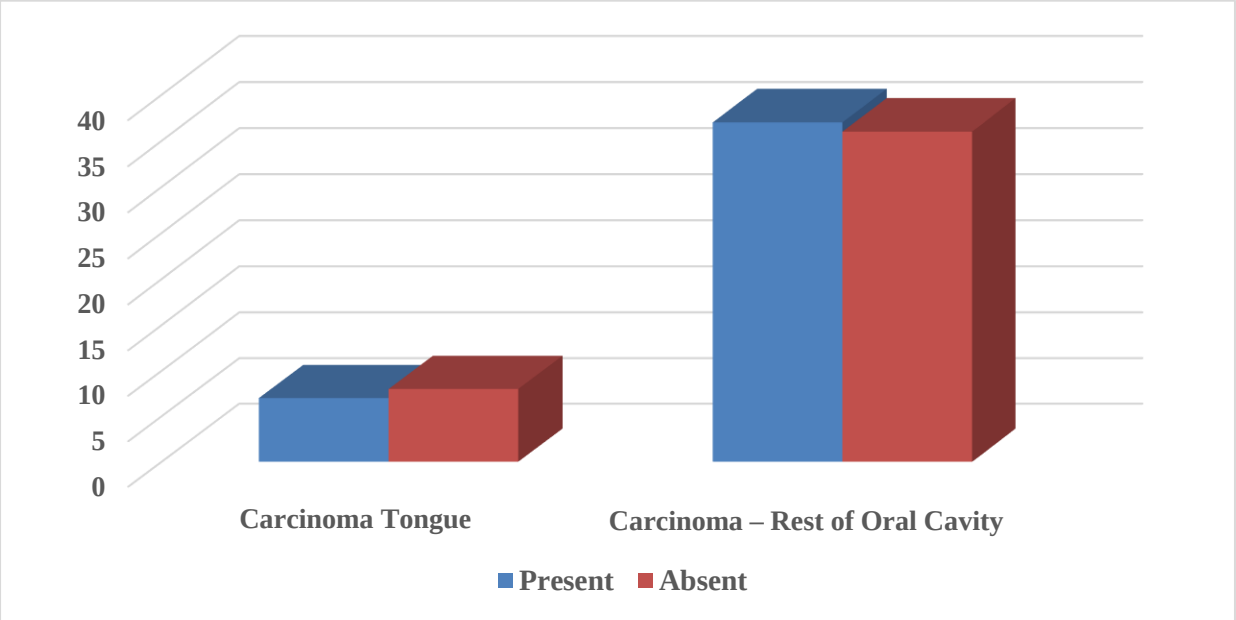

CHART 11: DISTRIBUTION OF SUBJECTS BASED ON PATHOLOGICAL NODAL INVOLVEMENT

Based on HPE report, 50% of the patients were noted to have pathological involvement of lymph node which was similar to number of patients with clinically palpable lymph nodes (48.9%)

5.12 NUMBER OF POSITIVE LYMPH NODES ON HPE REPORT

TABLE 16: DISTRIBUTION OF PATIENTS WITH RESPECT TO NUMBER OF INVOLVED LYMPH NODES ON HISTOPATHOLOGY (pN)

Number of LNs Involved	Carcinoma Tongue	Carcinoma – Rest of Oral Cavity Subset	Total	Percentage (%)
0	8	36	44	50
1	3	16	19	21.6
2	2	5	7	7.9
3	2	11	13	14.7
≥ 4	0	5	5	5.8
TOTAL	15	73	88	100

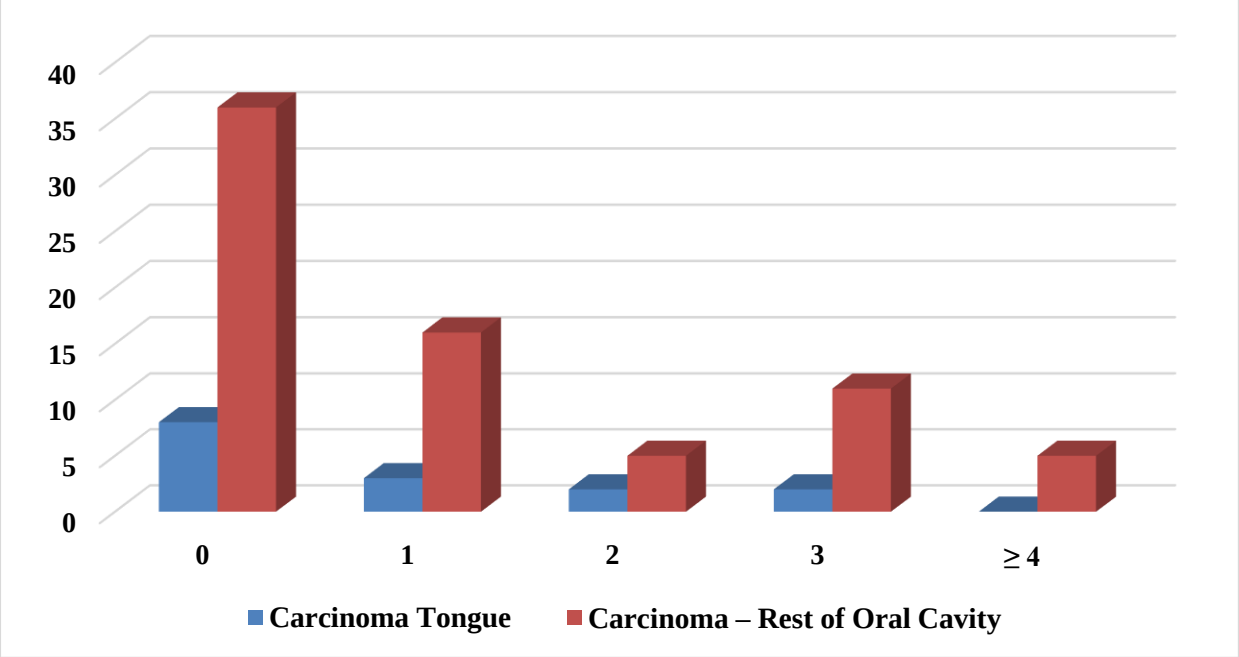

CHART 12: DISTRIBUTION OF SUBJECTS BASED ON NUMBER OF INVOLVED LYMPH NODES ON HPE REPORT

Half the subjects in the study had ≥ 1 lymph node involved when the surgical specimen was examined histologically.

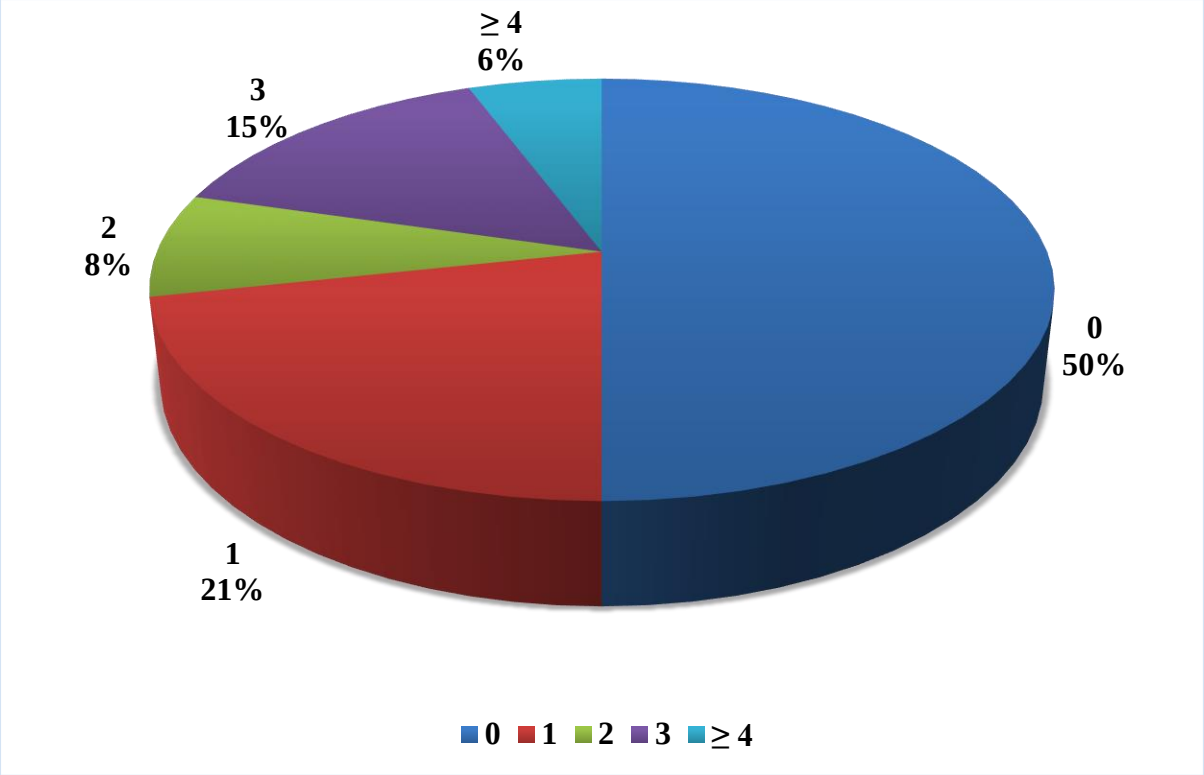

CHART 13: PIE CHART SHOWING DISTRIBUTION OF SUBJECTS BASED ON NUMBER OF INVOLVED LYMPH NODES ON HPE REPORT

50% of patients had no lymph node involvement by malignant cells when examined histopathologically

5.13 CLINICAL STAGING OF ORAL CAVITY CARCINOMA

TABLE 17: DISTRIBUTION OF PATIENTS WITH RESPECT TO CLINICAL STAGING OF CARCINOMA

STAGE OF CARCINOMA	Carcinoma Tongue	Carcinoma - Rest of Oral Cavity Subset	Total	Percentage (%)
STAGE I	0	0	0	0
STAGE II	3	19	22	25
STAGE III	7	29	36	40.9
STAGE IVA	4	22	26	29.5
STAGE IVB	1	3	4	4.6
TOTAL	15	73	88	100.0

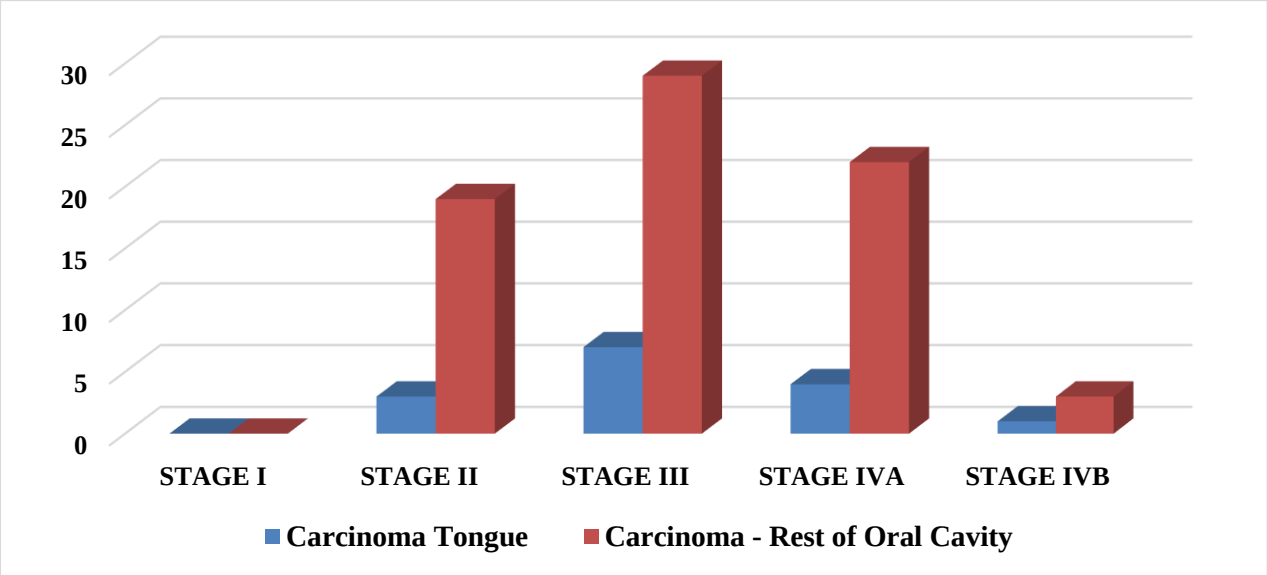

CHART 14: DISTRIBUTION OF SUBJECTS BASED ON CLINICAL STAGING OF ORAL CAVITY CARCINOMA

3/4th of the patients in this study were noted to have Stage III and Stage IV disease based on clinical examination.

None of the patients in this study belonged to Stage I

5.14 PATHOLOGICAL STAGING OF ORAL CAVITY CARCINOMA

TABLE 18: DISTRIBUTION OF PATIENTS WITH RESPECT TO PATHOLOGICAL STAGING OF CARCINOMA

STAGE OF CARCINOMA	Carcinoma Tongue	Carcinoma - Rest of Oral Cavity Subset	Total	Percentage (%)
STAGE I	0	0	0	0
STAGE II	2	17	19	21.6
STAGE III	8	21	29	32.9
STAGE IVA	4	28	32	36.4
STAGIVB	1	7	8	9.1
TOTAL	15	73	88	100.0

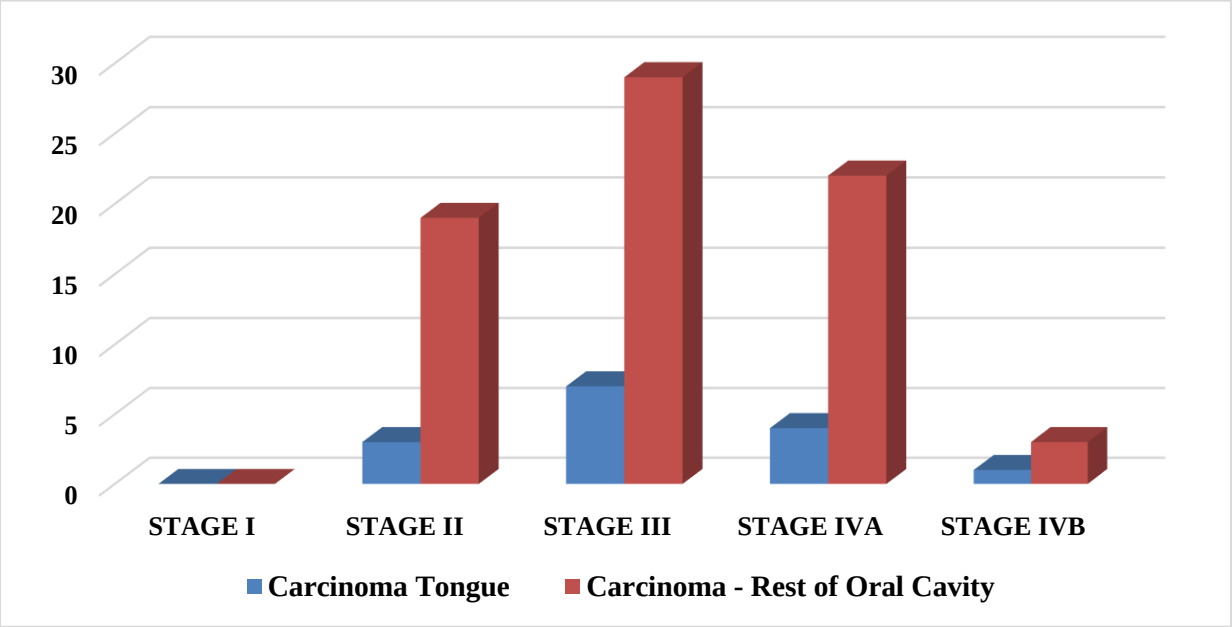

CHART 15: DISTRIBUTION OF SUBJECTS BASED ON PATHOLOGICAL STAGING OF ORAL CAVITY CARCINOMA

Based on final HPE report, 3/4th of the patients were noted to have stage III and IV disease.

None of the patients belonged to Stage I based on pathological study.

5.15 OPERATIVE PROCEDURE PERFORMED

TABLE 19: DISTRIBUTION OF PATIENTS BASED ON SURGICAL MANAGEMENT

OPERATIVE PROCEDURE	Total	Percentage (%)
WIDE EXCISION	4	4.5
BITE RESECTION	4	4.5
COMPOSITE RESECTION	59	67
MAXILLECTOMY	8	9.1
SUBTOTAL GLOSSECTOMY	5	5.6
HEMI GLOSSECTOMY	10	11.3
TOAL	88	100

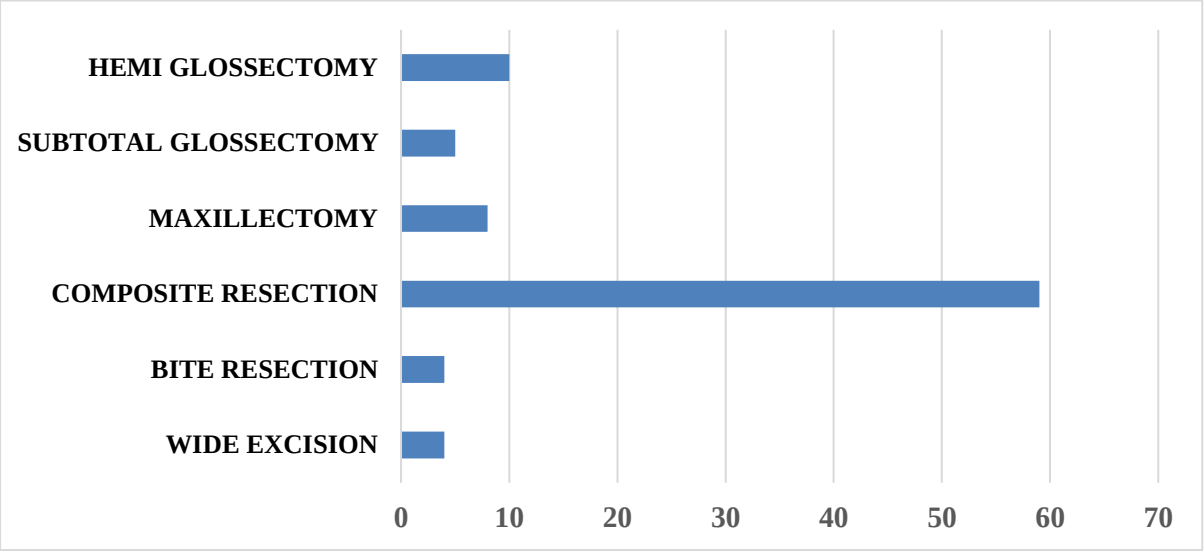

CHART 16: DISTRIBUTION OF SUBJECTS BASED ON OPERATIVE PROCEDURE PERFORMED

Composite Resection was the most commonly performed surgical procedure in our study (67%).

5.16 ADJUVANT TREATMENT

TABLE 20: DISTRIBUTION OF SUBJECTS BASED ON ADJUVANT TREATMENT RECEIVED

ADJUVANT MODALITY	Carcinoma Tongue	Carcinoma – Rest of Oral Cavity Subset	Total	Percentage (%)
Only Radiotherapy	8	39	47	61.1
Combined CT and RT	7	23	30	38.9
TOTAL	15	62	77	100

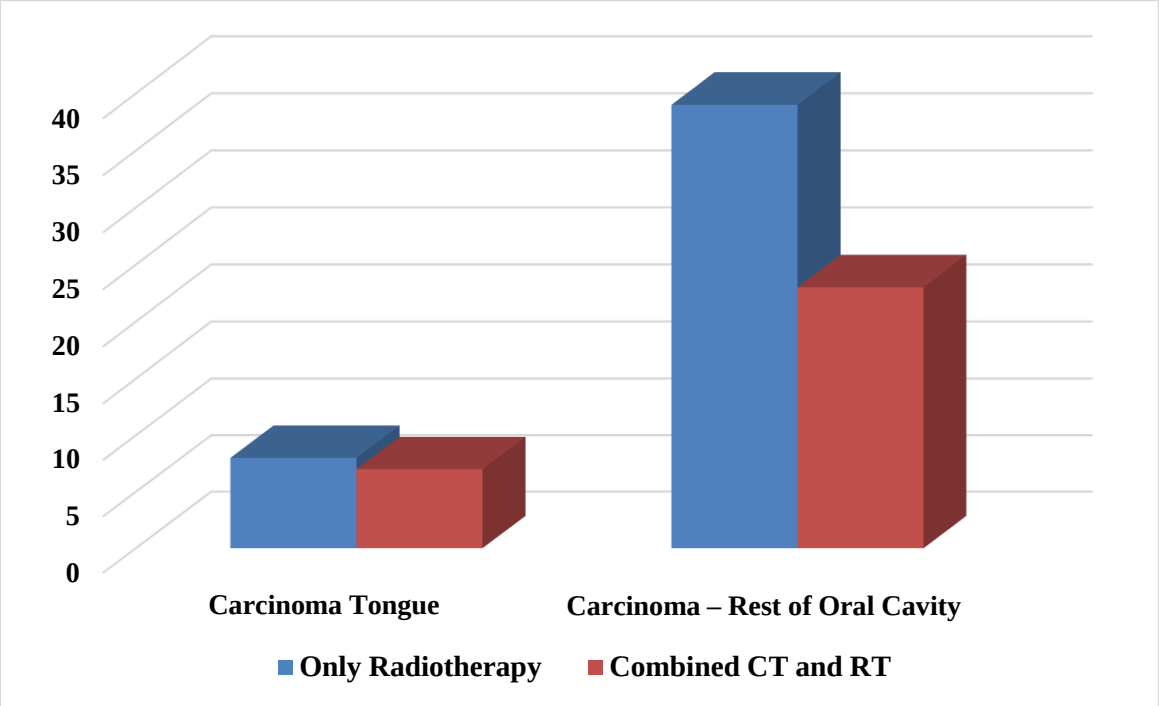

CHART 17: DISTRIBUTION OF SUBJECTS BASED ON ADJUVANT TREATMENT RECEIVED

Out of the 88 patients who were operated for oral malignancies, 77 patients (87.5%) required adjuvant treatment in the form of radiotherapy and chemotherapy.

100% of patients who presented with carcinoma tongue required post operative RT and CT.

5.17 DIFFERENTIATION OF CARCINOMA ON HPE

TABLE 21: DISTRIBUTION OF SUBJECTS WITH RESPECT TO DIFFERENTIATION OF CARCINOMA ON HPE

Level of Differentiation	Carcinoma Tongue	Carcinoma – Rest of Oral Cavity Subset	Total	Percentage (%)
Well Differentiated	5	50	55	62.5
Moderately Differentiated	10	22	32	36.4
Poorly Differentiated	0	1	1	1.1
TOTAL	15	73	88	100.0

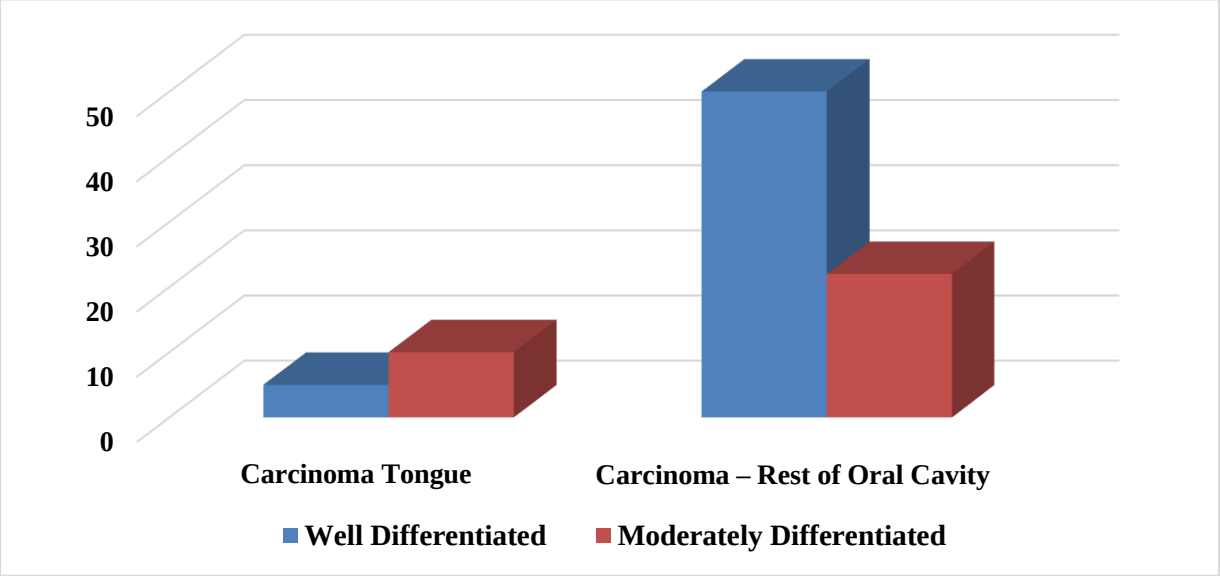

CHART 18: GRAPH SHOWING DISTRIBUTION OF SUBJECTS BASED ON PATHOLOGICAL TUMOUR DIFFERENTIATION

Nearly 2/3rd of the patients had well differentiated oral cavity malignancy on HPE with one patient having a poorly differentiated upper GB sulcus carcinoma.

5.18 VASCULAR/ NEURAL/ BONE INVASION ON HPE

TABLE 22: DISTRIBUTION OF PATIENTS BASED ON VASCULAR/ NEURAL/ BONE INVASION/ EXTRANODAL EXTENSION OF MALIGNANCY

Invasion	Carcinoma Tongue	Carcinoma – Rest of Oral Cavity Subset	Total
Neural	1	11	12
Vascular	0	4	4
Bone	3	6	9
Extranodal Extension	2	8	10
TOTAL	6	29	35

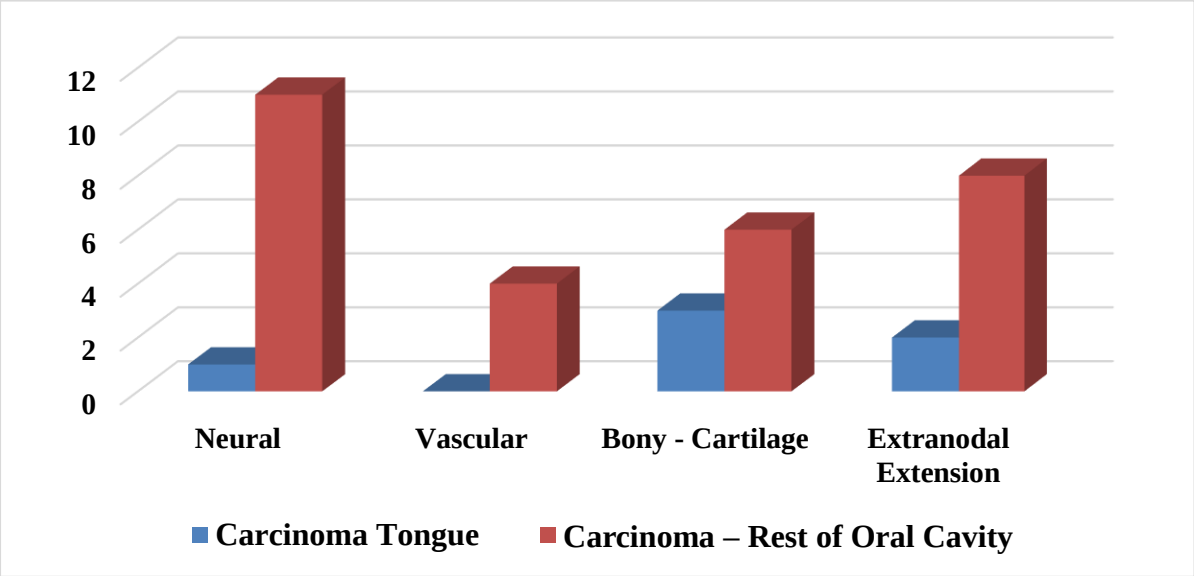

CHART 19: DISTRIBUTION OF SUBJECTS BASED ONNEURAL/VASCULAR/ BONE INVASION & EXTRANODAL EXTENSION

35 patients out of a total of 88 (39.7%) showed evidence of neural/vascular/ bone invasion/ extranodal extension on HPE.

Most common type of invasion noted on HPE was Neural (12/35) accounting for 34%

None of the patients with Tongue Carcinoma had Vascular Invasion on HPE.

5.19 DISEASE RECURRENCE

TABLE 23: DISTRIBUTION OF SUBJECTS ACCORDING TO RECURRENCE

Disease Status	Carcinoma Tongue	Carcinoma – Rest of Oral Cavity Subset	Total	Percentage (%)
Recurrence	2	4	6	6.8
Disease Free	11	55	66	75
Lost to Follow Up	1	8	9	10.3
Dead	1	6	7	7.9
TOTAL	15	73	88	100.0

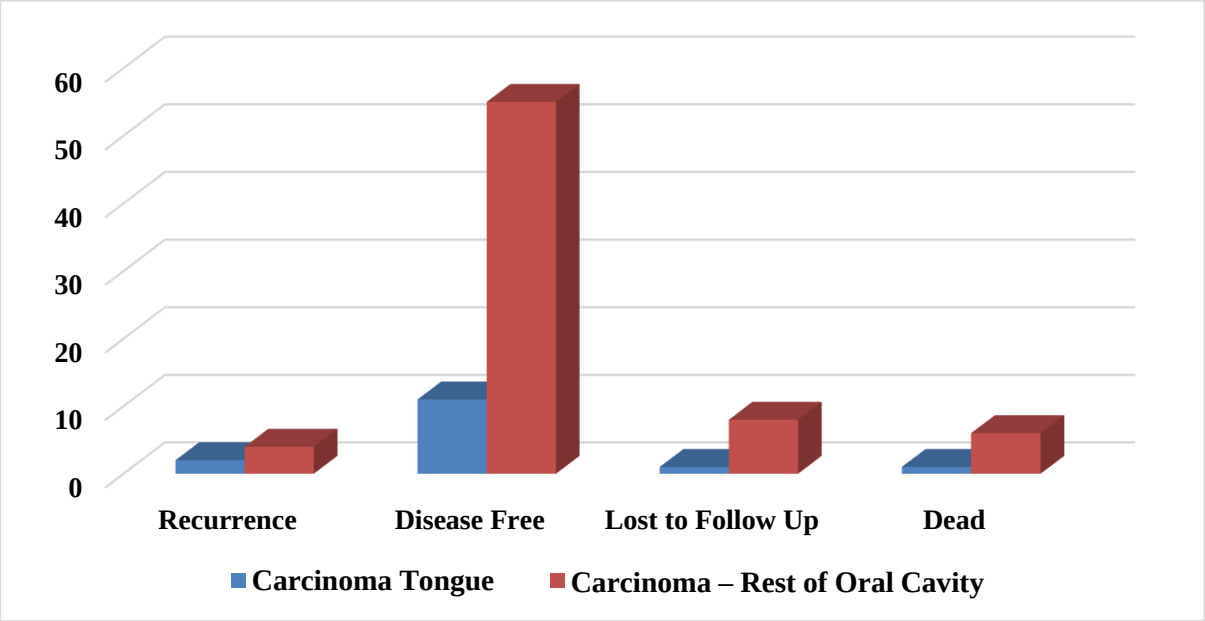

CHART 20: GRAPH SHOWING DISTRIBUTION OF SUBJECTS ACCORDING TO RECURRENCE

A total of 6 patients had recurrence among the total 88 patients while 75% patients remained disease free.

Mortality due to disease was noted in nearly 8% patients

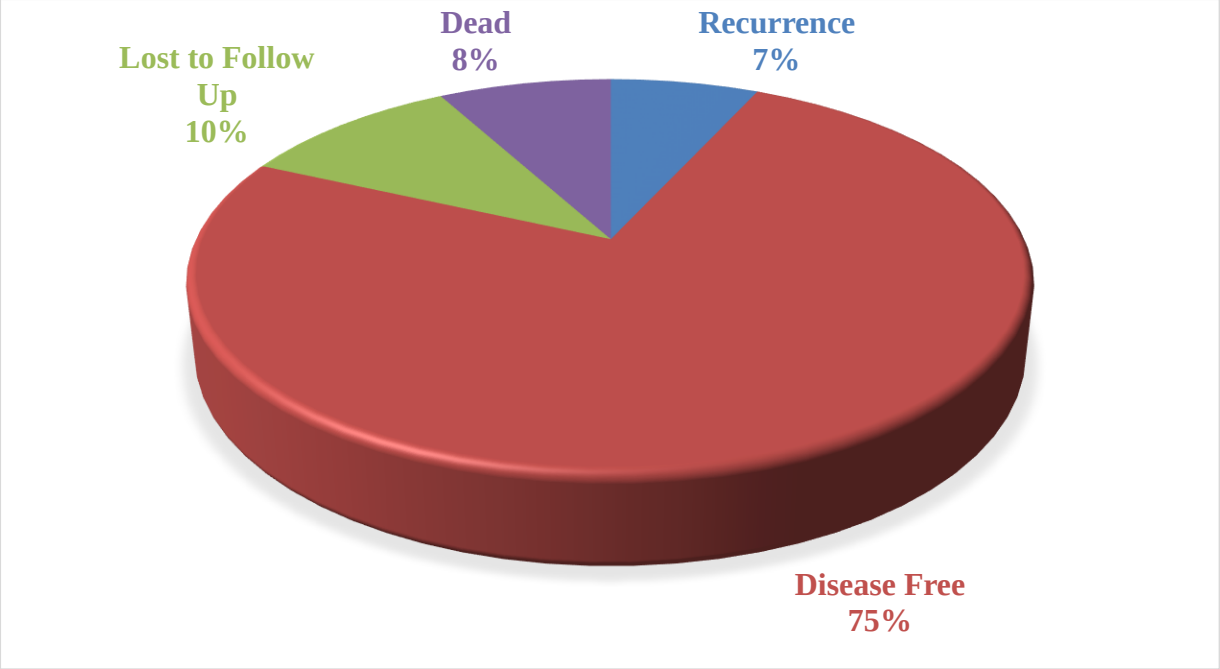

CHART 21: PIE CHART SHOWING OVERALL DISTRIBUTION OF PATIENTS BASED ON RECURRENCE

Out of the 7 patients who died in the study, 4 had mortality secondary to recurrence at a local/ distant site while the other 3 died due to other causes.

5.20 SITE OF DISEASE RECURRENCE

TABLE 24: DISTRIBUTION OF SUBJECTS ACCORDING TO SITE OF RECURRENCE

Site of Recurrence	Alive	Dead	Total	Percentage (%)
LOCAL	2	1	3	30
REGIONAL	1	1	2	20
LOCO-REGIONAL	1	0	1	10
DISTANT	2	2	4	40
TOTAL	6	4	10	100.0

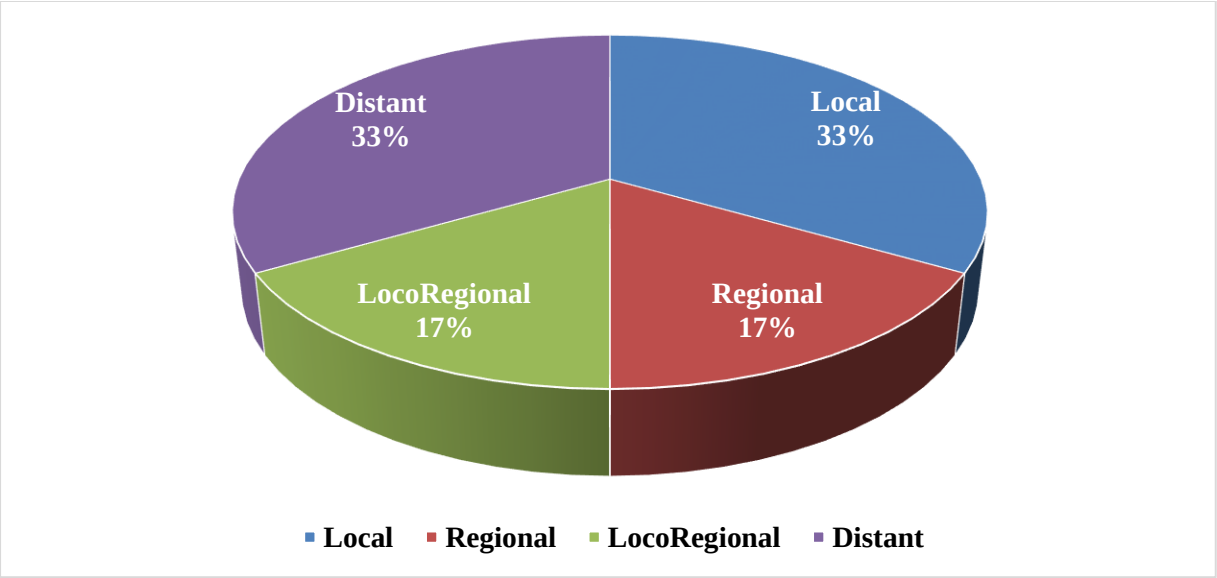

CHART 22: PIE CHART SHOWING SITE OF RECURRENCE AMONG ALIVE SUBJECTS

Among patients who are alive, 6 patients had recurrence with 33% patients have distant recurrence.

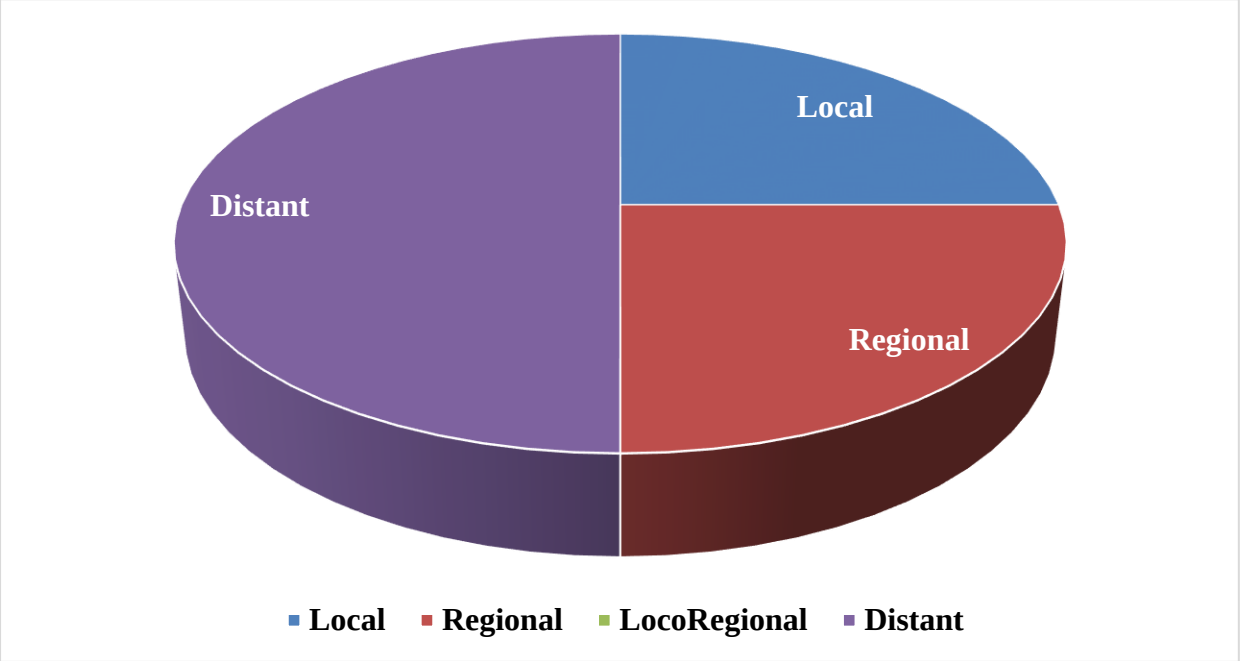

CHART 23: PIE CHART SHOWING SITE OF RECURRENCE AMONG DEAD SUBJECTS

Out of the 7 patients who died in the study, 4 had mortality secondary to recurrence at a local/ distant site. 2 patients had distant metastasis – to the lung and intracranial extension.

5.21 DEPTH OF INVASION OF TUMOUR ON HISTOPATHOLOGY

a) TABLE 25: DISTRIBUTION OF SUBJECTS ACCORDING TO DEPTH OF INVASION (DOI) OF TUMOUR ON HPE – CARCINOMA TONGUE

DOI – CARCINOMA TONGUE	Number of Patients	Percentage (%)
≤ 4mm	1	6.6
> 4 mm	14	93.4
TOTAL	15	100

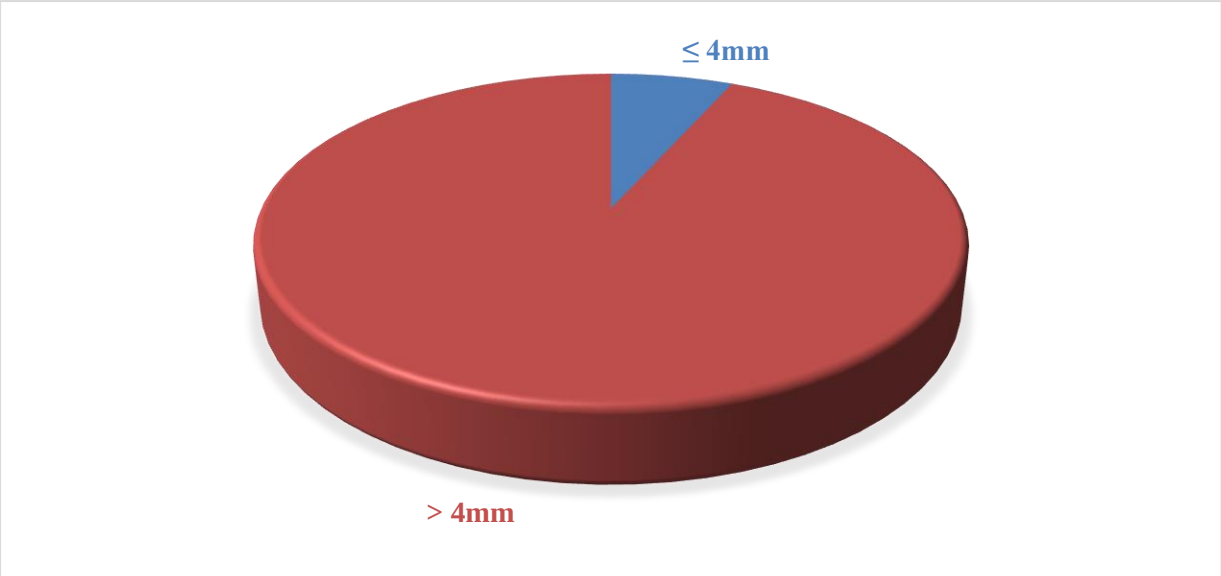

CHART 24: PIE CHART SHOWING DISTRIBUTION OF PATIENTS WITH CARCINOMA TONGUE BASED ON DEPTH OF INVASION (DOI)

93.4% patients with Carcinoma Tongue had > 4mm depth of invasion of tumour on HPE report – suggestive of aggressive behavior of malignancy.

b) TABLE 26: DISTRIBUTION OF SUBJECTS ACCORDING TO DEPTH OF INVASION (DOI) OF TUMOUR ON HPE – CARCINOMA REST OF ORAL CAVITY SUBSET

DOI – CARCINOMA REST OF ORAL CAVITY SUBSET	Number of Patients	Percentage (%)
≤ 5mm	15	20.5
>5 mm	58	79.5
TOTAL	73	100

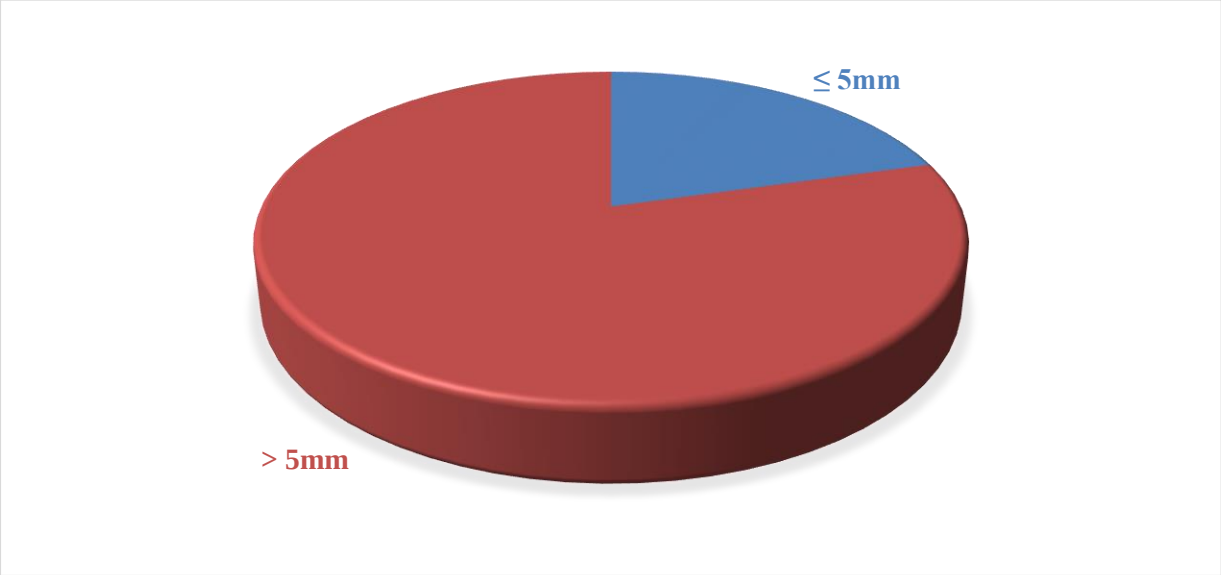

CHART 25: PIE CHART SHOWING DISTRIBUTION OF PATIENTS WITH CARCINOMA REST OF ORAL CAVITY SUBSET BASED ON DEPTH OF INVASION (DOI)

79.5% patients with Carcinoma Rest of Oral Cavity had > 5mm depth of invasion of tumour on HPE report – suggestive of aggressive behavior of malignancy.

5.22 NEUTROPHIL TO LYMPHOCYTE (N: L) RATIO

TABLE 27: DISTRIBUTION OF SUBJECTS BASED ON N: L RATIO

Cutoff N:L ratio of ≥ 2.93 taken in this study.

N:L Ratio	Carcinoma Tongue	Carcinoma – Rest of Oral Cavity Subset	Total	Percentage (%)
< 2.93	11	49	60	68.2
≥ 2.93	4	24	28	31.8
TOTAL	15	73	88	100.0

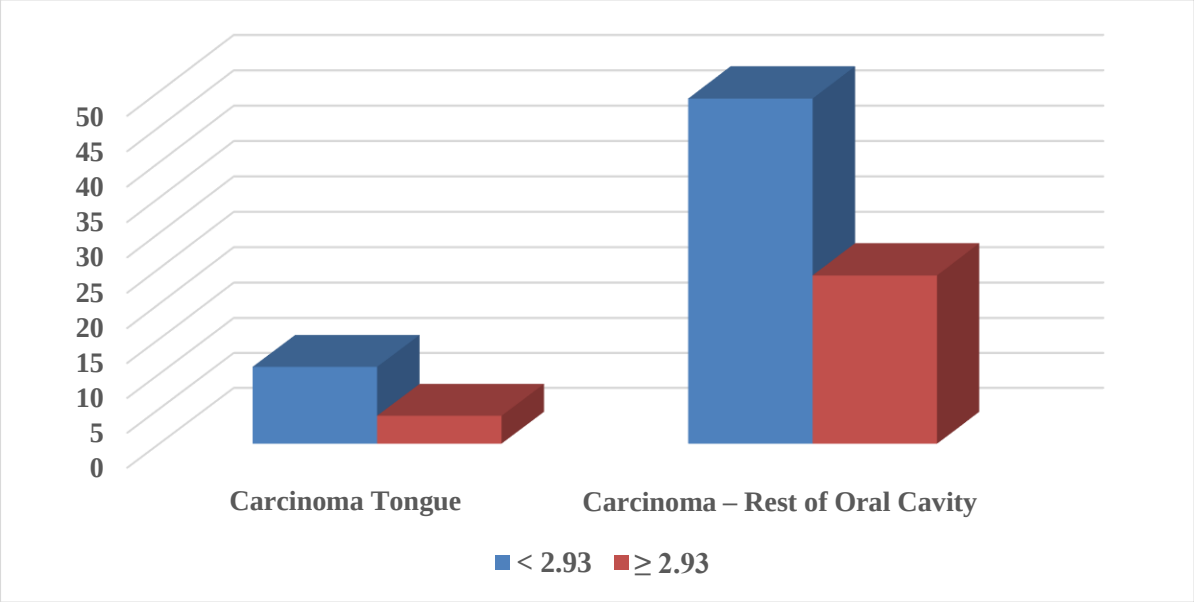

CHART 26: GRAPH REPRESENTING THE DISTRIBUTION OF SUBJECTS BASED ON N: L RATIO (CUT OFF RATIO 2.93)

68% of patients involved in this study had a N:L ratio below the cut off value of 2.93

The remaining 32% patients had N:L ratio above 2.93 which included 27% patients from Carcinoma Tongue group and 33% patients from Rest of Oral Cavity Carcinoma group.

5.23 NEUTROPHIL TO LYMPHOCYTE (N: L) RATIO IN PATIENTS WITH CARCINOMA TONGUE – BASED ON LYMPH NODE (N) STATUS

TABLE 28: DISTRIBUTION OF SUBJECTS WITH CARCIOMA TONGUE BASED ON MEAN N: L RATIO AND LYMPH NODE STATUS

LYMPH NODE STATUS	MEAN N: L RATIO	STANDARD DEVIATION	p Value
POSITIVE	3.88	3.53	**0.112**
NEGATIVE	2.2	1.08	

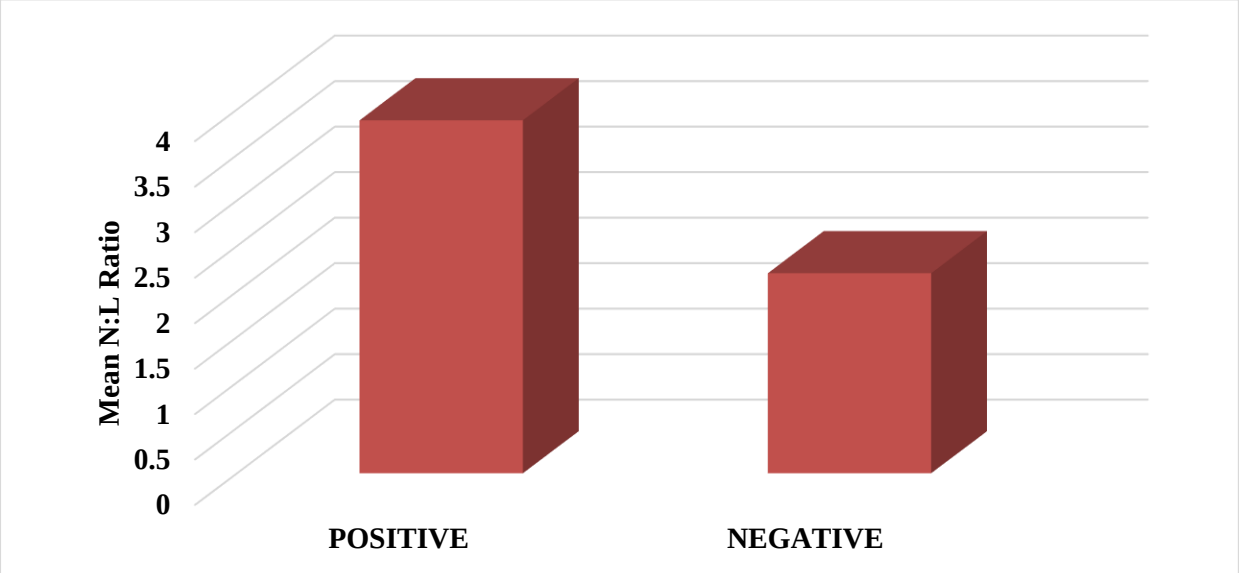

CHART 27: CHART REPRESENTING ASSOCIATION BETWEEN MEAN N: L RATIO & LYMPH NODE STATUS – CARCINOMA TONGUE

P value was noted to be 0.112 - there was no statistically significant relationship between NLR and Lymph node status in patients with Carcinoma Tongue.

5.24 NEUTROPHIL TO LYMPHOCYTE (N: L) RATIO IN PATIENTS WITH CARCINOMA REST OF ORAL CAVITY SUBSET – BASED ON LYMPH NODE (N) STATUS

TABLE 29: DISTRIBUTION OF SUBJECTS WITH CARCIOMA REST OF ORAL CAVITY SUBSET BASED ON MEAN N: L RATIO AND LYMPH NODE STATUS

LYMPH NODE STATUS	MEAN N: L RATIO	STANDARD DEVIATION	p Value
POSITIVE	2.77	2.17	**0.183**
NEGATIVE	5.21	8.8	

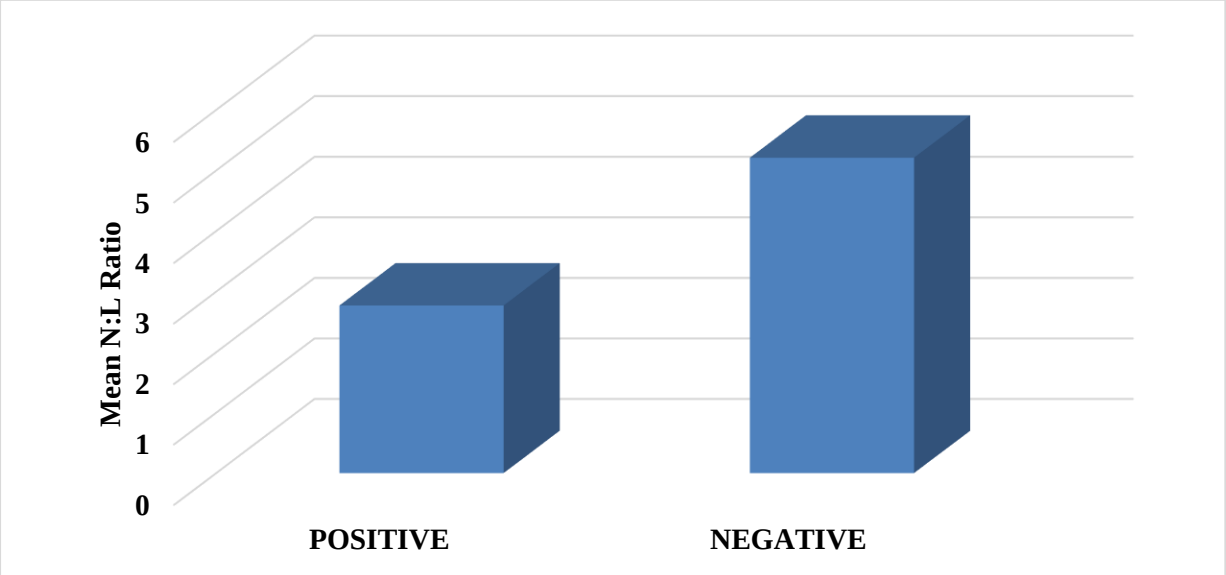

CHART 28: CHART REPRESENTING ASSOCIATION BETWEEN MEAN N: L RATIO & LYMPH NODE STATUS – CARCINOMA REST OF ORAL CAVITY SUBSET

P value was noted to be 0.183 - there was no statistically significant relationship between NLR and Lymph node status in patients with Carcinoma Rest of Oral Cavity.

TABLE 30: COMPARATIVE TABLE OF CLINICOPATHOLOGICAL PARAMETERS BASED ON pN LYMPH NODE STATUS

		pN0 Group (n=44)	pN+ Group (n=44)
GENDER	Male	15	19
	Female	29	25
AGE (YEARS)	Mean	56.1	54.2
SUBSET INVOLVED	Carcinoma Tongue	8	7
	Carcinoma – Rest of Oral Cavity	36	37
DOI (mm)	Mean	11.1	12.3
N:L RATIO	Mean	4.65	2.9
BONE INVASION	Present	2	7
	Absent	42	37
VASCULAR INVASION	Present	0	4
	Absent	44	40
PERINEURAL INVASION	Present	7	5
	Absent	37	39

5.25 NEUTROPHIL TO LYMPHOCYTE (N: L) RATIO IN PATIENTS WITH CARCINOMA TONGUE – BASED ON NEURAL INVASION ON HPE

TABLE 31: COMPARISON OF N: L RATIO ACCORDING TO NEURAL INVASION AMONG SUBJECTS WHO HAD CARCINOMA TONGUE

NEURAL INVASION	MEAN N: L RATIO	STANDARD DEVIATION	p Value
POSITIVE	1.60	-	**0.597**
NEGATIVE	3.09	2.6	

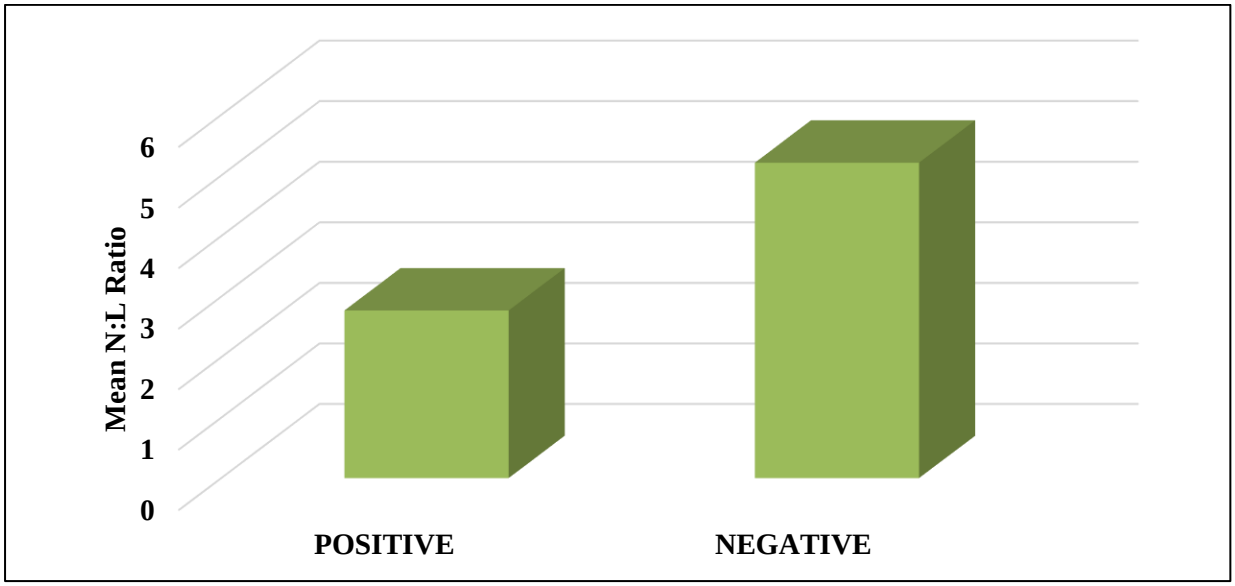

CHART 29: CHART REPRESENTING ASSOCIATION BETWEEN MEAN N: L RATIO & NEURAL INVASION – CARCINOMA TONGUE

P value was noted to be 0.597 - there was no statistically significant relationship between mean NLR and Neural invasion status in patients with Carcinoma Tongue.

5.26 NEUTROPHIL TO LYMPHOCYTE (N: L) RATIO IN PATIENTS WITH CARCINOMA REST OF ORAL CAVITY – BASED ON NEURAL INVASION ON HPE

TABLE 32: COMPARISON OF N: L RATIO ACCORDING TO NEURAL INVASION AMONG SUBJECTS WHO HAD CARCINOMA REST OF ORAL CAVITY

NEURAL INVASION	MEAN N: L RATIO	STANDARD DEVIATION	p Value
POSITIVE	2.73	1.1	**0.547**
NEGATIVE	4.14	6.9	

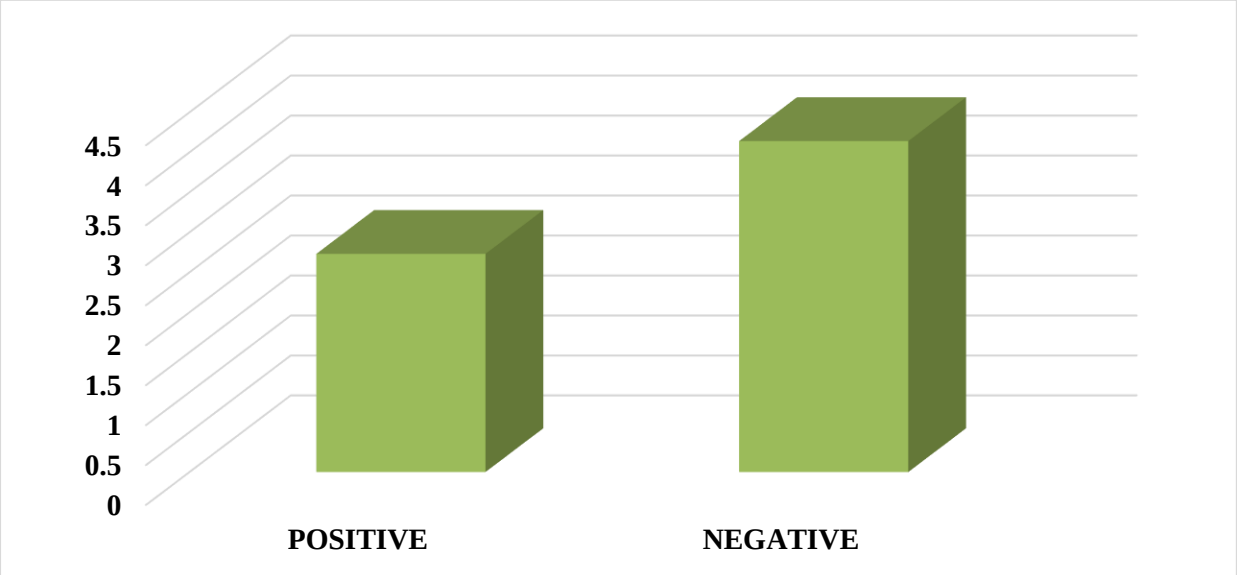

CHART 30: CHART REPRESENTING ASSOCIATION BETWEEN MEAN N: L RATIO & NEURAL INVASION – CARCINOMA REST OF ORAL CAVITY

P value was noted to be 0.547 - there was no statistically significant relationship between mean NLR and Neural invasion status in patients with Carcinoma Rest of Oral Cavity.

5.27 NEUTROPHIL TO LYMPHOCYTE (N: L) RATIO IN PATIENTS WITH CARCINOMA TONGUE – BASED ON BONE/ CARTILAGE INVASION ON HPE

TABLE 33: COMPARISON OF N:L RATIO ACCORDING TO BONE INVASION AMONG SUBJECTS WHO HAD CARCINOMA TONGUE

BONE INVASION	MEAN N: L RATIO	STANDARD DEVIATION	p Value
POSITIVE	2.16	0.6	**0.556**
NEGATIVE	3.20	2.8	

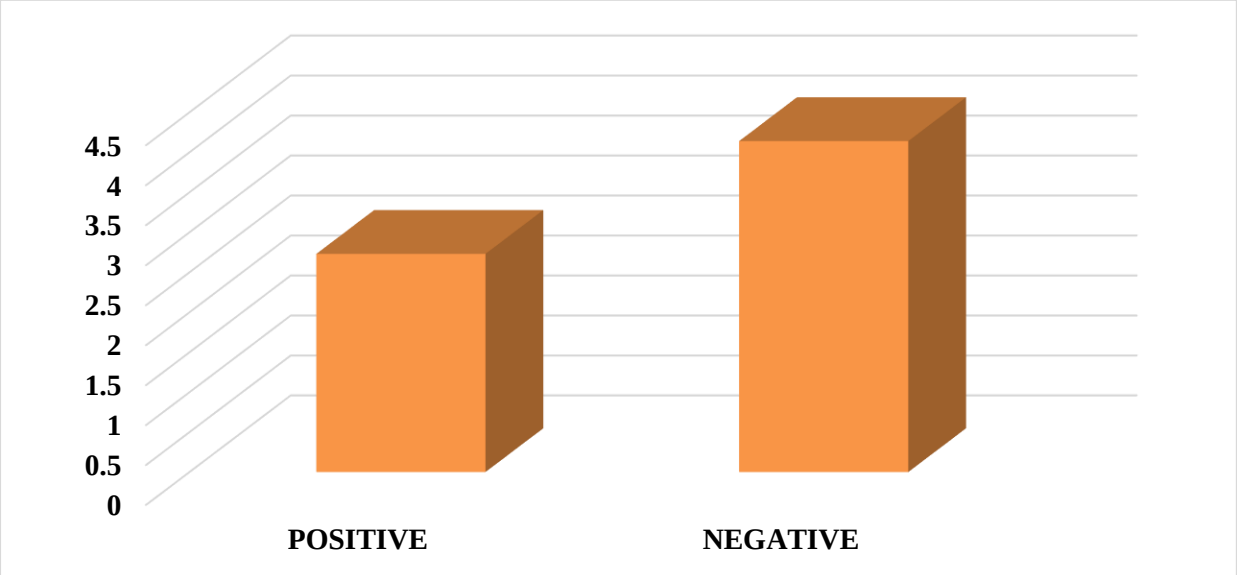

CHART 31: CHART REPRESENTING ASSOCIATION BETWEEN MEAN N: L RATIO & BONE INVASION – CARCINOMA TONGUE

P value was noted to be 0.556 - there was no statistically significant relationship between mean NLR and Bone/ Cartilage invasion status in patients with Carcinoma Tongue.

5.28 NEUTROPHIL TO LYMPHOCYTE (N: L) RATIO IN PATIENTS WITH CARCINOMA REST OF ORAL CAVITY – BASED ON BONE/ CARTILAGE INVASION ON HPE

TABLE 34: COMPARISON OF N:L RATIO ACCORDING TO BONE INVASION AMONG SUBJECTS WHO HAD CARCINOMA REST OF ORAL CAVITY

BONE INVASION	MEAN N: L RATIO	STANDARD DEVIATION	p Value
POSITIVE	6.18	8.4	**0.388**
NEGATIVE	3.77	6.3	

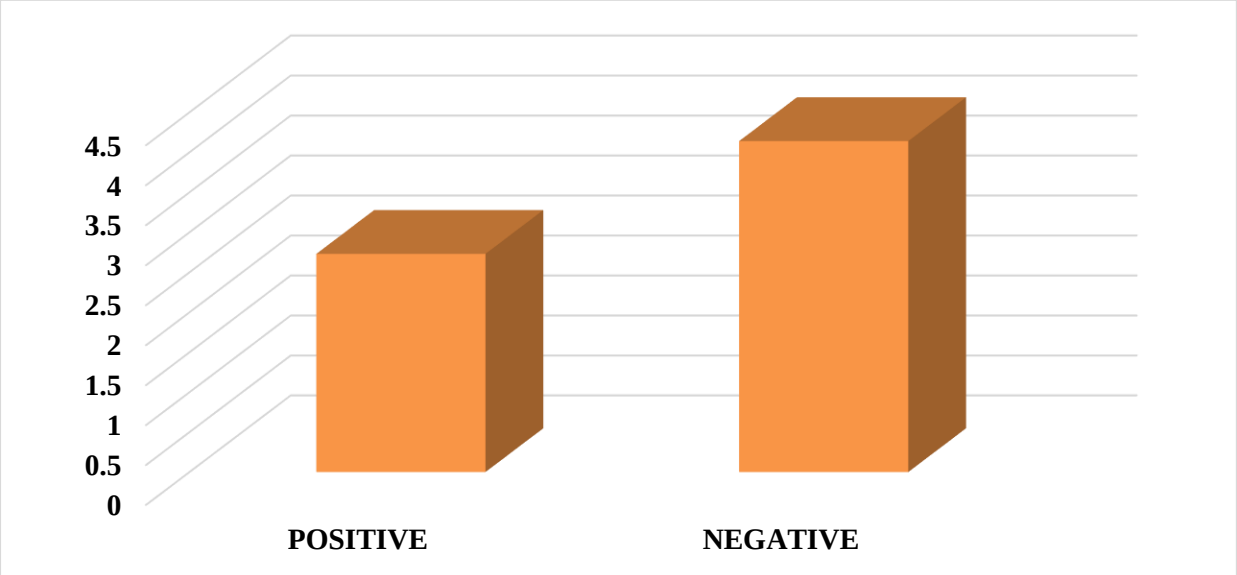

CHART 32: CHART REPRESENTING ASSOCIATION BETWEEN MEAN N: L RATIO & BONE/ CARTILAGE INVASION – CARCINOMA REST OF ORAL CAVITY

P value was noted to be 0.388 - there was no statistically significant relationship between mean NLR and Bone/ Cartilage invasion status in patients with Carcinoma Rest of Oral Cavity.

5.29 NEUTROPHIL TO LYMPHOCYTE (N: L) RATIO IN PATIENTS WITH CARCINOMA TONGUE – BASED ON VASCULAR INVASION ON HPE

None of the patients belonging to Carcinoma Tongue group showed Vascular Invasion on Histopathological Examination Report.

5.30 NEUTROPHIL TO LYMPHOCYTE (N: L) RATIO IN PATIENTS WITH CARCINOMA REST OF ORAL CAVITY – BASED ON VASCULAR INVASION ON HPE

TABLE 35: COMPARISON OF N: L RATIO ACCORDING TO VASCULAR INVASION AMONG SUBJECTS WHO HAD CARCINOMA REST OF ORAL CAVITY

VASCULAR INVASION	MEAN N: L RATIO	STANDARD DEVIATION	p Value
POSITIVE	2.47	2.3	**0.639**
NEGATIVE	4.05	6.6	

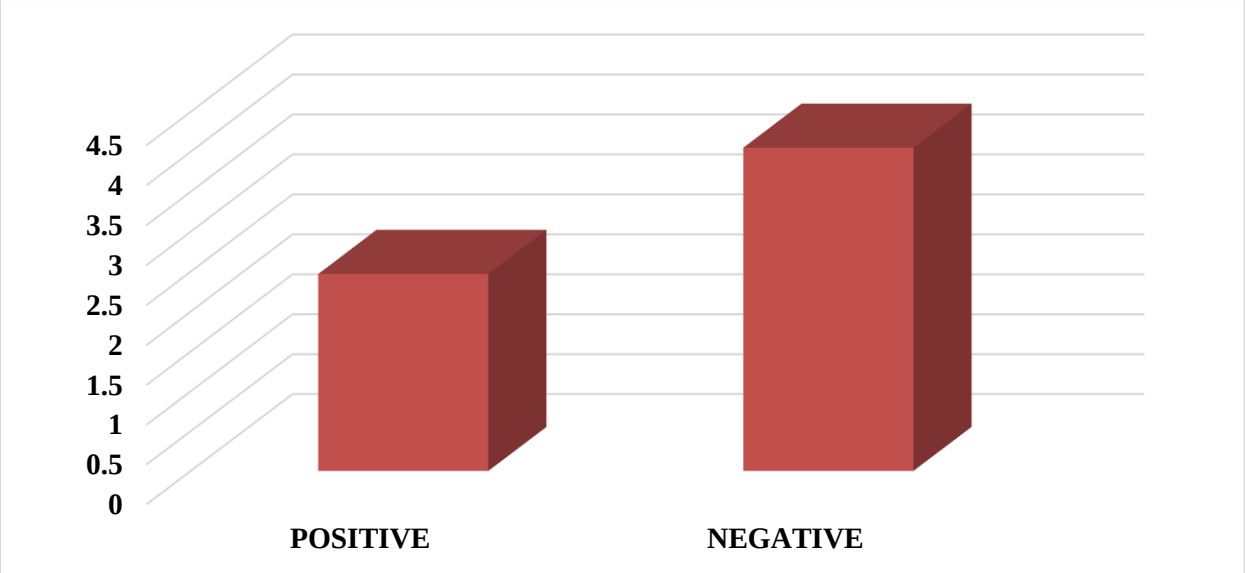

CHART 33: CHART REPRESENTING ASSOCIATION BETWEEN MEAN N:L RATIO & VASCULAR INVASION – CARCINOMA REST OF ORAL CAVITY

P value was noted to be 0.639 - there was no statistically significant relationship between mean NLR and Vascular invasion status in patients with Carcinoma Rest of Oral Cavity.

5.31 NEUTROPHIL TO LYMPHOCYTE (N: L) RATIO IN PATIENTS WITH CARCINOMA TONGUE – BASED ON RECURRENCE AMONG SUBJECTS

TABLE 36: COMPARISON OF N: L RATIO ACCORDING TO DISEASE FREE AMONG SUBJECTS WHO HAD CARCINOMA TONGUE

DISEASE RECURRENCE	MEAN N: L RATIO	STANDARD DEVIATION	p Value
PRESENT	2.8	0.9	**0.416**
ABSENT	3.28	2.9	

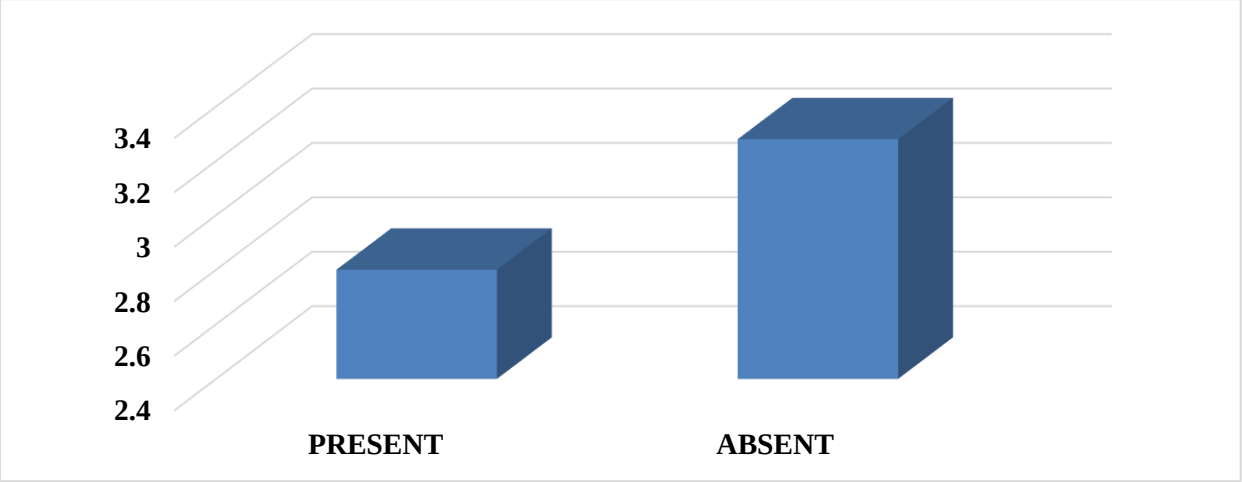

CHART 34: CHART REPRESENTING ASSOCIATION BETWEEN MEAN N: L RATIO & RECURRENCE STATUS – CARCINOMA TONGUE

P value was noted to be 0.416 - there was no statistically significant relationship between mean NLR and Disease Recurrence status in patients with Carcinoma Tongue.

5.32 NEUTROPHIL TO LYMPHOCYTE (N: L) RATIO IN PATIENTS WITH CARCINOMA REST OF ORAL CAVITY – BASED ON RECURRENCE AMONG SUBJECTS

TABLE 37: COMPARISON OF N: L RATIO ACCORDING TO RECURRENCE AMONG SUBJECTS WHO HAD CARCINOMA REST OF ORAL CAVITY

DISEASE RECURRENCE	MEAN N: L RATIO	STANDARD DEVIATION	p Value
PRESENT	1.78	0.6	**0.248**
ABSENT	4.28	7.2	

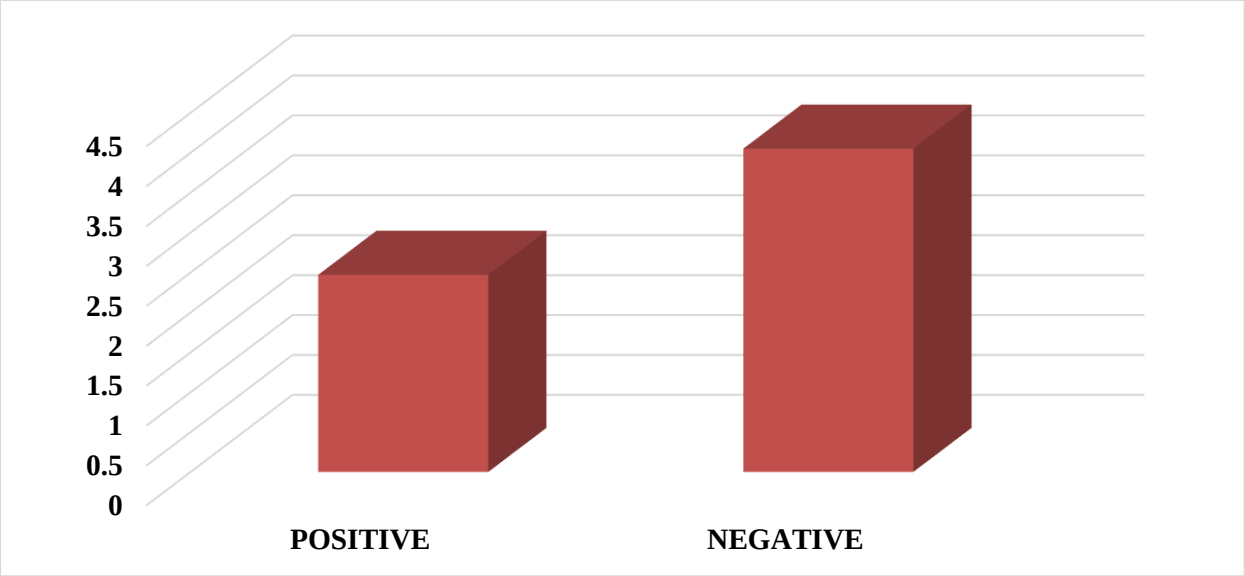

CHART 35: CHART REPRESENTING ASSOCIATION BETWEEN MEAN N: L RATIO & RECURRENCE STATUS – CARCINOMA REST OF ORAL CAVITY

P value was noted to be 0.248 - there was no statistically significant relationship between mean NLR and Disease Recurrence status in patients with Carcinoma Rest of Oral Cavity.

5.33 NEUTROPHIL TO LYMPHOCYTE (N: L) RATIO IN PATIENTS WITH CARCINOMA TONGUE – BASED ON PATHOLOGICAL T STAGING

TABLE 38: DISTRIBUTION OF SUBJECTS WITH CARCIOMA TONGUE BASED ON N: L RATIO AND PATHOLOGICAL T STAGE

CARCINOMA TONGUE	<2.93	≥2.93	Total	Percentage (%)
T2	5	0	5	33.3
T3	4	3	7	46.7
T4a	2	1	3	20
TOTAL	11	4	15	100

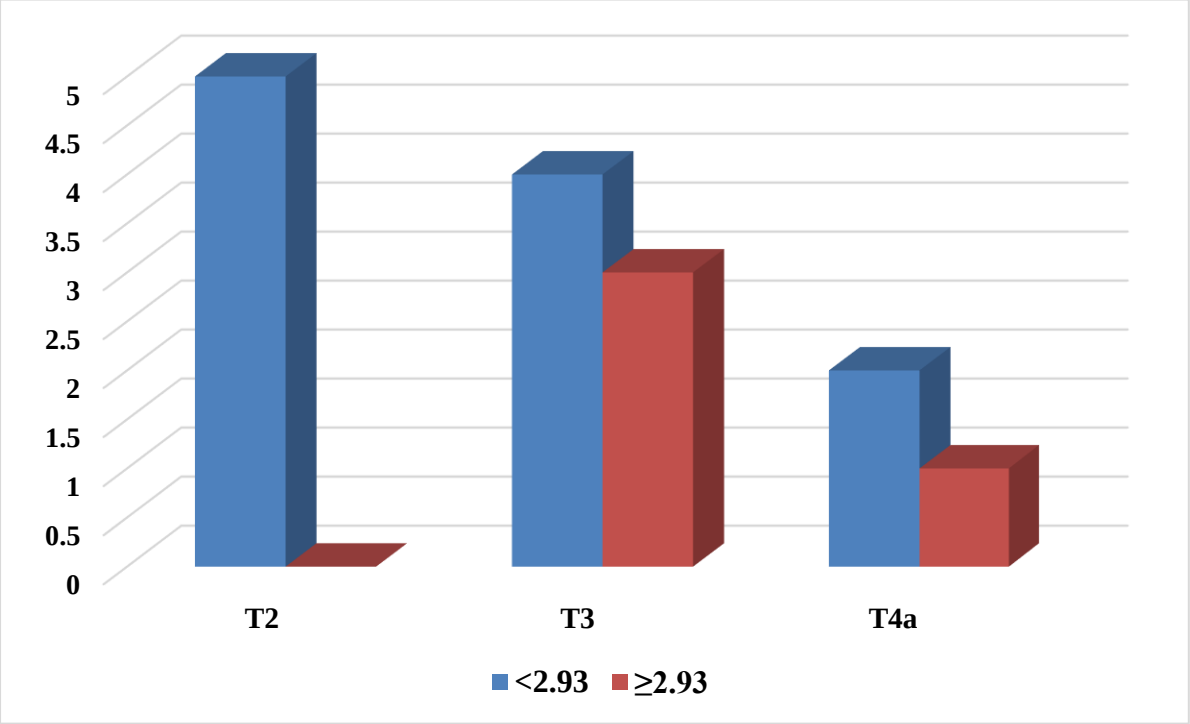

CHART 36: CHART REPRESENTING ASSOCIATION BETWEEN N:L RATIO (CUT OFF 2.93) AND PATHOLOGICAL T STAGING IN SUBJECTS WITH CARCINOMA TONGUE

Out of the 4 patients with Carcinoma Tongue who had N:L ratio above 2.93 – 75% belonged to T3 pathological stage while 25% belonged to pT4a stage.

5.34 NEUTROPHIL TO LYMPHOCYTE (N: L) RATIO IN PATIENTS WITH CARCINOMA REST OF THE ORAL CAVITY – BASED ON PATHOLOGICAL T STAGING

TABLE 39: DISTRIBUTION OF SUBJECTS WITH CARCIOMA REST OF ORAL CAVITY BASED ON N: L RATIO AND PATHOLOGICAL T STAGE

CARCINOMA REST OF ORAL CAVITY	<2.93	≥2.93	Total	Percentage (%)
T2	23	9	32	43.8
T3	12	4	16	21.9
T4a	14	11	25	34.3
TOTAL	49	24	73	100

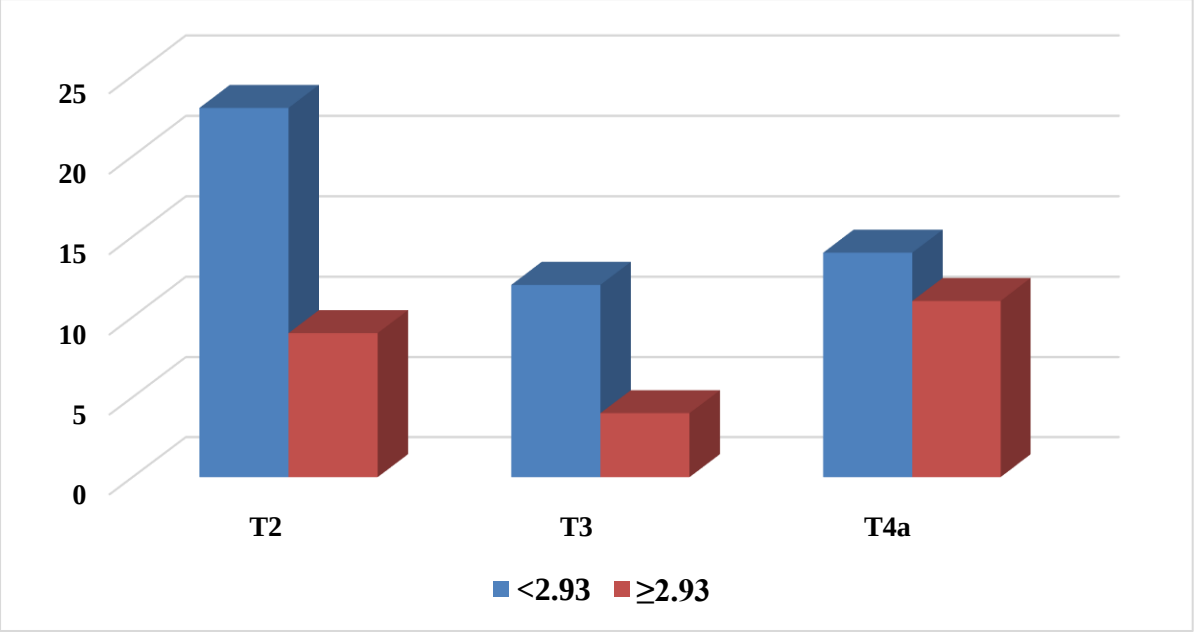

CHART 37: CHART REPRESENTING ASSOCIATION BETWEEN N:L RATIO (CUT OFF 2.93) AND PATHOLOGICAL T STAGING IN SUBJECTS WITH CARCINOMA REST OF ORAL CAVITY

Out of the 24 patients with Carcinoma Rest of Oral Cavity who had N:L ratio above 2.93 – 46% belonged to T4a pathological stage.

5.35 NEUTROPHIL TO LYMPHOCYTE (N: L) RATIO IN PATIENTS WITH CARCINOMA TONGUE – BASED ON PATHOLOGICAL LYMPH NODE STATUS

TABLE 40: DISTRIBUTION OF SUBJECTS WITH CARCIOMA TONGUE BASED ON N: L RATIO AND PATHOLOGICAL LN STATUS

LYMPH NODE STATUS	<2.93	≥2.93	Total	Percentage (%)
POSITIVE	4	3	7	46.7
NEGATIVE	7	1	8	53.3
TOTAL	11	4	15	100

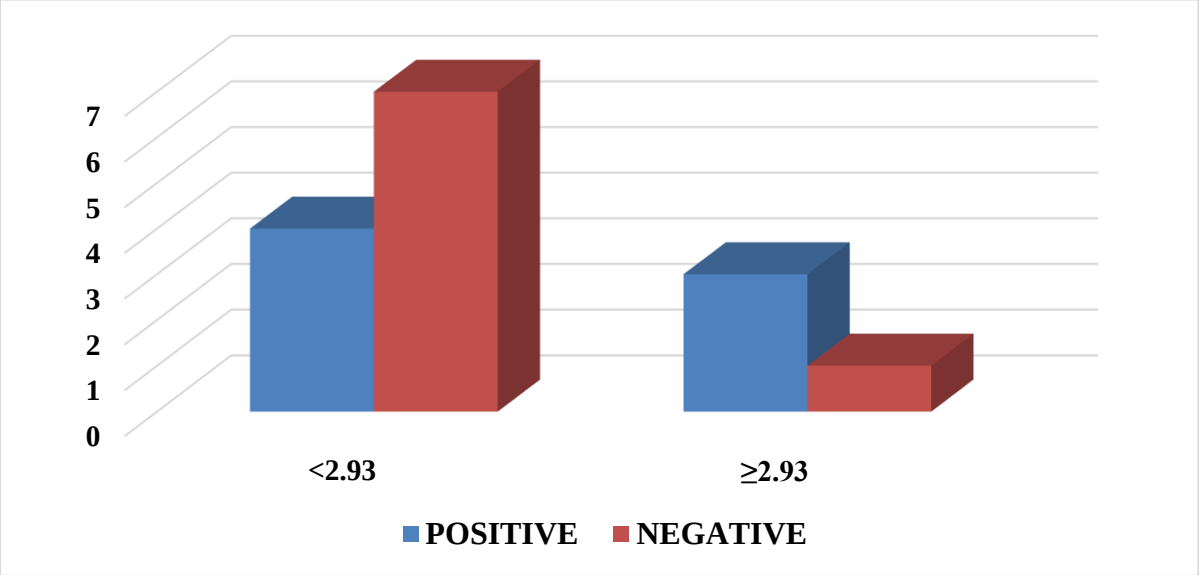

CHART 38: CHART REPRESENTING ASSOCIATION BETWEEN N:L RATIO (CUT OFF 2.93) AND PATHOLOGICAL LYMPH NODE STATUS IN SUBJECTS WITH CARCINOMA TONGUE

Out of the 7 patients with Carcinoma Tongue who had positive lymph node involvement pathologically – 43% had NLR ≥ 2.93.

5.36 NEUTROPHIL TO LYMPHOCYTE (N: L) RATIO IN PATIENTS WITH CARCINOMA REST OF ORAL CAVITY – BASED ON PATHOLOGICAL LYMPH NODE STATUS

TABLE 41: DISTRIBUTION OF SUBJECTS WITH CARCIOMA REST OF ORAL CAVITY BASED ON N: L RATIO AND PATHOLOGICAL LN STATUS

LYMPH NODE STATUS	<2.93	≥2.93	Total	Percentage (%)
POSITIVE	25	12	37	50.7
NEGATIVE	24	12	36	49.3
TOTAL	49	24	73	100

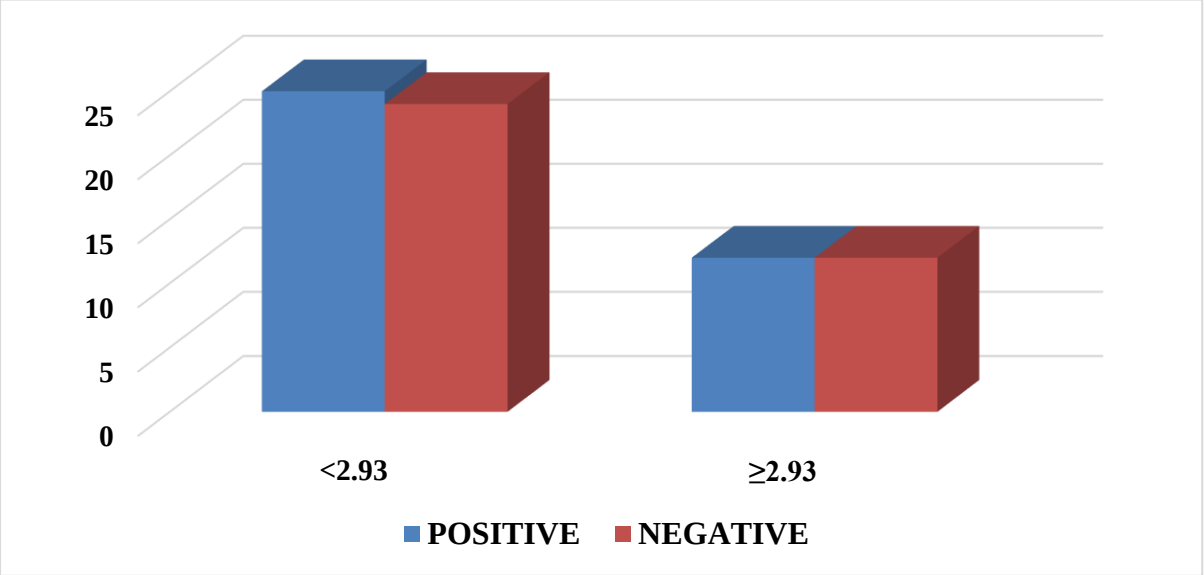

CHART 39: CHART REPRESENTING ASSOCIATION BETWEEN N:L RATIO (CUT OFF 2.93) AND PATHOLOGICAL LYMPH NODE STATUS IN SUBJECTS WITH CARCINOMA REST OF ORAL CAVITY

Out of the 37 patients with Carcinoma Rest of Oral Cavity who had positive lymph node involvement pathologically – 32.4% had NLR ≥ 2.93.

5.37 RELATION BETWEEN NEUTROPHIL TO LYMPHOCYTE (N: L) RATIO ≥2.93 AND DEPTH OF INVASION IN PATIENTS WITH CARCINOMA TONGUE

TABLE 42: DISTRIBUTION OF SUBJECTS WITH CARCIOMA TONGUE BASED ON N: L RATIO ≥2.93 AND DEPTH OF INVASION

CARCINOMA TONGUE	N: L Ratio ≥2.93		Total	Percentage (%)
	DOI ≤ 4mm	DOI > 4mm		
T2	1	0	1	20
T3	0	3	3	60
T4a	0	1	1	20
TOTAL	1	4	5	100

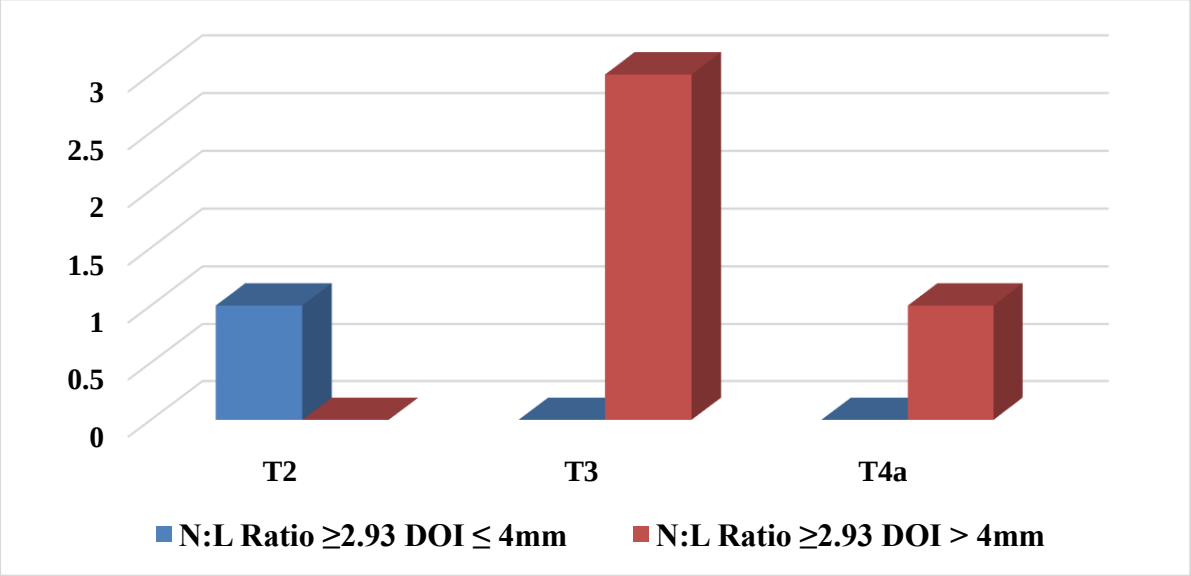

CHART 40: CHART REPRESENTING ASSOCIATION BETWEEN N:L RATIO ≥2.93 AND DEPTH OF INVASION IN SUBJECTS WITH CARCINOMA TONGUE

Out of the 15 patients with Carcinoma Tongue, 5 patients had N:L Ratio ≥ 2.93 - 80% of the these patients had DOI > 4mm on HPE report.

Further all the 4 patients were noted to have T3 and above disease stage.

TABLE 43: DISTRIBUTION OF SUBJECTS WITH CARCIOMA TONGUE BASED ON N: L RATIO AND DEPTH OF INVASION

N: L Ratio	DOI ≤ 4mm	DOI >4mm	Total	Percentage (%)
< 2.93	1	10	11	73.3
≥ 2.93	0	4	4	26.7
TOTAL	1	14	15	100

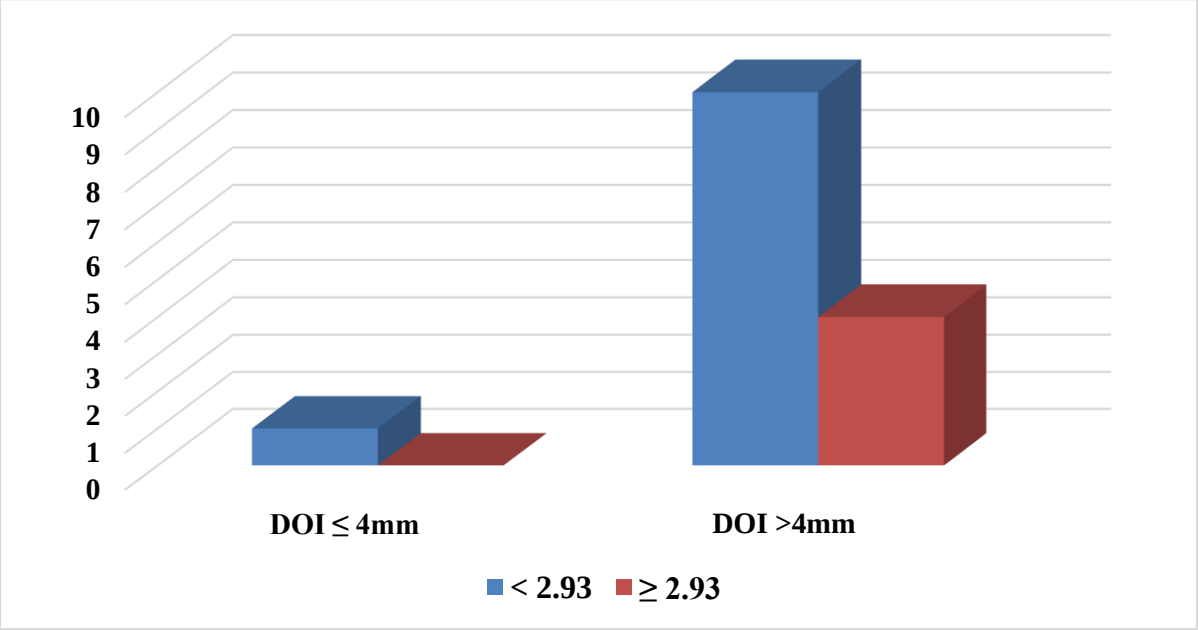

CHART 41: CHART REPRESENTING ASSOCIATION BETWEEN N:L RATIO AND DEPTH OF INVASION IN SUBJECTS WITH CARCINOMA TONGUE

93.3% patients with Carcinoma Tongue had Depth of Invasion ≥ 4mm on HPE report.

28% of these patients had a N: L Ratio ≥ 2.93.

5.38 RELATION BETWEEN NEUTROPHIL TO LYMPHOCYTE (N: L) RATIO ≥2.93 AND DEPTH OF INVASION IN PATIENTS WITH CARCINOMA REST OF ORAL CAVITY

TABLE 44: DISTRIBUTION OF SUBJECTS WITH CARCIOMA REST OF ORAL CAVITY BASED ON N: L RATIO ≥2.93 AND DEPTH OF INVASION

CARCINOMA REST OF ORAL CAVITY	N: L Ratio ≥2.93		Total	Percentage (%)
	DOI ≤ 5mm	DOI > 5mm		
T2	3	6	9	37.5
T3	0	4	4	16.7
T4a	0	11	11	45.8
TOTAL	3	21	24	100

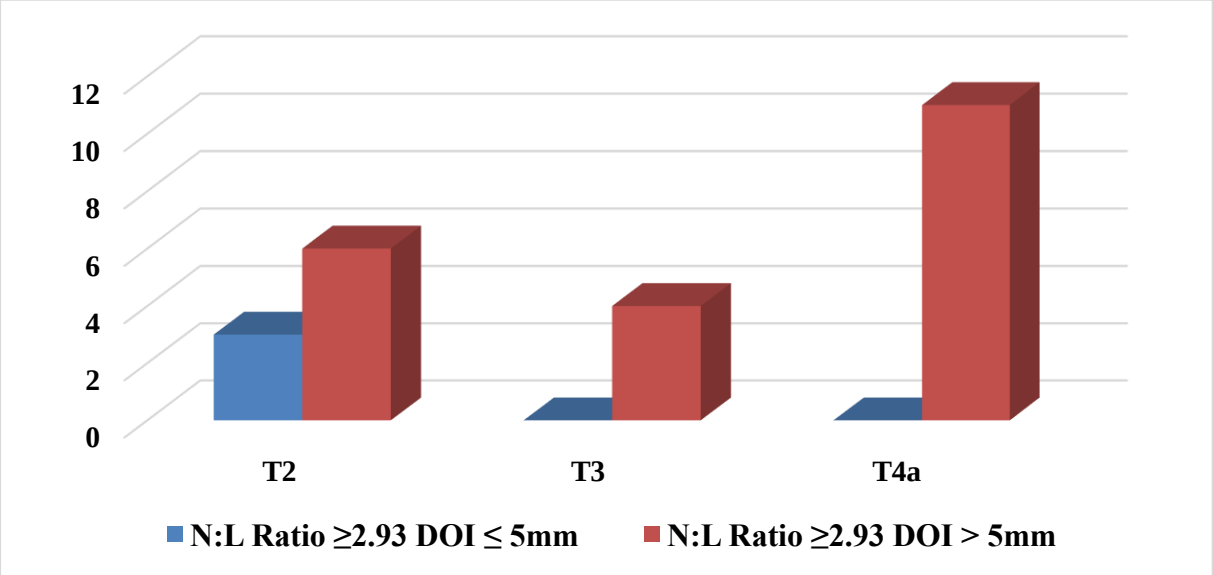

CHART 42: CHART REPRESENTING ASSOCIATION BETWEEN N: L RATIO ≥2.93 AND DEPTH OF INVASION IN SUBJECTS WITH CARCINOMA REST OF ORAL CAVITY

Out of the 73 patients with Carcinoma Rest of Oral Cavity, 24 patients had N: L Ratio ≥ 2.93 - 87.5% of these patients had DOI ≥ 5mm on HPE report.

Further 62.5% of the patients with ≥ 2.93 N: L Ratio were noted to have T3 and above disease stage.

TABLE 45: DISTRIBUTION OF SUBJECTS WITH CARCINOMA REST OF ORAL CAVITY BASED ON N: L RATIO AND DEPTH OF INVASION

N: L Ratio	DOI ≤ 5mm	DOI > 5mm	Total	Percentage (%)
< 2.93	12	37	49	67.1
≥ 2.93	3	21	24	32.9
TOTAL	15	58	73	100

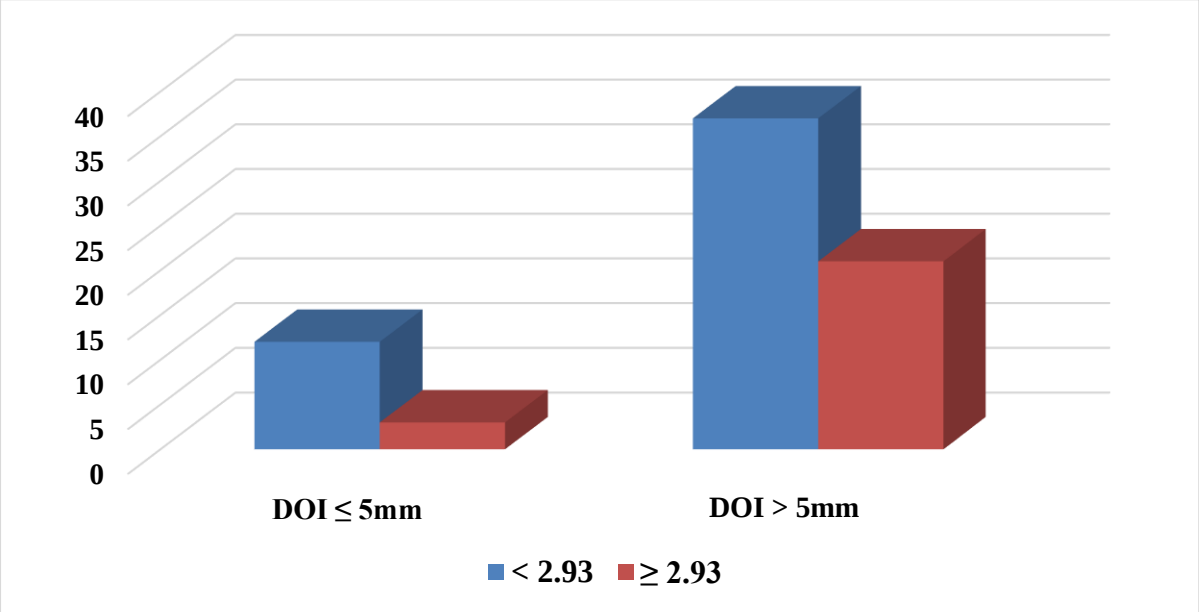

CHART 43: CHART REPRESENTING ASSOCIATION BETWEEN N:L RATIO AND DEPTH OF INVASION IN SUBJECTS WITH CARCINOMA REST OF ORAL CAVITY

P value was noted to be 0.467 – no statistically significant relationship noted between the DOI and NLR ratio (based on cut off 2.93) for subjects with Carcinoma Rest of Oral Cavity.

36.2% of patients with DOI > 5mm had a N:L ratio ≥ 2.93 – however this was not statistically significant.

5.39 RELATION BETWEEN N: L RATIO AND NEURAL/ VASCULAR/ BONE/ EXTRANODAL INVASION – CARCINOMA TONGUE

TABLE 46: DISTRIBUTION OF SUBJECTS WITH CARCIOMA TONGUE BASED ON N: L RATIO AND TYPE OF INVASION

CARCINOMA TONGUE		NLR < 2.93		NLR ≥ 2.93		Total	P Value
		N	%	N	%		
Extranodal Extension	PRESENT	2	18.2	0	0	15	**1.00**
	ABSENT	9	81.8	4	100		
Neural Invasion	YES	1	9.1	0	0	15	**1.00**
	NO	10	90.1	4	100		
Bone Invasion	YES	3	27.3	0	0	15	**0.516**
	NO	8	72.7	4	100		
Vascular Invasion	YES	0	0	0	0	15	-
	NO	11	100	4	100		

5.40 RELATION BETWEEN N:L RATIO AND NEURAL/ VASCULAR/ BONE/ EXTRANODAL INVASION – CARCINOMA REST OF ORAL CAVITY

TABLE 47: DISTRIBUTION OF SUBJECTS WITH CARCIOMA REST OF ORAL CAVITY BASED ON N:L RATIO AND TYPE OF INVASION

CARCINOMA REST OF ORAL CAVITY		NLR < 2.93		NLR ≥ 2.93		Total	P Value
		N	%	N	%		
Extranodal Extension	PRESENT	8	16.3	0	0	73	**0.047**
	ABSENT	41	83.7	24	100		
Neural Invasion	YES	7	14.3	4	16.7	73	**1.00**
	NO	42	85.7	20	83.3		
Bone Invasion	YES	4	8.2	2	8.3	73	**1.00**
	NO	45	91.8	22	91.7		
Vascular Invasion	YES	3	6.1	1	4.2	73	**1.00**
	NO	46	93.9	23	95.8		

Patients belonging to Carcinoma Rest of Oral Cavity group with N: L Ratio of < 2.97 were noted to statistically significant Extranodal Invasion with p value < 0.05

5.41 AVERAGE DISEASE FREE INTERVAL IN PATIENTS WITH RECURRENCE

TABLE 48: DISTRIBUTION OF SUBJECTS WITH DISEASE RECURENCE WITH RESPECT TO DISEASE FREE INTERVAL

DISEASE FREE INTERVAL	Carcinoma Tongue	Carcinoma Rest of Oral Cavity	Total	Percentage (%)
< 12 Months	1	3	4	66.7
≥ 12 Months	1	1	2	33.3
TOTAL	2	4	6	100

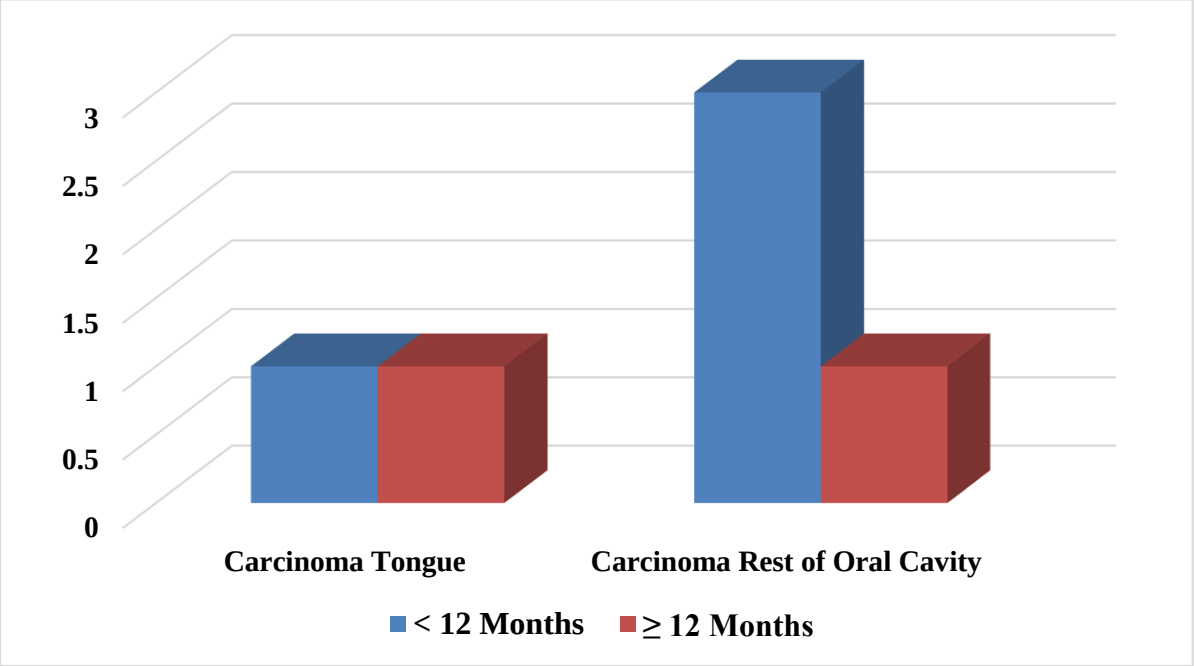

CHART 44: CHART REPRESENTING DISEASE FREE INTERVAL BEFORE ONSET OF RECURRENCE IN SUBJECTS BELONGING TO BOTH GROUPS

5.42 PROBABILITY OF DISEASE-FREE SURVIVAL WITH DURATION OF TIME

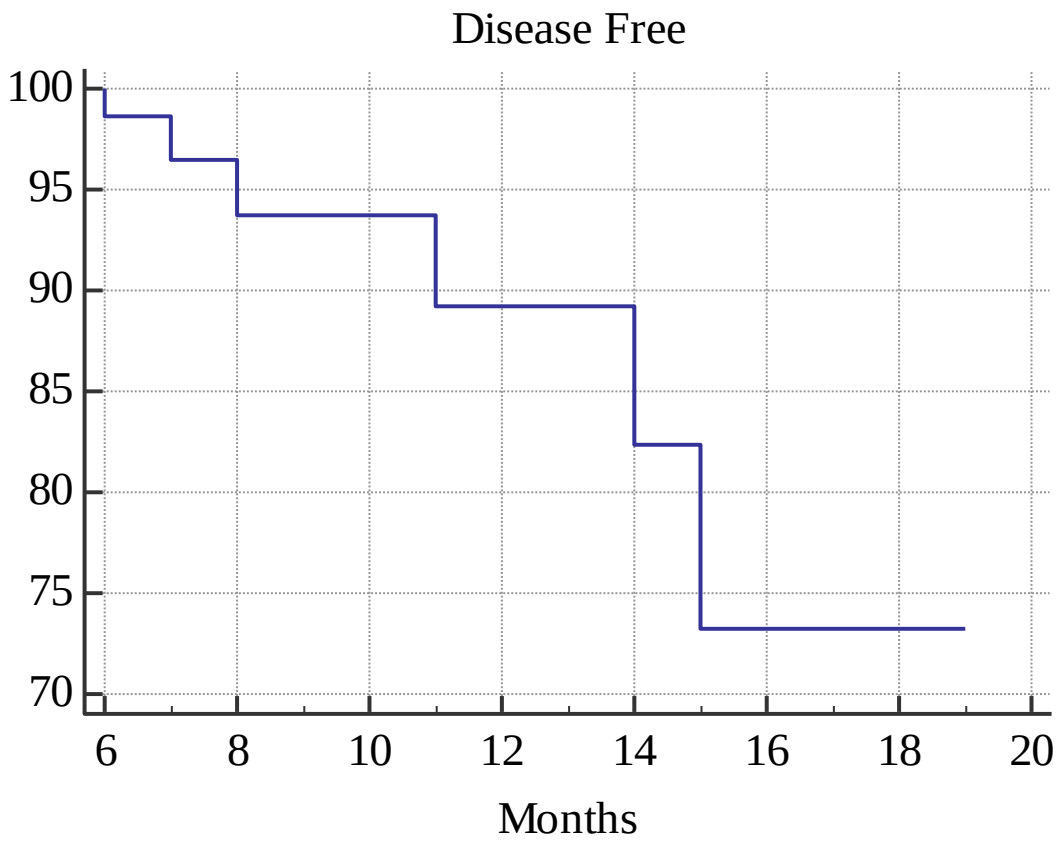

CHART 45: CHART REPRESENTING PROBABILITY OF DISEASE-FREE SURVIVAL

5.43 PROBABILITY OF DEATH SECONDARY TO DISEASE WITH DURATION OF TIME

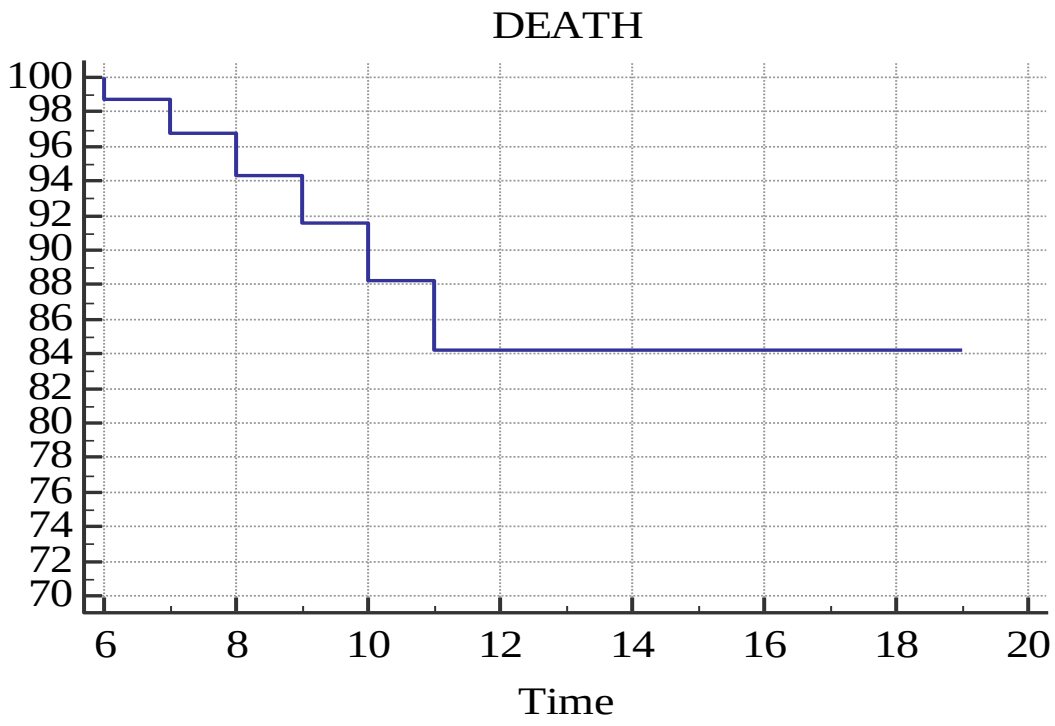

CHART 46: CHART REPRESENTING PROBABILITY OF DEATH

5.44 CORRELATION OF BETWEEN DEPTH OF INVASION AND NLR AMONG SUBJECTS WHO HAD CARCINOMA TONGUE

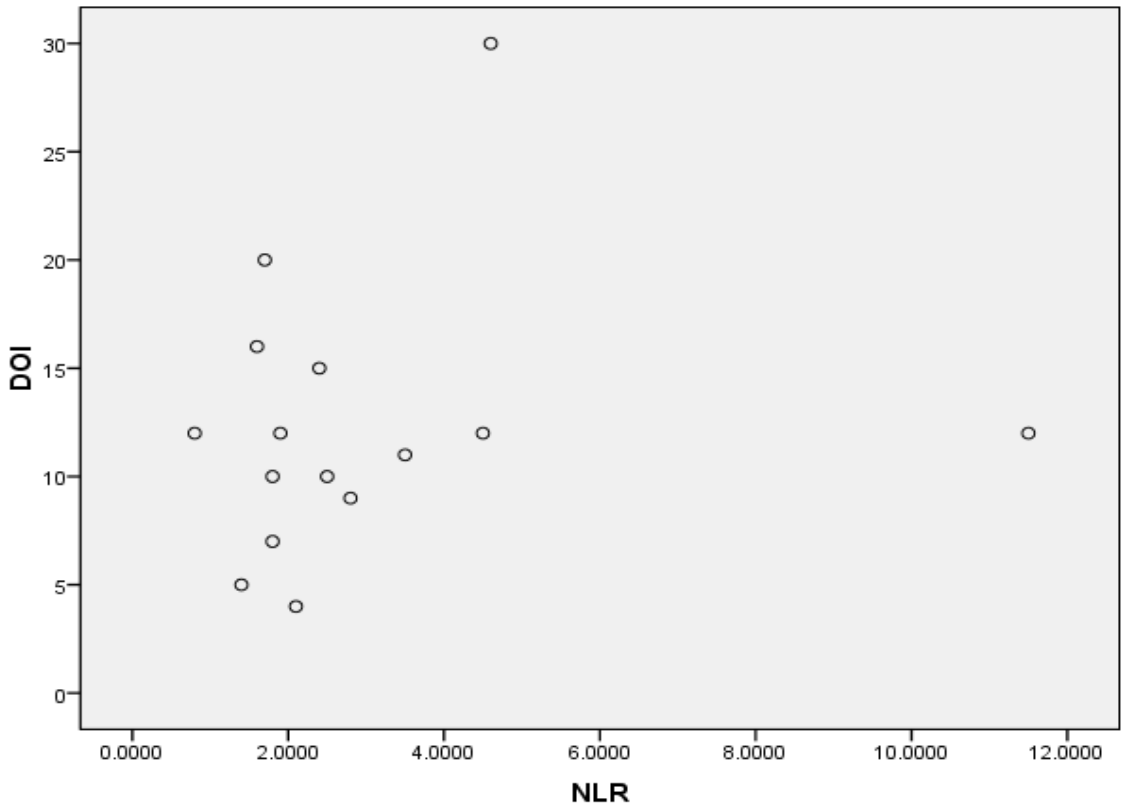

CHART 47: SCATTER PLOT SHOWING DEPTH OF INVASION WITH NLR AMONG SUBJECTS WHO HAD CARCINOMA TONGUE.

Pearson's r value is 0.1687

A positive correlation is noted between DOI and N:L Ratio in patients belonging to Carcinoma Tongue Group – however the relationship is weak.

5.45 CORRELATION OF BETWEEN DISEASE FREE PERIOD AND NLR AMONG SUBJECTS WHO HAD CARCINOMA TONGUE

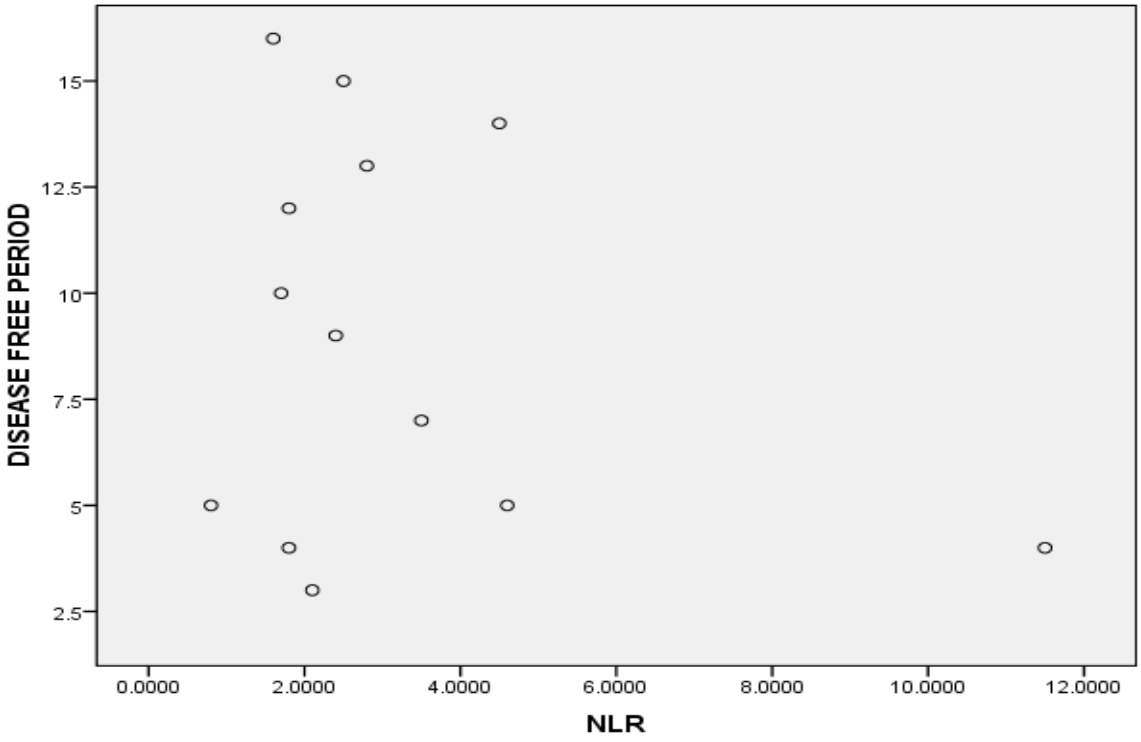

CHART 48: SCATTER PLOT SHOWING DISEASE FREE PERIOD WITH NLR AMONG SUBJECTS WHO HAD CARCINOMA TONGUE

Pearson's r value is -0.268

A negative correlation is noted between Disease Free Period and N: L Ratio in patients belonging to Carcinoma Tongue Group – however the relationship is weak.

5.46 CORRELATION OF BETWEEN DEPTH OF INVASION AND NLR AMONG SUBJECTS WHO HAD CARCINOMA REST OF ORAL CAVITY

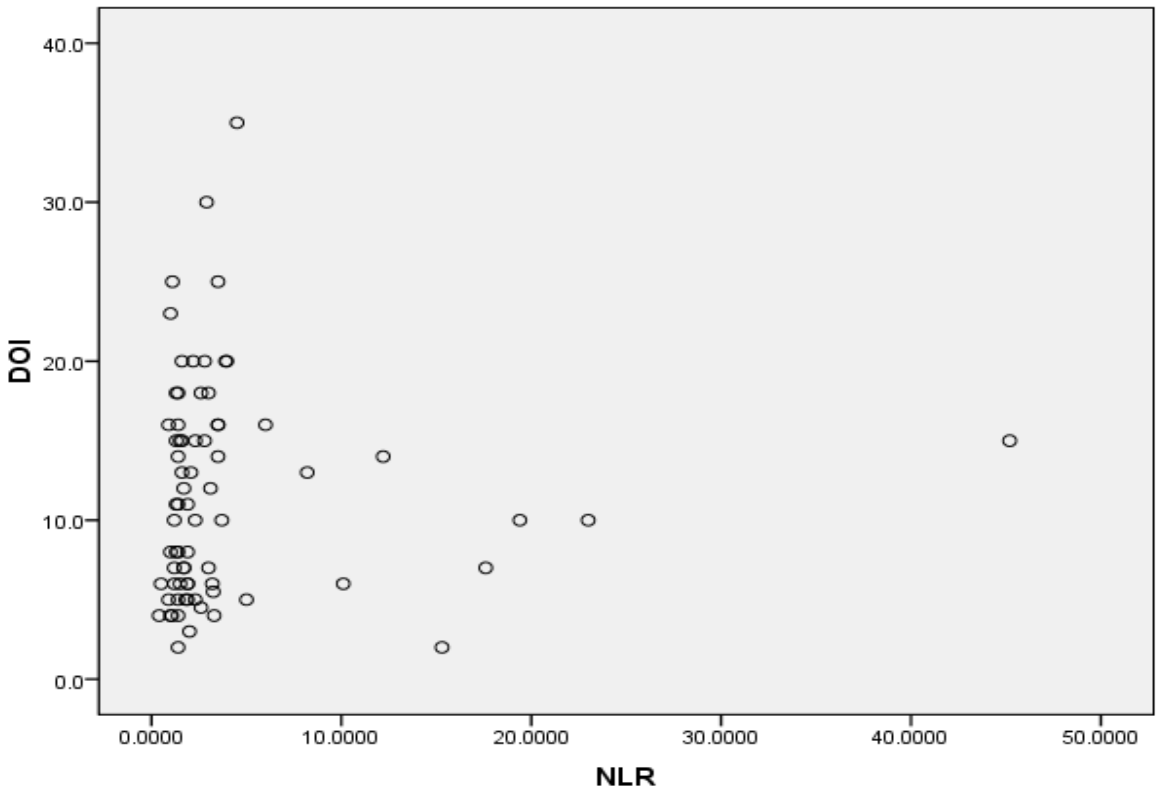

CHART 49: SCATTER PLOT SHOWING DEPTH OF INVASION WITH NLR AMONG SUBJECTS WHO HAD CARCINOMA REST OF ORAL CAVITY

Pearson's r value is 0.013

A positive correlation is noted between DOI and N: L Ratio in patients belonging to Carcinoma Rest of Oral Cavity Group – however the relationship is weak.

5.47 CORRELATION OF BETWEEN DISEASE FREE PERIOD AND NLR AMONG SUBJECTS WHO HAD CARCINOMA REST OF ORAL CAVITY

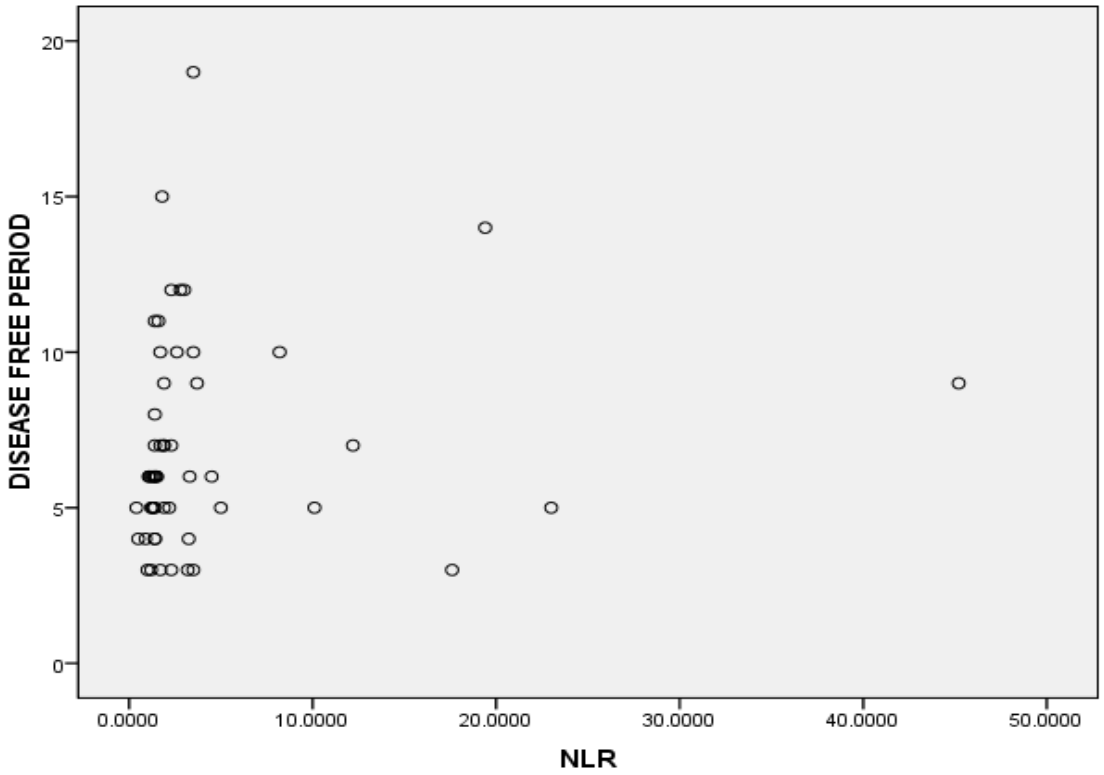

CHART 50: SCATTER PLOT SHOWING DISEASE FREE PERIOD WITH NLR AMONG SUBJECTS WHO HAD CARCINOMA REST OF ORAL CAVITY.

Pearson's r value is 0.116

A positive correlation is noted between Disease Free Period and N:L Ratio in patients belonging to Carcinoma Rest of Oral Cavity Group – however the relationship is weak.

5.48 ODDS RATIO CALCULATION – CARCINOMA TONGUE

a) TABLE 49: ODDS RATIO FOR N: L RATIO AND PATHOLOGICAL LYMPH NODE INVOLVEMENT

N: L RATIO	POSITIVE LYMPH NODE	NEGATIVE LYMPH NODE
< 2.93	4	7
≥ 2.93	3	1

ODDS RATIO – 0.19

5.49 ODDS RATIO CALCULATION – CARCINOMA REST OF ORAL CAVITY

a) TABLE 50: ODDS RATIO FOR N: L RATIO AND PATHOLOGICAL LYMPH NODE INVOLVEMENT

N: L RATIO	POSITIVE LYMPH NODE	NEGATIVE LYMPH NODE
< 2.93	25	24
≥ 2.93	12	12

ODDS RATIO – 1.04

b) TABLE 51: ODDS RATION FOR N: L RATIO AND DEPTH OF INVASION (DOI)

N: L RATIO	DOI ≤ 5mm	DOI > 5mm
< 2.93	12	37
≥ 2.93	3	21

ODDS RATIO – 2.27

Discussion

6. DISCUSSION

Thers is a growing interest in researching the relationship of inflammatory markers and cancer. Several studies performed previously in literature showed an unfavorable prognosis in patients with OSCC with elevated N: L Ratio. Oral cancer is an aggressive form of malignancy. Further it has been known to be associated with increased incidence of occult LN metastasis and deeper invasion – suggestive of a poorer prognosis.

Presence of inflammation as depicted by elevated N: L ratio has been associated with poor survivability after diagnosis with OSCC. Systemic inflammatory response can be easily measured by detecting circulating neutrophils and lymphocytes and calculating the N: L Ratio.

Neutrophils are known to aid in promoting tumour progression. Lymphocytes on the other hand can greatly inhibit the progression and proliferation of the tumour. There are three primary phenotypes that may be distinguished based on the location of T cells inside the tumour matrix:

- IMMUNE EXCLUDED: Defined as the presence of a significant number of immune cells in the stroma rather than the tumour parenchyma.

- IMMUNE INFLAMED: Identified by a significant infiltration of immune cells around the tumour cells.

- IMMUNE DESERT: A condition in which the stroma or parenchyma of the tumour is completely devoid of immune cells.

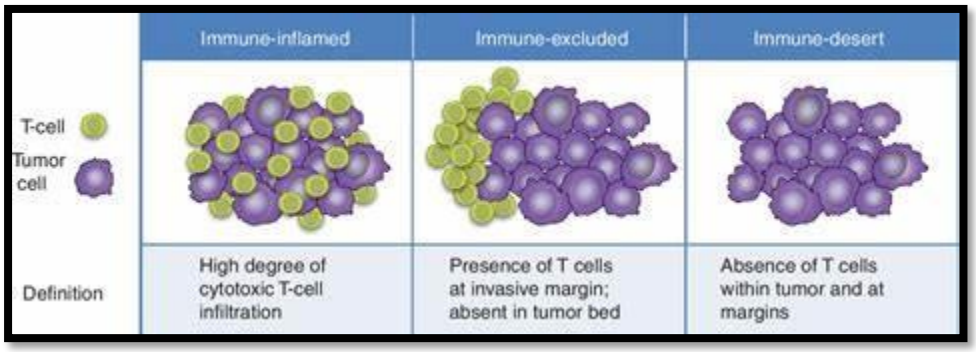

FIGURE 17: TUMOUR PHENOTYPE BASED ON PRESENCE OF T CELLS

Patients with an immune-desert phenotype for squamous cell carcinoma of the tongue had a worse survival rate, according to research by Troiano et al. Lymphokine-activated killer cells may be suppressed by an increase in neutrophils and/or a decrease in lymphocytes, which might be a potential cause of poor survival in cancer patients. The N: L Ratio is a reliable predictor for tumour prognosis. N: L Ratio has been demonstrated in several studies to play a prognostic function for OSCC.

A number of parameters have been used over time for prognostication of OSCC – Depth of Invasion (DOI in mm), Presence of pathological lymph node involvement, Presence of Neural/ Vascular/ Bone-Cartilage invasion and Presence of Extranodal Extension (ENE).

Depth of Invasion (DOI) has been included in the TNM classification as per AJCC 8th Edition, emphasizing its importance as a prognostic marker. DOI can be determined on preoperative CT imaging of head and neck and confirmed by analysis of the post operative HPE specimen.

This study was done to determine the predictive value of the N: L Ratio, specifically by looking at its impact on the previously listed parameters. Additionally, we separated the patients into the Carcinoma Tongue and Rest of the Oral Cavity groups depending on the subset of the oral cavity involved. This was done because for the rest of the oral cavity subgroup, greater aggressiveness was seen with DOI > 5mm whereas increased aggressiveness for Carcinoma Tongue was noted with DOI > 4mm; and also tongue malignancy have aggressive metastasis than other subsites. Further, a N: L Ratio threshold of 2.93 was taken into consideration after reviewing international literature; patients with N: L Ratio > 2.93 were thought to be more aggressive and, as a result, to have a worse prognosis than those with N: L Ratio of < 2.93 **(4)**

Null Hypothesis considered for our study was – **"N: L Ratio can be used as a reliable marker for Prognostication in OSCC prior to intervention"**, which can then further be utilized to determine the treatment plan of the patient – need for neoadjuvant and adjuvant CT/ RT.

We had a total of 88 patients who fulfilled the inclusion and exclusion criteria – 15 patients presented with Carcinoma Tongue (accounting for 17%) while the remaining 73 patients (83%) had Carcinoma involving the Other Subsets of the Oral Cavity.

1 death was noted in Carcinoma Tongue patient with 6 deaths in Carcinoma involving the other subsets of the Oral Cavity.

Majority of patients belonged to the 46 to 60 years age group with mean age noted to be 55.15 years to corresponds to world literature. Further patients who had Carcinoma Tongue had a younger mean age when compared to the other subsets.

Nearly 2/3rd of the patients who presented with OSCC were females which maybe secondary to the prevalent habit of chewing quid among the female population in Kolar. However, male preponderance was seen in case of Carcinoma Tongue. As per the National Cancer Registry, incidence of oral cancer in India is very high with 44.8 males and 23.7 females in 100000 population diagnosed with OSCC. **(64)** Majority of these patients present with advanced disease process due to prevalence of poverty, lack of knowledge and neglect among the poplulation.

Most common subset involved in OSCC in our study was noted to be Buccal Mucosa accounting for nearly 40% of all cases, predominantly due to extensive use of quid in Kolar. However, in India cancer of the tongue is noted to be the most common form of OSCC followed by buccal mucosa. **(64)**

The average duration of presentation of symptom was noted to be 5 to 6 months in our study, which was noted to be similar to the average duration of presentation in patients with OSCC in Indian Literature.

Based on clinical examination nearly 43.2% of the patients belonged to cT2 stage. This finding was confirmed based on pathological examination of the resected specimen which showed 42% patients belonging to pT2 stage. Further 50% of the patients included in our study had no metastatic involvement of lymph nodes on HPE while the remaining 50% patients showed regional lymph node involvement.

Based on HPE report 45% of patients belonged to Stage IV of which 36% belonged to Stage IVA and 9% were noted to have Stage IVB. Stage IVB disease usually develops due to spread of disease to infratemporal fossa. 62.5% patients were noted to have well differentiated malignancy on HPE while the remaining 37.5% patients had moderate to poorly differentiated malignancy with poorly differentiated malignancy accounting for 1.1% of the cases.

87.5% patients required adjuvant treatment in the form of RT or Combined CT/ RT due to poor prognostic factor in HPE or due to locally advanced disease.

A total of 12 patients showed Neural Invasion on HPE report of which 11 patients belonged to the Rest of the Oral Cavity Subset group (8 patients belonged had carcinoma buccal mucosa).

None of the patients with Carcinoma Tongue had Vascular Invasion on HPE. Further, 9 patients showed invasion of the Bone/ Cartilage and 10 patients had extranodal extension of malignancy.

13.3% patients who presented with Carcinoma Tongue had recurrence while 8.2% of the patients with malignancy involving the other subsets of the oral cavity presented with recurrence. Most common site of recurrence in both group of patients was noted to be Distant Recurrence.

Among patients with Carcinoma Tongue, 1 patient was noted to have DOI ≤ 4mm on HPE while the remaining 93% patients had > 4mm DOI – majority of the patients had aggressive nature of the tumour. In case of Carcinoma involving the other subsets of the oral cavity, 80% of the patients were noted to have DOI > 5mm on examination of the resected specimen, suggestive of aggressive nature of the tumour.

As per the study conducted by **Salzano G et al. in 2020 [(4)]** N: L Ratio > 2.93 was associated with increased incidence of occult cervical metastasis and aggressive nature of malignancy. On comparing the DOI with cut-off N: L Ratio of 2.93, it was noted that in patients with Carcinoma Tongue, out of the 11 patients who had DOI > 4mm on HPE, only 4 patients (accounting to 26.7%) had a NLR above 2.93, while the majority of the patients with DOI > 4mm (10 patients) had NLR <2.93. Similarly in patients with Carcinoma involving the Rest of the Oral Cavity, out of the 80% patients who had DOI > 5mm, only 1/3rd of the patients had NLR beyond the pre-decided cut-off of 2.93. Hence as per our study, NLR and DOI did not show any correlation to determine the aggressiveness of the tumour.

Further a positive correlation was noted between NLR and DOI as per the study conducted by **Salzano G et al. in 2020 [(4)]** which was confirmed by our study - Using Pearson's Correlation Coefficient Calculator, a weak but positive correlation was noted between DOI and NLR for patients belonging to Carcinoma Tongue group, i.e., with increase in NLR value an increase in the DOI was noted. Similarly, a weak but positive correlation was noted between DOI and NLR for patients belonging to Carcinoma Rest of Oral Cavity group as well.

On comparing pN status with NLR, it was noted that patients that in patients with Carcinoma Tongue, the mean NLR was noted to be higher for those with pN+ status as compared to those with a pN0 status – suggesting that patients with higher NLR ratio have a higher risk of involvement of lymph nodes. However, this finding was noted to statistically insignificant as

p value was noted to be 0.112. Further, it was noted that out of the 7 patients who had pN+ status, 43% of patients had NLR ≥ 2.93. **Wu et al., in 2017 [65]** demonstrated an increase in the incidence of cervical lymph node metastasis with a N: L Ratio of 2.95, a finding which also noted in our study.

On the other hand, in patients with Carcinoma involving the rest of the oral cavity, a negative correlation was noted between NLR and pN+ status – patients with pN+ status were noted to have a lower mean NLR when compared to patients with pN0 status. However, this correlation was also statistically insignificant (p value 0.183). Out of 37 patients who has pN+ status, 32% patients had NLR ≥ 2.93. **Petrescu MO et al., 2020**, in their study of 205 patients noted an increasing incidence of cervical lymph node metastasis with N: L ratio between 2.93 to 2.95. **[57]**

The mean DOI in patients with Carcinoma Tongue with pN0 status was noted to be 12.12mm which was almost similar to the DOI in this subset of patients with pN+ status – 12.57mm. On the other hand, a higher DOI was noted in patients with Carcinoma Rest of Oral Cavity with pN+ status – 12.27mm when compared to those with pN0 status – 10.83mm. **Salzano G et al. 2020 [4]** in their review had demonstrated an increased DOI in patients with pN+ status when compared to pN0 group (7.28mm versus 3.36mm) **[4]**

On comparing the presence of Neural/ Vascular/ Bone invasion with NLR in patients with Carcinoma Tongue, a negative correlation was again noted – patients with no neural and bone-cartilage invasion were noted to have a higher mean NLR. These findings were however not statistically significant. Similarly on comparing the presence of Neural/ Vascular/ Bone invasion with NLR in patients with Carcinoma Rest of the Oral Cavity, a positive correlation was noted between mean NLR and those who had bone invasion – these patients had a higher mean NLR when compared to those who had no bone- cartilage invasion. The p value was noted to be 0.388 which was statistically insignificant. **Wu et al., in 2017 [65]** had noted a positive correlation between NLR > 2.95 and predictors of cervical LN metastasis such as perineural invasion.

On comparing the recurrence rate in both the group of patients with mean NLR, again a negative correlation was noted – patients with higher mean NLR were noted to have lower recurrence rates when compared to those with lower mean NLR.

On comparing DOI with NLR cut-off of 2.93, the following findings were noted – in patients belonging to Carcinoma Tongue group, out of the 14 patients who had DOI > 4mm on HPE of the resected specimen, 29% patients had NLR ≥ 2.93 cut-off. On the other hand, in patients belonging to Carcinoma Rest of Oral Cavity group, out of the 58 patients who had DOI > 5mm on HPE of the resected specimen, 36% patients had NLR ≥ 2.93 cut-off.

A total of 6 patients were noted to have recurrence of disease out of the total of 88 patients – out of which 67% patients presented with recurrence within 12 months of surgery. It was noted that the risk of recurrence was higher in patients with a higher DOI when compared to those lower DOI. The mean DOI in patients with Carcinoma Rest of the Oral Cavity who had recurrence was noted to be 16.75mm while DOI for patients who remained disease free was noted to be 10.44mm – this finding was noted to statistically significant with a p value of 0.028. Studies conducted by **Tam et al., in 2019 [66] and Moore et al., in 1986 [67]** demonstrated a better DSS in patients with lower DOI on HPE report.

Further, on comparing the relationship between disease free period and NLR it was noted that in patients belonging to Carcinoma Tongue, disease free period was more in those patients who had a lower NLR – however, this correlation was weak. The same finding was however not noted in patients belonging to Carcinoma Rest of Oral Cavity Group – disease free period increased with increase in NLR. **Hasegawa T et al., [3]** in their study of 433 patients reported a higher N: L Ratio to be associated with reduced DSS and Overall Survival.

The findings of our study did not corelate with majority of the world literature wherein NLR was noted to be a reliable prognostic marker in determining the aggressiveness of OSCC. Based on our study, NLR was noted to have a weak but positive correlation with DOI in patients belonging to both Carcinoma Tongue and Carcinoma Rest of the Oral Cavity. Also, patients with Carcinoma Tongue who had pN+ status were noted to have a higher NLR ratio when compared to those with pN0 status.

An increased NLR ratio maybe associated with increased incidence of pathological lymph node involvement in selective subsets of the OSCC. Further, an increase in NLR ratio may also be associated with increased DOI as noted in our study – however the results were statistically insignificant to substantiate our claim.

LIMITATIONS:

- A small study population of patients may affect the results of the study.
- The study was a single centre research – a multicentre study is indicated.
- Unequal distribution of patients between the two groups could have negatively affected the results of study.
- NLR cut-off of 2.93 taken in this study was from the study performed by Giovani Salzano et al., in their study published in 2021. This cut-off may not be suitable for the Indian population. Hence a NLR cut-off for the Indian population need to be determined.

Due to the above-mentioned limitations of this study, results of the study cannot be generalized.

Summary

7.SUMMARY

Malignancies of the Head & Neck contribute for approximately 30% of cancers in India. Approximately half of them are oral malignancies.Tobacco chewing, betel leaf quid and areca nut, viruses (e.g., HPV), reduced nutritional adequacy, a record of head and neck carcinoma, fungal infection (Candida) are the principal etiological and predisposing factors for OSCC.

Predictive biomarkers are useful in planning of treatment as they give critical data regarding the broad outcome of the patient. However, biomarkers (molecular) requiring specimens as tissues for processing remains tough on patients, since sample collection is an invasive procedure.

According to current research, a relationship exists between inflammatory micro-environment of tumor and systemic response by the tumour. Increased neutrophil counts and/or reduced lymphocyte counts may inhibit killer cells (lymphokine activated). This is plausible mechanism for tumour aggression. Cancer induced lymphocytopenia may signify a generalized state of immunological decline. NLR may reveal the coexistence of two conflicting inflammatory and immunological pathways in cancer patients. The inflammatory response to a tumour promotes angiogenesis, causes DNA damage, and delays apoptosis, facilitating tumour cell proliferation and increasing the risk of spreading. Recent research has found links between systemic inflammation and tumour prognosis. As a result, NLR is an established marker of systemic inflammation.

Until recently, the AJCC staging of oral malignancies exclusively took into account the tumor's 2-dimensional size. The most current AJCC Staging (8^{th} Edition) takes into account tumour depth, which is the third dimension and is more relevant in tumour dissemination. “Depth of invasion is the extent of cancer progression into the tissue beneath an epithelial surface.”

The depth of invasion influences disease spread and therefore prognosis, and the cutoff limit evaluated for aggressive behavior in tongue carcinoma is 4mm and for buccal mucosa is 5mm, beyond which tumour is aggressive and has a higher probability of metastasis to lymph nodes.

In this study, we intend to assess predictive potentiality of NLR to estimate the invasiness, regional spread to lymph nodes,severity of malignancy, survival rates (disease specific and overall survival)of patients by correlating preoperative NLR with histopathological DOI and lymph node metastasis.

The objectives of our study are:

1)To document Neutrophil: Lymphocyte ratio preoperatively in patients with T2-T4 staged Carcinoma of Oral Cavity(Squamous cell)

2) To document depth of invasion and number and oncological level of metastatic lymph nodes if any after HPE of the resected tumor of above mentioned subset of patients.

3)To determine whether the Neutrophil: Lymphocyte ratio has a correlation with above mentioned parameters for prognostication of Oral carcinoma.

The study was conducted as a prospective, cross-sectional study. It included 88 patients with biopsy proven OSCC staged T2-T4, as per AJCC Classification (8^{th} Edition), at Sri Devaraj Urs Medical College, R.L.Jalappa Hospital & Research Centre, Tamaka, Kolar from January 2021 to August 2022.

A complete blood count(CBC) will be done and NLR ratio will be estimated preoperatively. After surgery (Primary resection of tumour with neck dissection and reconstruction) the resected specimen will be examined histopathologically and size, depth of invasion, number of metastatic lymph nodes will be documented. Cut-off limit for NLR was taken as 2.93, and depth of invasion was taken 4mm for tongue cancer and 5mm for other subsites of oral cavity.

Postoperatively hospital's multidisciplinary tumour board established postoperative adjuvant therapy plans based on histopathological examination.

Follow-up was done on a regular basis (second monthly) for the first six months, then every six months through phone or clinical follow-up on out-patient basis.

Our study included 88 patients who fulfilled the inclusion and exclusion criteria – 15 patients with Carcinoma Tongue and 73 patients had Carcinoma involving the Other Subsets of the Oral Cavity. Majority of patients belonged to the 46 to 60 years age group with mean age noted to be 55.15 years.

Nearly 2/3rd of the patients who presented with OSCC were females which maybe secondary to the prevalent habit of chewing quid. However, male preponderance was seen in case of Carcinoma Tongue.

Most common subset involved in OSCC in our study was noted to be Buccal Mucosa.

A total of 12 patients showed Perineural Invasion on HPE report of which 11 patients belonged to the Other subsites group.

13.3% patients who presented with Carcinoma Tongue had recurrence while 8.2% in patients with malignancy involving the other subsites of the oral cavity.

Among patients with Carcinoma Tongue, 1 patient was noted to have DOI ≤ 4mm on HPE while the remaining 93% patients had > 4mm DOI – majority of the patients had tumor aggressiveness. In case of Carcinoma involving the other subsets of oral cavity, 80% of patients had DOI > 5mm on examination of the resected specimen, suggestive of tumor aggressiveness.

Patients with Carcinoma Tongue, the mean NLR was noted to be higher for those with pN+ status as compared to those with a pN0 status.In patients with Carcinoma involving other subsites, no correlation was seen between NLR and pN+ status.A higher DOI was noted in patients with Carcinoma Rest of Oral Cavity with pN+ status – 12.27mm when compared to those with pN0 status – 10.83mm

On comparing the presence of perineural/ Vascular/ Bone invasion with NLR in patients with Carcinoma Tongue, a negative correlation was again noted – patients with no perineural and bone invasion were noted to have a higher mean NLR.

On comparing DOI with NLR cut-off of 2.93, patients belonging to Carcinoma Tongue group, out of the 14 patients who had DOI > 4mm on HPE of the resected specimen, 29% patients had NLR ≥ 2.93 cut-off. In patients belonging to Carcinoma Rest of Oral Cavity group, out of the 58 patients who had DOI > 5mm on HPE of the resected specimen, 36% patients had NLR ≥ 2.93 cut-off.

A total of 6 patients were noted to have recurrence of disease out of the total of 88 patients – out of which 67% patients presented with recurrence within 12 months of surgery. It was noted that the chance of recurrence was more in patients with a higher DOI when compared to those lower DOI.

Further, on comparing the relationship between disease free period and NLR it was noted that in patients belonging to Carcinoma Tongue, disease free period was more in those patients who had a lower NLR – however, this correlation was weak. The same finding was however not noted in patients belonging to Carcinoma Rest of Oral Cavity Group.

NLR was noted to have a weak but positive correlation with DOI in patients belonging to both Carcinoma Tongue and Carcinoma Rest of the Oral Cavity. Also, patients with Carcinoma Tongue who had pN+ status were noted to have a higher NLR ratio when compared to those with pN0 status.Larger multi-institutional study comparing NLR with DOI and lymph node metastasis are needed for evidence and based on that NLR can be proven as a predictive marker in Oral Squamous Cell Carcinoma of oral cavity.

Conclusion

8.CONCLUSION

A prospective, cross-sectional study was performed in patients who presented with OSCC between January 2021 and August 2022 to corelate the association of NLR with DOI and cervical lymph node metastasis for better prognostication of oral malignancies. Ours was one of the few studies in literature which attempted to corelate the association between preoperative NLR and postoperative DOI status on HPE.

- An increased incidence of OSCC was noted in Kolar, with majority of the patients being females which could be accounted to the high prevalence of consumption of quid.
- Inadequate knowledge, poverty and negligence could explain the higher incidence of advanced disease at the time of presentation to OPD.
- NLR is being used as emerging prognostic marker in OSCC for early identification of patients with advanced disease.
- Our study was able to demonstrate a weak but positive corelation between an increasing NLR and incidence of pN+ status particularly in patients with Carcinoma Tongue.
- A similar weak and positive corelation was also noted between increasing NLR and DOI in patients with OSCC.
- Increased incidence of bone invasion was noted with higher NLR in patients with Carcinoma including the subsets other than the tongue.
- A larger multi-institutional study may be required to substantiate the prognostic role of NLR in patients with OSCC.

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Annexures

ANNEXURE – 1

STUDY PERFORMA

CORELATION OF NEUTROPHIL- LYMPHOCYTE RATIO WITH DEPTH OF INVASION AND LYMPH NODE METASTASIS IN ORAL CANCERS

1. **Patient Characteristics**:

Demographics-

Hospital Number		
Age (years)		
Gender	Male	Female
Occupation		
Comorbidities (if any)		
Consumption of Tobacco (in any form)		
Date of Admission	Date of Surgery	
Date of Discharge		

Chief Complaints-

Presenting Complaints	Ulcer or Growth in the Oral Cavity Swelling or Mass in the Neck Restricted Mouth Opening Excessive Salivation Difficult in Swallowing
Duration of Each Complaint	
Presence of any Risk Factors	

History of similar complaints in the past	YES	NO
History of any biopsy from neck swelling/ oral cavity growth in the past	YES	NO

2. <u>Personal History</u>:

Diet		
Appetite (Loss of Appetite/ Weight)		
Addictive Habits	Smoking/ Tobacco in other forms	Alcohol
Pack Years (for smoking)		
Quid/ Gutkha/ Betel Leaves	Duration	
	Side of Oral Cavity where Quid is kept	

2. <u>General Physical Examination</u>:

Level of Consciousness	
Built and Nourishment Status	
Karnofsky Performance Scale	
Vitals	Temperature
	Blood Pressure
	Pulse Rate
	Respiratory Rate and SpO

3. <u>Local Examination of Tumour:</u>

Mouth Opening (Mallampati Classification)	I	II	III	IV
Trismus	I	II	III	IV

Site of Growth/ Ulceration – Subset Involved				
Dimensions	Breadth		Length	
Type of Growth	Verrucous	Ulcerative	Ulceroproliferative	Infiltrative
Extent of Growth/ Ulceration				
Skin Involvement	YES		NO	
Overlying Skin Pinchable	YES		NO	
Bleeds to Touch/ Discharge Present	YES		NO	
Tenderness associated with Growth/ Ulcer	YES		NO	
Bone Involvement	YES		NO	

4. <u>Neck Examination:</u>

Palpable Cervical Lymph Nodes	YES	NO
Level of Lymph Node Involved		
Size		
Consistency		
Fixity of the Lymph Node	Mobile	Fixed
Skin Involvement over LN	YES	NO
Overlying Skin Pinchable	YES	NO
Laryngeal Framework		
Laryngeal Crepitus	YES	NO
Other Lymph Node Involvement (non cervical)		

5. <u>Systemic Examination:</u>

Cardiovascular System	
Respiratory System	
Per Abdomen Examination	

Central Nervous System	

6. Investigations:

Preoperative Incisional Biopsy/ Core Needle Biopsy/ FNAC Report			
Neutrophil: Lymphocyte (N:L) Ratio			
CE Computed Tomography (CECT) Head and Neck Report			
Final Historpathological Report	FINAL DIAGNOSIS:		
	DOI (mm):		
	Lymph Node Imvolvement and Number Involved:		
	Extranodal Extension:		
	Neural Invasion	Bone – Cartilage Invasion	Vascular Invasion

7. Operative Details:

- Date of Surgery: _____
- Type of Surgery Performed: _____
- Reconstruction (if any): _____

8. Follow Up of Patient:

- Duration of Follow Up: _____
- Disease Free Period: _____
- Recurrence (if any): _____
- Death (if any) and Cause: _____

ANNEXURE – 2

PATIENT INFORMATION SHEET - ENGLISH

STUDY TITLE:

CORELATION OF NEUTROPHIL- LYMPHOCYTE RATIO WITH DEPTH OF INVASION AND LYMPH NODE METASTASIS IN ORAL CANCERS

Your participation in this study is entirely voluntary. It is your choice whether to participate or not. If you agree to participate in this study, a blood investigation (complete blood count – CBC), length and breadth of the tumour will be documented clinically along with preoperative imaging. After surgery, the resected specimen will be examined histopathologically and size, depth of invasion, number and oncological level of metastatic lymph nodes will be documented for the purpose of this study.

Your participation will help us to use the outcome of this study for future subjects. Your participation in this study will not put you at any additional risk

All information collected from you will be strictly confidential and will not be disclosed to any outsider. The information collected through this study will be used for research purpose. This information will not reveal your identity. This study has been reviewed by the central ethical committee.

There is no compulsion to participate in this study. Further, you are at the liberty to withdraw from this study at any time if you wish to do so. Your treatment aspect will not be affected if you don't wish to participate in the study. You are required to sign this Patient Information Sheet (PIS) only of you voluntarily agree to participate in this study. A copy of this document will be given to you for your information.

For any further clarification you are free to contact the Principal Investigator.

PRINIPAL INVETIGATOR’S NAME: Dr. DIANA ANN JOSE

MOBILE NUMBER: 9841637903

EMAIL ID: dianaann31592@gmail.com

ANNEXURE – 3

PATIENT INFORMATION SHEET - KANNADA

ರೋಗಿಯಮಾಹಿತಿಪತ್ರ

ಅಧ್ಯಯನದವಿಷಯ:ಮೌಖಿಕಕ್ಯಾನ್ಸರ್ಗಳಲ್ಲಿ ಆಕ್ರಮಣಮತ್ತುದುಗ್ಧರಸಗ್ರಂಥಿಯಒಳಹರಿವಿನೊಂದಿಗೆನ್ಯೂಟ್ರೋಫಿಲ್-ಲಿಂಫೋಸೈಟ್ಅನುಪಾತದಪರಸ್ಪರಸಂಬಂಧ

ಅಧ್ಯಯನಸ್ಥಳ:ಶ್ರೀದೇವರಾಜ್ಅರಸ್ಉನ್ನತಶಿಕ್ಷಣಮತ್ತುಸಂಶೋಧನಾಸಂಸ್ಥೆ,
ಟಮಕ, ಕೋಲಾರ - 563101.

ಈಅಧ್ಯಯನದಉದ್ದೇಶವನ್ನುಅರ್ಥಮಾಡಿಕೊಳ್ಳಲುನಿಮಗೆಸಹಾಯಮಾಡುತ್ತದೆ "ಮೌಖಿಕಕ್ಯಾನ್ಸರ್ಗಳಲ್ಲಿ ಆಕ್ರಮಣಮತ್ತುದುಗ್ಧರಸಒಳಹರಿವಿನಆಳದೊಂದಿಗೆನ್ಯೂಟ್ರೋಫಿಲ್-ಲಿಂಫೋಸೈಟ್ಅನುಪಾತವನ್ನುಸರಿಪಡಿಸುವುದು.
ಈಅಧ್ಯಯನದಉದ್ದೇಶವನ್ನುಅರ್ಥಮಾಡಿಕೊಳ್ಳಲುಸಹಾಯಮಾಡುವುದು. ಈಸಂಶೋಧನಾ ಅಧ್ಯಯನದಲ್ಲಿಸ್ವಯಂಪ್ರೇರಿತವಾಗಿಪಾಲ್ಗೊಳ್ಳಲುನಿಮ್ಮನ್ನುಆಹ್ವಾನಿಸಲಾಗಿದೆ, ಅಧ್ಯಯನದಉದ್ದೇಶ, ಕಾರ್ಯವಿಧಾನ, ಪ್ರಯೋಜನಗಳುಮತ್ತುಅಸ್ವಸ್ಥತೆಗಳನ್ನುನೀವುಓದುವುದುಮತ್ತುಅರ್ಥಮಾಡಿಕೊಳ್ಳುವುದು ಮುಖ್ಯವಾಗಿದೆ.

1. ಈಅಧ್ಯಯನದಉದ್ದೇಶವೇನು?
   ನ್ಯೂಟ್ರೋಫಿಲ್-
   ಲಿಂಫೋಸೈಟ್ಅನುಪಾತವನ್ನುಹಿಸ್ಟೋಲಾಜಿಕ್ಆಳದಆಕ್ರಮಣಮತ್ತುಮೌಖಿಕಕ್ಯಾನ್ಸರ್ನಲ್ಲಿದುಗ್ಧರಸಗ್ರಂಥಿಯಒಳಗೊಳ್ಳುವಿಕೆಯೊಂದಿಗೆಮೌಲ್ಯಮಾಪನಮಾಡಲು

2. ವಿವಿಧತನಿಖೆಗಳನ್ನುಬಳಸಲಾಗುತ್ತಿದೆ? ಯಾವುದೇಸಂಬಂಧಿತಅಪಾಯಗಳಿವೆಯೇ?
   ಪೂರ್ವಭಾವಿಯಾಗಿ: ಸಿಬಿಸಿ, ಪೆರಿಫೆರಲ್ಸ್ಮೀಯರ್
   ಶಸ್ತ್ರಚಿಕಿತ್ಸೆಯನಂತರದ: ಮರುಹೊಂದಿಸಲಾದಗೆಡ್ಡೆಯಹಿಸ್ಟೊಪಾಥೋಲಾಜಿಕಲ್ವರದಿ
ಸಂಪೂರ್ಣವಾಗಿಈಅಧ್ಯಯನದಲ್ಲಿ ಒಳಗೊಂಡಿರುವವಿವಿಧತನಿಖೆಗಳೊಂದಿಗೆಯಾವುದೇಅಪಾಯಗಳಿಲ್ಲ.

3. ಭಾಗವಹಿಸುವವನಾಗಿನನಗೆಏನುಪ್ರಯೋಜನ?
   ಈಸಂಶೋಧನಾಅಧ್ಯಯನದಲ್ಲಿಭಾಗವಹಿಸುವುದರಿಂದಚಿಕಿತ್ಸೆಯಅಂತಿಮಫಲಿತಾಂಶವುಬದಲಾಗುವುದಿಲ್ಲ.
   ಆದಾಗ್ಯೂ,
   ಈಅಧ್ಯಯನದಿಂದಪಡೆದಜ್ಞಾನದಪರಿಣಾಮವಾಗಿಭವಿಷ್ಯದಲ್ಲಿರೋಗಿಗಳುಪ್ರಯೋಜನಪಡೆಯಬಹುದು.
   ಸಂಶೋಧನಾಅಧ್ಯಯನದಸಮಯದಲ್ಲಿನಡೆಸುವಯಾವುದೇಕಾರ್ಯವಿಧಾನಗಳಿಗೆನಿಮಗೆಹೆಚ್ಚುವರಿಶುಲ್ಕವಿಧಿಸಲಾಗುವುದಿಲ್ಲ.
   ಈಅಧ್ಯಯನದಲ್ಲಿನೀವುಭಾಗವಹಿಸುವುದುಸಂಪೂರ್ಣವಾಗಿಸ್ವಯಂಪ್ರೇರಿತವಾಗಿದೆ.ನೀವುಅಧ್ಯಯನದಲ್ಲಿಭಾಗವಹಿಸಲುನಿರಾಕರಿಸಬಹುದುಅಥವಾಭಾಗವಹಿಸುವಮೊದಲುನೀವುಅರ್ಹತೆಪಡೆದಯಾವುದೇಪ್ರಯೋಜನಗಳ ದಂಡಅಥವಾನಷ್ಟವಿಲ್ಲದೆನೀವುಯಾವುದೇಸಮಯದಲ್ಲಿಅಧ್ಯಯನದಲ್ಲಿಭಾಗವಹಿಸುವುದನ್ನುನಿಲ್ಲಿಸಬಹುದು.

ಗೌಪ್ಯತೆ
ನಿಮ್ಮವೈದ್ಯಕೀಯಮಾಹಿತಿಯನ್ನುಅಧ್ಯಯನದವೈದ್ಯರುಮತ್ತುಸಿಬ್ಬಂದಿಗೌಪ್ಯವಾಗಿಡಲಾಗುವುದುಮತ್ತು ಸಾರ್ವಜನಿಕವಾಗಿಲಭ್ಯವಿರುವುದಿಲ್ಲ.
ನಿಮ್ಮಮೂಲದಾಖಲೆಗಳನ್ನುನಿಮ್ಮವೈದ್ಯರುಅಥವಾನೈತಿಕವಿಮರ್ಶೆಮಂಡಳಿಪರಿಶೀಲಿಸಬಹುದು.
ಹೆಚ್ಚಿನಮಾಹಿತಿಗಾಗಿಸಂಪರ್ಕಿಸಿ
ಪ್ರಧಾನತನಿಖಾಧಿಕಾರಿ: **Dr. ಡಯಾನಾಆನ್ಸೋಸ್**
ಮೊಬೈಲ್ಸಂಖ್ಯೆ: **9841637903**
ಇಮೇಲ್ವಿಳಾಸ: **dianaann31592@gmail.com**

ANNEXURE – 4

INFORMED CONSENT FORM

Title of the Project: **CORELATION OF NEUTROPHIL- LYMPHOCYTE RATIO WITH DEPTH OF INVASION AND LYMPH NODE METASTASIS IN ORAL CANCERS**

I have the read the foregoing information, or it has been read to me. I have had the opportunity to ask questions about it and any question that I have asked has been answered to my satisfaction. I consent voluntarily to participate in this research.

_____ _____

Signature/ Thumb Impression of Subject Date of Signature

_____

Print name of the subject in capitals

For Illiterate:

I have witnessed the accurate reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely for his/ her participation in this study.

_____ _____

Signature/ Thumb Impression of Witness Date of Signature

_____

Print name of the witness in Capitals

Statement by the Researcher/ Person taking the consent

I have accurately read out the Patient Information Sheet to the potential participant to the best of my ability. I confirm that the participant was given the opportunity to ask questions about the study, and all the questions sked by the participant have been correctly answered to the best of my ability. I confirm that the individual has not been coerced into giving the consent, and that the consent has been given freely and voluntarily.

A copy of this Informed Consent Form (ICF) has been provided to the participant.

_____ _____

Signature/ Thumb Impression of Researcher Date of Signature

Taking the Consent

_____

Print name of Researcher Taking the Consent

ANNEXURE – 5

INFORMED CONSENT FORM - KANNADA

ಶ್ರೀ ದೇವರಾಜ್ ಅರಸ್ ಉನ್ನತ ಶಿಕ್ಷಣ ಮತ್ತು ಸಂಶೋಧನಾ ಸಂಸ್ಥೆ,

ಟಮಕ, ಕೋಲಾರ - 563101.

ತಿಳಿವಳಿಕೆ ಸಮ್ಮತಿ ನಮೂನೆ

ಅಧ್ಯಯನದ ಹೆಸರು - ಮೌಖಿಕ ಕ್ಯಾನ್ಸರ್ಗಳಲ್ಲಿ ಆಕ್ರಮಣ ಮತ್ತು ದುರ್ಗಾರಸ ಮೆಟಾಸ್ಟಾಸಿಸ್ನ ಆಳದೊಂದಿಗೆ ನ್ಯೂಟ್ರೋಫಿಲ್-ಲಿಂಫೋಸೈಟ್ ಅನುಪಾತದ ಪರಸ್ಪರ ಸಂಬಂಧ "

ನಾನು ಮೇಲಿನ ಮಾಹಿತಿಯನ್ನು ಓದಿದ್ದೇನೆ, ಅಥವಾ ಅದನ್ನು ನನಗೆ ಓದಲಾಗಿದೆ. ಅದರ ಬಗ್ಗೆ ಪ್ರಶ್ನೆಗಳನ್ನು ಕೇಳುವ ಅವಕಾಶ ನನಗೆ ಸಿಕ್ಕಿದೆ ಮತ್ತು ನಾನು ಕೇಳಿದ ಯಾವುದೇ ಪ್ರಶ್ನೆಗಳಿಗೆ ನನ್ನ ತೃಪ್ತಿಗೆ ಉತ್ತರಿಸಲಾಗಿದೆ. ಈ ಸಂಶೋಧನೆಯಲ್ಲಿ ಪಾಲ್ಗೊಳ್ಳಲು ನಾನು ಸ್ವಯಂ ಪ್ರೇರಣೆಯಿಂದ ಒಪ್ಪುತ್ತೇನೆ.

ಭಾಗವಹಿಸುವವರ ಮುದ್ರಣ ಹೆಸರು: _____

ಭಾಗವಹಿಸುವವರ ಸಹಿ / ಹೆಬ್ಬೆರಳು ಮುದ್ರಣ: _____

ದಿನಾಂಕ: _____

ಅನಕ್ಷರಸ್ಥರಿಗೆ -

ಸಂಭಾವ್ಯ ಪಾಲ್ಗೊಳ್ಳುವವರಿಗೆ ಒಪ್ಪಿಗೆಯ ರೂಪವನ್ನು ನಿಖರವಾಗಿ ಓದುವುದಕ್ಕೆ ನಾನು ಸಾಕ್ಷಿಯಾಗಿದ್ದೇನೆ ಮತ್ತು ವ್ಯಕ್ತಿಯು ಪ್ರಶ್ನೆಗಳನ್ನು ಕೇಳುವ ಅವಕಾಶವನ್ನು ಹೊಂದಿದ್ದಾನೆ. ವ್ಯಕ್ತಿಯು ಮುಕ್ತವಾಗಿ ಒಪ್ಪಿಗೆ ನೀಡಿದ್ದಾನೆ ಎಂದು ನಾನು ಖಚಿತಪಡಿಸುತ್ತೇನೆ.

ಸಾಕ್ಷಿಯ ಮುದ್ರಣ ಹೆಸರು: _____

ಸಾಕ್ಷಿಯ ಸಹಿ / ಹೆಬ್ಬೆರಳು ಮುದ್ರಣ: _____

ದಿನಾಂಕ: _____

ಒಪ್ಪಿಗೆ ಪಡೆಯುವ ಸಂಶೋಧಕ / ವ್ಯಕ್ತಿಯ ಹೇಳಿಕೆ:

ನನ್ನ ಸಾಮರ್ಥ್ಯದಿಂದ ಸಂಭಾವ್ಯ ಭಾಗವಹಿಸುವವರಿಗೆ ಮಾಹಿತಿ ಹಾಳೆಯನ್ನು ನಾನು ನಿಖರವಾಗಿ ಓದಿದ್ದೇನೆ. ಅಧ್ಯಯನದ ಬಗ್ಗೆ ಪ್ರಶ್ನೆಗಳನ್ನು ಕೇಳಲು ಭಾಗವಹಿಸುವವರಿಗೆ ಅವಕಾಶ ನೀಡಲಾಗಿದೆ ಎಂದು ನಾನು ಖಚಿತಪಡಿಸುತ್ತೇನೆ,

ಮತ್ತು ಭಾಗವಹಿಸುವವರು ಕೇಳಿದ ಎಲ್ಲಾ ಪ್ರಶ್ನೆಗಳಿಗೆ ಸರಿಯಾಗಿ ಮತ್ತು ನನ್ನ ಸಾಮರ್ಥ್ಯಕ್ಕೆ ಉತ್ತರಿಸಲಾಗಿದೆ. ಒಪ್ಪಿಗೆ ನೀಡುವಂತೆ ವ್ಯಕ್ತಿಯನ್ನು ಒತ್ತಾಯಿಸಲಾಗಿಲ್ಲ ಎಂದು ನಾನು ಖಚಿತಪಡಿಸುತ್ತೇನೆ, ಮತ್ತು ಒಪ್ಪಿಗೆ ಇದೆ

ಮುಕ್ತವಾಗಿ ಮತ್ತು ಸ್ವಯಂಪ್ರೇರಣೆಯಿಂದ ನೀಡಲಾಗಿದೆ.

MASTER CHART

KEY TO MASTER CHART:

- UHID – Unique Hospital Identification Number
- GBS – Gingivo Buccal Sulcus
- MRND – Modified Radical Neck Dissection
- PMMC – Pectoralis Major Myo-Cutaneous Flap
- SOHND – Supra Omohyoid Neck Dissection
- SND – Selective Neck Dissection
- ITF – Infra Temporal Fossa
- FND – Functional Neck Dissection
- DOI – Depth of Invasion
- # - Cycles of Radiotherapy