ULTRASOUND GUIDED FINE NEEDLE ASPIRATION CYTOLOGY IN EVALUATION OF CERVICAL LYMPH NODE METASTASIS IN ORAL SQUAMOUS CELL CARCINOMA

By

DR.ARGHA BARUAH

Dissertation submitted to
SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION AND RESEARCH, KOLAR, KARNATAKA

DOCTOR OF MEDICINE

IN

PATHOLOGY

Under the guidance of

DR. TN SURESH
Professor of Pathology

DEPARTMENT OF PATHOLOGY

SRI DEVARAJ URS MEDICAL COLLEGE

TAMAKA,KOLAR

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Professor and HOD

DEPARTMENT OF ENT

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IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF MEDICINE

IN

PATHOLOGY

Under the Co-Guidance of

DR. ANIL KUMAR SAKALECHA

Professor

DEPARTMENT OF RADIODIOGNOSIS

SRI DEVARAJ URS MEDICAL COLLEGE

TAMAKA,KOLAR

2018

DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation entitled **"Ultrasound guided Fine needle aspiration cytology in evaluation of cervical lymph node metastasis in Oral Squamous cell carcinoma"** is a bonafide and genuine research work carried out by me under the guidance of **Dr. T N Suresh** ,Professor , Department of Pathology ,Sri Devaraj Urs Medical College ,Tamaka, Kolar.

Date: (**Dr. ARGHA BARUAH)**

Place: Kolar Signature of the Candidate

CERTIFICATE BY THE GUIDE

This is to certify that the dissertation entitled **"Ultrasound guided Fine needle aspiration cytology in evaluation of cervical lymph node metastasis in Oral squamous cell carcinoma"** is a bonafide research work done by **Dr. ARGHA BARUAH** in partial fulfilment of the requirement for the degree of **DOCTOR OF MEDICINE** in **PATHOLOGY.**

Date:
Place: Kolar

Signature of the Guide
Dr. T N SURESH
Professor
Department Of Pathology

.

CERTIFICATE BY THE CO-GUIDE

This is to certify that the dissertation entitled **"Ultrasound guided Fine needle aspiration cytology in evaluation of cervical lymph node metastasis in Oral squamous cell carcinoma"** is a bonafide research work done by **Dr. ARGHA BARUAH** in partial fulfilment of the requirement for the degree of **DOCTOR OF MEDICINE** in **PATHOLOGY.**

Date:
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Signature of the Co-Guide
Dr. SM Azeem Mohiyuddin
Professor and HOD
Department Of ENT

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Date:
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Signature of the Co-Guide
Dr. Anil Kumar Sakalecha
Professor
Department Of Radiodiagnosis

ENDORSEMENT BY THE HOD, PRINCIPAL / HEAD OF THE INSTITUTION

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Dr. CSBR PRASAD
Seal and Signature of the HOD

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Place: Kolar.

Dr. ML HARENDRA KUMAR
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ETHICS COMMITTEE CERTIFICATE

This is to certify that the ethical committee of Sri Devaraj URS Medical College, Tamaka, Kolar has unanimously approved the dissertation work of Dr. Argha Baruah,a post graduate student in the department of pathology of Sri Devaraj URS Medical College,entitled **"Ultrasound guided Fine needle aspiration cytology in evaluation of cervical lymph node metastasis in Oral squamous cell carcinoma"** to be submitted to the Sri Devaraj URS Academy of Higher Education and Research, Tamaka,Kolar, Karnataka.

Signature	Signature
Member Secretary	Principal

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DATE: **SIGNATURE OF THE CANDIDATE**

PLACE: KOLAR **DR ARGHA BARUAH**

LIST OF ABBREVIATIONS

SL NO.	ABBREVIATION	EXPANSION
1.	FNAC	Fine Needle Aspiration Cytology
2.	H&E	Haematoxylin & Eosin
3.	PAP	Papanicolaou Stain
4.	DPX	Distyrene Plasticizer Xylene
5.	CT	Computed Tomography
6.	LN	Lymph Node
7.	MRI	Magnetic Resonance Imaging
8	SCC	Squamous Cell Carcinoma
9.	USG	Ultrasound
10.	AAR	Age Adjusted Rate
11.	HPV	Human Papilloma Virus
12.	BMI	Body Mass Index
13.	OSF	Oral Submucous Fibrosis
14.	DNA	Deoxyribonucleic Acid

15.	EGFR	Epidermal Growth Factor Receptor
16.	TGF-α	Transforming Growth Factor-alpha
17.	miRNA	Micro ribonucleic acid
18.	ENE	Extranodal extension
19.	TNM	Tumor Nodes and Metastasis
20.	AJCC	American Joint Commitee on Cancer
21.	DOI	Depth Of Invasion
22.	PPV	Positive Predictive Value
23.	NPV	Negative Predictive Value
24.	END	Elective Neck Dissection
25.	HPE	Histopathological examination

ABSTRACT

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INTRODUCTION:

Oral cancer is the most frequent malignancy of head and neck region. Cancer of oral cavity are the sixth most common cancers globally.[1] It is a major problem and is regarded as one of the top three types of cancer in India. [2] Around 20 per 100000 Indian population are affected by oral cancer. [3]Oral cancer is the most common cancer in our geographic area, Kolar. [4] 90-95% of oral cancer is squamous cell carcinoma.[2]

The presence of metastatic lymph nodes greatly affects the outcome .[5] The 5-year survival for a patient is halved if a single ipsilateral neck node is involved, and is reduced by three-quarters if bilateral nodes are present. Spread of the disease outside the capsule reduces survival by a further 50%. [6] So the ratio of metastasis to lymph nodes reflects the aggressiveness of tumor and is important for assessing prognosis as well as to decide upon how extensive treatment the patient has to undergo.

Lymph nodes are bean shaped structures distributed throughout the human body and are enlarged in infections, immune disorders, lymphoma and in metastasis. However,regional lymphadenopathy in a known carcinoma patient is not always due to metastatic tumour, and not every nodule represents a lymph node. So confirmation of metastatic node is very important before deciding for any management plan for the patient and to reduce unnecessary morbidity or mortality due to neck dissection or radiation.

One of the most important problems in selecting patients for elective neck dissection is confirmatory diagnostic methods to classify a patient as neck positive or negative.

Fine needle aspiration cytology(FNAC) is used for diagnosis of metastasis in the lymph node.[7] FNAC procedure is very cost effective, simple, and free of complications, well tolerated by the patient, done on an outpatient basis and repeatable.The biggest challenge of FNAC is its difficulty for taking the representative sample which can be minimized by ultrasound. [7] Role of ultrasound guided FNAC in staging of cervical lymph node status in Oral Squamous Cell carcinoma not yet become a routine part of preoperative evaluation.[8]

In this study we evaluated the role of Ultrasound guided FNAC in preoperative assessment of cervical lymph node metastasis in oral squamous cell carcinoma by comparing with histopathological examination which is the gold standard .

OBJECTIVE OF THE STUDY:

To perform ultrasound guided fine needle aspiration cytology and to evaluate its efficacy in preoperative assessment of cervical lymph node metastasis in oral squamous cell carcinoma by comparing it with histopathological diagnosis

MATERIALS AND METHODS :

All biopsy proven oral squamous cell carcinoma patients with palpable lymph nodes who had undergone neck dissection from R L Jalappa Hospital and Research Centre during the period of January 2016 to June 2017 were included in the study.

Cervical lymph node with ultrasonographic features such as round shape, absence of echogenic hilum, sharp nodal borders, presence of intranodal necrosis and peripheral vascularity suggestive of metastatic cervical lymph nodes were aspirated. USG guided FNAC under aseptic precautions was done using a 23 gauge needle.

Minimum of three slides were prepared from the aspirated material.

One slide was air dried and remaining two slides were fixed in alcohol.

One air dried slide was stained with Giemsa.

The two alcohol fixed slides were stained first with methylene blue and if they show enough cellular material they were considered satisfactory. Then they were stained with haematoxylin , eosin and PAP stain.

The FNAC diagnosis was correlated with the histological findings.

Results:

In the present study most of the cases belonged to the age group of 40 to 70 years with a male to female ratio of 1:2.1.The most common presenting complaint was ulcer in the mouth (75.70%) Most common predisposing factor was betel quid chewing(98.6%).The common site of carcinoma of oral cavity was Buccal mucosa (70%) . Ulceroexophytic tumor was seen in 70.0% cases.Most of the cases presented clinically at a late stage ie. Stage IV (65.8%). On cervical lymph node aspiration 29 cases(41.4%) showed positivity for malignant squamous cells while 41 cases(58.6%) were negative for malignant cells .On histopathological study of neck dissection specimen ,31 cases (44.3%) showed cervical lymph node metastasis. 39 cases(55.7%)were negative for tumor deposit in lymph node.Out of 31 metastatic cases on HPE, 29(93.5%)were positive in cytology and 2 (6.5%) were negative .Remaining 39 cases were negative for metastasis both on HPE and cytology . There was significant association between cytology and HPE findings. **(p value <0.001)**.Sensitivity of USG guided FNAC was **93.55%,**specificity was **100%**,positive predictive value was **100%** ,negative predictive value was **95.12%** and diagnostic accuracy was **97.14%.**

6.5% showed false negative results on USG guided FNAC. This is due to aspiration from nonrepresentative node. Statistical correlation found between size of lymph node and cervical lymph node metastasis (**p = 0.021**). There was no statistical correlation found between tumor site ,tumor size,level of lymph node,differentiation of tumor with cervical lymph node metastasis.Chances of lymph node metastasis were higher if size of lymph node is >30mm.

Conclusion:

Oral cancer is one of the most common cancer. Metastatic spread to cervical lymph nodes greatly affects the patient outcome. Accurate preoperative diagnosis of cervical metastasis is important to reduce the unnecessary morbidity or mortality due to neck dissection or radiation .

High diagnostic accuracy of FNAC combined with advantage of ultrasound guidance helps in accurate diagnosis of cervical lymph node metastasis in oral cancers.Ultrasound guided FNAC should be recommended in the preoperative assessment of cervical lymph node metastasis in Oral squamous cell carcinoma.

Keyword : FNAC, cytology, Oral cancer, Ultrasound guided, squamous cell carcinoma, lymph node metastasis.

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INTRODUCTION

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The presence of metastatic lymph nodes greatly affects the outcome .[5] The 5-year survival for a patient is halved if a single ipsilateral neck node is involved, and is reduced by three-quarters if bilateral nodes are present. Spread of the disease outside the capsule reduces survival by a further 50%. [6] So the ratio of metastasis to lymph nodes reflects the aggressiveness of tumor and is important for assessing prognosis as well as to decide upon how extensive treatment the patient has to undergo.

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OBJECTIVE OF THE STUDY

AIMS AND OBJECTIVES

To perform ultrasound guided fine needle aspiration cytology and to evaluate its efficacy in preoperative assessment of cervical lymph node metastasis in oral squamous cell carcinoma(SCC) by comparing it with histopathological diagnosis.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Incidence and Epidemiology:

Oral carcinoma is the sixth most common cancer in the world. [1] In a worldwide study on incidence of 27 types of cancers, it was found that oral cancer mortality rates ranges between 1 and 15 per 100,000 persons and depends on the stage of the disease. In Eastern European countries, such as the Czech Republic, Hungary, and the Slovak Republic, mortality rates exceed 10 per 100000. Amongst the countries ,having some reliable cancer registries ,oral cancer incidence is highest in India and lowest in Belarus.[2]

Incidence of oral cancer and mortality is also high in Papua New Guinea, Taiwan and China, where chewing of betel quids with or without tobacco or areca nut chewing is common, as well as in Eastern Europe, France, and parts of South America (Brazil and Uruguay), where tobacco smoking and alcohol consumptions are high.[2]

90-95% of oral cancer in India is squamous cell carcinoma .[3] It is a major problem and is regarded as one of the top three types of cancer in the country. [2] Around 20 per 100000 Indian population are affected by oral cancer . In India among all cancers 30-35% involve head and neck and half among them involve oral cavity.[9] In a study it was found that in India, due to oral cancer each day 5 people die every hour .[3]

It has been predicted by the international agency for research on cancer that by India's incidence of cancer will increase from 1 million in 2012 to more than 1.7 million in 2035.[2] As cancer registration is not compulsory in India, many cases remain unrecorded and are lost

on follow up .Hence available incidence and prevalence rate can be regarded as "Tip of iceberg" situation.

A recent study on cancer in Bangalore showed frequency of lip cancer in males was 0.1%, tongue cancer was 3.8% and buccal mucosa with lower alveolar complex was 3.1%. In case of females the frequency of lip cancer was 0.1%, tongue cancer was 0.9% and buccal mucosa with lower alveolar complex was 4.4%.[10]

In the geographical area of Kolar ,squamous cell carcinoma is the most common and frequent malignancy of head and neck region and constitutes 29.66% of total cancer incidence.[4]

Demographic profile and risk factors :

Changes in lifestyle and increase in life expectancy have increased the incidences of oral cancer

1)Age:

Oral cancer is common in elderly persons of 50 to70 years of age .Incidence of oral cancer increases by age.[11] Sankaranarayan and his colleagues reported that in India ,the peak-age of occurrence is in the fifth decade of life which is a decade earlier than that described in the western literature. [12] Off late in India young individuals are often seen with oral cancer. Consumption of tobacco in different forms between the age of 15-49 by 57% men and 11%

women led to oral cancer at an earlier age .[11] In Kerala ,a 10 years cohort study reveals the increase of oral cancer in younger age group ,less than 50 years of age.[12]

2)Gender:

In all age groups, men are more susceptible than women. In India, due to behavioural and lifestyle patterns men are two to four times vulnerable to oral cancer than women . [13]However, in southern India high incidence rates are seen amongst women because of chewing tobacco .[14]

Although in females use of tobacco products and alcohol is less in comparison to males, a rising trend has been observed in females in recent years. Moreover , it was found that women who chew tobacco 10 or more times a day have 9.2 times more risk than that of non-tobacco chewers irrespective of age .[14]

Males of East Khasi Hills District from Meghalaya had the highest Age adjusted incidence rates(AAR)of 11.7 of tongue cancer followed by Ahmedabad (10.4). Among Females ,Bhopal had the highest AAR (3.7) of tongue cancer followed by Ahmedabad (3.4). Nagpur and Kamrup Urban District are jointly in the third place with an AAR of 3.2. [10]

Males of Ahmedabad showed the highest AAR (18.1) of mouth cancer followed by Bhopal (14.3) whereas, females of East Khasi Hills District of Meghalaya had the highest AAR (9.1).[10]

3)Low income:

A study demonstrated that oral cancer is related to low income because it is of interrelated factors like nutrition, health care, living condition and risk behaviours which lead to the development of oral cancer.[13] Most of the people does not have access to well organized and well regulated cancer care system . Moreover, as oral cancer is mostly diagnosed at later stages, therefore result is poor treatment outcomes and high costs and morbidity.

4)Tobacco:

More than 90% of oral cancer cases reported are due to use of tobacco products.[15] Various forms of tobacco are used all over India. Betel liquid, processed or unprocessed tobacco, aqueous calcium hydroxide (slaked lime) and pieces of areca nut wrapped in the leaf of piper betel vine leaf are the forms used. Gutka, zarda (boiled tobacco), mawa (tobacco,lime and areca), gadhaku (tobacco and molasses), kharra , mishri(burned tobacco) and khainni(tobacco and lime) are also the consumed form of tobacco. In India , chewing Tobacco (smokeless tobacco) used is mostly of Nicotiana rustica species which contains high concentration of nitrosamines- a highly carcinogenic substance, while most smoking tobacco is Nicotiana tabacum. Sun/air cured chewing tobacco whether processed, unprocessed, or in manufactured form is cheapest and used in different parts of India.[16]

In Karnataka, chewing tobacco is found as bundles of long strands of leaves (hogesoppu) or as powdered sticks (kaddipudi) that can be used with lime, areca nut, or in a betel quid (paan) .[16] Though preference of chewing tobacco over smoking is related to literacy , income of an individual and sociocultural norm, yet in India, smoking by women is still not taken easily. Therefore chewing tobacco is the preferred choice among women.

Bidi smokers are 4 times at risk of developing oral cancer compared to non-smokers. Reason behind this could be poor combustibility, nicotine and tar content of bidi which exceeds that of cigarette.[17] Sanghvi and his colleagues observed the risk ratio for oral cancers and found four-fold increase in chewers, two-fold in smokers, and four-fold in chewers who also smokes.[18] A study states that in comparison to tobacco chewing, tobacco used in the form of smoking has 5.19 times increased risk and cause precancerous lesion on palate.[19] Patients having both smoking and tobacco chewing habit are 8.4 times prone to oral cancer compared to those who do not have tobacco consumption.

A cross-sectional study on reverse smoking and its association with premalignant and malignant lesions of the palate conducted in Andhra Pradesh states that reverse smoking, significantly induced more lesions than conventional chutta smoking and determines subsequent palatal cancer .[20] In India states like Uttar Pradesh, Jharkhand and Bihar have higher prevalence of oral cancer.[21] Use of smokeless tobacco (pan parag, zarda etc) is on rise in north India ,especially states like Uttar Pradesh, Bihar and Assam.

5)Occupational Risks:

Exposure to excessive solar radiation and ultraviolet light cause lip cancers. Sulfur dioxide, asbestos, pesticide exposures, and mists from strong inorganic acids and burning of fossil fuels can also lead to oral cancer.[22]

6)Alcohol consumption

In a prospective study it was found that alcohol consumption greatly increases the risks of oral cancer and is related to duration of alcohol consumption .[23] Farmers and laborers consume country liquor - a form of locally brewed alcohol which is cheap and easily

available. Epidemiological evidence establishes the synergistic role played by alcohol with tobacco. Andre and his colleagues observed a deleterious effect of alcohol consumption even with nonsmokers or casual smokers.[23]

7)Oral hygiene

Poor oral hygiene also causes cancer of oral cavity. In a study ,it was found that more than 85% of oral cancer patients had poor oral hygiene. In India, poor oral hygiene related risk for developing oral cancer is around 32% for men and 64% for women . Patients wearing dentures for longer periods particularly ill fitting dentures are vulnerable to oral cancer.[24]

8)Viral Infections

Viruses play vital role in the development of malignant tumors of the squamous epithelia. The viruses implicated in oral cancer development ie. on oral squamous epithelium are human papilloma virus (HPV), herpes simplex virus and Epstein–Barr virus . International Agency of Research of Cancer (IARC) in 2012 declared that HPV 16 was associated with 95.5% of HPV- oral cancers.[25]

9)Genetic:

Genetic predisposition and epigenetic influence are an important risk factor in the development of Oral SCC. Copper and his colleagues , observed that first-degree relatives of 105 patients with head and neck cancer developed a second primary cancer in upper aero digestive tract. [26]

10)Immunosuppression:

Immunosuppressed persons are more susceptible to develop oral cancers. Immunosuppressed organ transplant patients are prone to lip cancers and and this can be due to increased exposure to solar radiation and other risk factors such as smoking. Epidemiological studies and cancer registries have shown that patients undergoing kidney, bone marrow, heart or liver transplantation and with HIV infection have high risk of head and neck malignancies .[27,28]

11)Fungal Infections:

Fungal infections mainly caused by, Candida albicans has been implicated in the pathogenesis of oral premalignant lesions. It superimpose leukoplakia, particularly nodular leukoplakia and predispose malignant transformation.

12)Diet and nutrition:

Frequent consumption of fruit and vegetables, particularly carrots, fresh tomatoes, and green peppers reduce the risk of oral cancer. Fish, vegetable oil, olive oil, bread, cereals, legumes, protein, fat, chicken, liver, shrimp, lobster, and fiber also have a protective effect.[29] Of course certain food groups such as cakes ,desserts, butter, eggs, soups, red meat, salted meat, cheese, pasta or rice, millet, and corn bread are associated with higher risk of oral cancer . Tondon and his colleagues observed that deficiency of vitamin A,C and E may contribute to oral cancers in India.[30]

13)Body mass Index:

Body mass index (BMI) is inversely associated with oral cancer. In a study it was found that paan chewers with low BMI has high risk of oral cancer .[29]

14)Associated conditions:

A number of conditions elevated the risk of developing oral SCC including Li Fraumeni syndrome, Plummer-Vinson syndrome, Bloom syndrome, Fanconi anemia, chemotherapy induced immunosuppression of organ transplantation, dyskeratosis congenita, xeroderma pigmentosum and discoid lupus erythematosus. [31]

Anatomy: [32]

The oral cavity is an internal area of the head that is created by the bony space between the base of the skull and its connection to the mandible at the temporomandibular joint. Oral cavity is divided into various parts as follows :

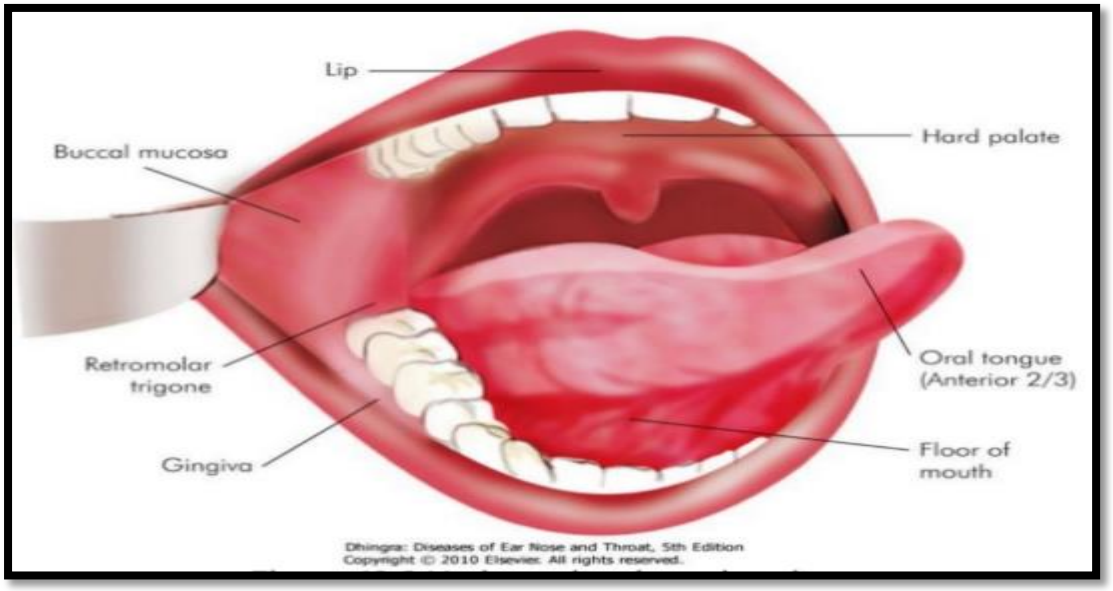


Figure1: Microphotograph of parts of oral cavity (Source: Dhingra: Disease of Ear Nose and Throat)

Borders: [32]

Anteriorly, the teeth limit the oral cavity proper.

Superiorly the hard palate from anterior to posterior form the roof of the cavity. Inferiorly the anterior 2/3rd of tongue and below it the floor of the mouth with its many appendages make up an unstable and moveable base.

Posteriorly, the cavity continues into oropharynx, with the anterior pillars of tonsils marking the end of the cavity proper and the junction of hard and soft palate and circumvallate papillae of tongue curving slightly downwards.

LYMPHNODES:

Lymph nodes are bean shaped structure. A lymph node is enclosed in a fibrous capsule and is made up of an outer cortex and an inner medulla.

There are 800 lymph nodes in the human body, 300 are in the neck. Cervical lymph nodes are subjected to a number of different pathological conditions including tumours, infection and inflammation.

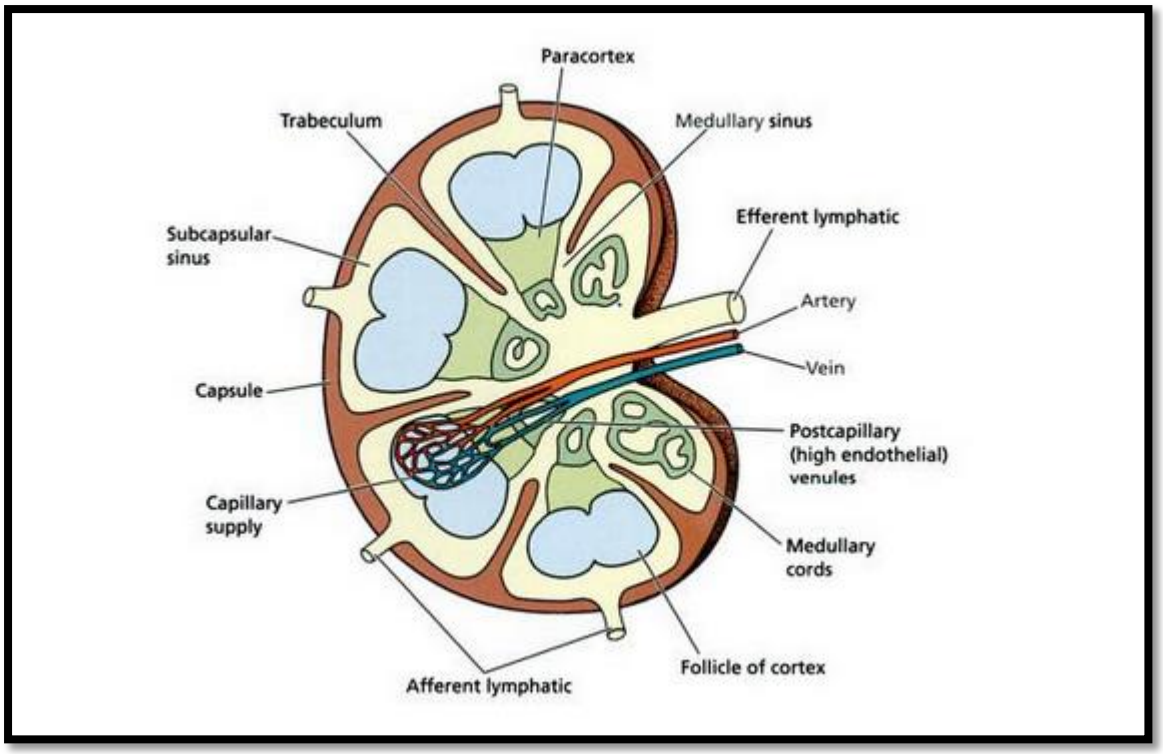


Figure 2: Microphotograph: Structure of lymphnode (Source of picture-Ioachim's Lymph Node pathology)

To establish a consistent and easily reproducible and a common language between the clinician and the pathologist, the Head and Neck Service at Memorial Sloan Kettering Cancer Center has described a leveling system of cervical lymphnodes.

Lymphnodes are divided into various levels:

- Level I: Submental and submandibular nodes
    - Level Ia: Submental nodes- found between the anterior belly of the digastric muscles and arch of hyoid bone.
    - Level Ib: Submandibular nodes-found around submandibular glands between the lower border of mandible and anterior and posterior belly of digastric muscle.

- Level II: Upper jugular nodes - Between posterior belly of digastric muscles superiorly and hyoid bone inferiorly
    - Level IIa: Anterior, medial, lateral or posterior to spinal accessory nerve.
    - Level IIb: Posterior to spinal accessory nerve
- Level III: Middle jugular nodes -present in anterior triangle of neck- between the hyoid bone and cricoid cartilage
- Level IV: Lower jugular nodes – present in anterior triangle between the cricoid cartilage and the clavicle
- Level V: All Posterior cervical nodes, posterior to the sternocleidomastoid muscle
    - Level VA: Spinal accessory nodes from skull base to inferior belly of omohyoid
    - Level VB: Between inferior belly of omohyoid and clavicle-in posterior triangle of neck
- Level VI: Visceral space lymph nodes - midline group of cervical nodes from hyoid to sternal manubrium, includes pre-laryngeal, pre -tracheal, and paratracheal subgroups
- Level VII: Superior mediastinal nodes - between carotid arteries from top of manubrium superiorly to innominate vein inferiorly

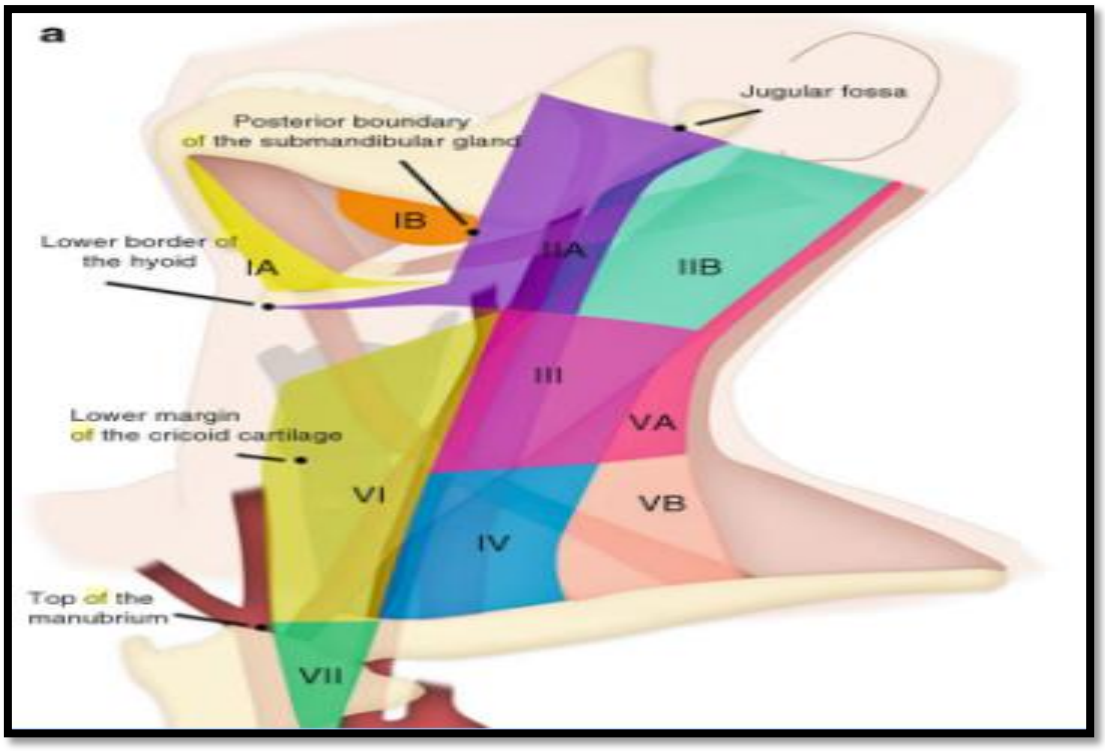


Figure 3: Microphotograph of level of lymphnode (Source: Atlas of lymphnode anatomy)

Lymphatic drainage of oral cavity :[34,35]

Buccal mucosa:

It drains to mostly Level Ib lymphnodes

Retromolar tongue:

It drains to Ib and II lymphnodes

Hard palate and upper alveolus:

It drains mostly to Level Ib lymphnodes

Floor of mouth

The lymphatic drainage of the floor of mouth is supplied by an anterior and posterior complex. The anterior complex drains the anterior half of the floor of mouth and anterior

portion of the sublingual gland. These lymphatic vessels terminate in the Group Ia nodes. The posterior group drains the posterior two-thirds of the mouth floor. The primary drainage is to drain the ipsilateral Group I lymph nodes. However, there is occasionally a direct lymphatic drainage to the Group II and III nodes .

Oral tongue(anterior 2/3RD)

A superficial and deep lymphatic network drains the oral tongue. The superficial network extends from the tip of the tongue to the circumvallate papillae and drains into the muscular network. There are three main components of the deep muscular lymphatic drainage pathway for the oral tongue.

1)The anterior pathway drains the tip of the oral tongue and primarily drains to Group I and sometimes skip metastasis to Level III.

2)The lateral (marginal) group drains the lateral one-third of the dorsum of the tongue from the tip to the circumvallate papillae. These lymphatic channels drain to Groups Ib mainly and rarely to II and III.

3)The central pathway drains the central two-thirds of the tongue. These vessels drain to the Group I nodes or course through a sublingual node and terminate in group III nodes. Investigators have also identified direct metastases to Group IV without involvement of groups I-III ("skip metastases").

Cross-drainage in the oral tongue is common, thereby placing both sides of the neck at risk for nodal metastases

Pathogenesis and carcinogenesis:

Like any other cancer oral carcinogenesis is a progressive disease and normal epithelium passes through stages starting from hyperkeratosis to dysplasia to carcinoma in situ finally transforming into invasive phenotypes.

Premalignant Lesions

1.Leukoplakia

Leukoplakia is described as a white patch which cannot be scraped out and cannot be characterized clinically or pathologically as any other disease.[37] Lesions arising from oral cavity are most likely to harbor dysplasia or progress to malignancy.[38] The rate of progression to malignancy has been reported to be between 3.6% and 17.5%. The malignant transformation in homogenous leukoplakia reaches up to 2-5% and in heterogenous leukoplakia reaches up to 20%. [39,41]

2.Erythroplakia

In Erythroplakia there forms a red patch that cannot be clinically or pathologically distinguished as any other definable disease Upon histological analysis, 30-35% of erythroplakic lesions undergo malignant transformation. [40]

4.Oral submucous fibrosis

Oral submucous fibrosis (OSF) is a chronic progressive condition found predominantly in people of Asian origin. OSF is caused due to the use of the areca nut and spices with resultant disruption of the extracellular matrix. Epithelial dysplasia has been described in 7-26% of OSF tissues. Long-term studies suggest a malignant transformation rate in

approximately 20-25% of these lesions particularly if chewable forms of tobacco like paan masala and gutkha is used. [42]

5.Lichen planus, discoid lupus erythematous, and epidermolysis bullosa

Although classified as potentially malignant conditions, the data regarding progression to malignancy for these conditions is controversial. Because of the difficulty in classifying and clinically distinguishing the varied lesions associated with these conditions, the potential for malignant transformation remains unclear.[39]

Tobacco and oral cancer

The most important carcinogens in tobacco smoke are the aromatic hydrocarbon benzopyrene and the tobacco-specific nitrosamines namely 4-(nitrosomethylamino)-1-(3-pyridyl)-1-butanone and N'-nitrosonicotine. They covalently bind with deoxyribonucleic acid (DNA) of keratinocyte stem cells forming DNA adducts.[43] These adducts are responsible for critical mutations which are involved in DNA replication.

Also the metabolism of these carcinogens involves oxygenation by P450 enzymes in cytochromes and conjugation by glutathione-S-transferase. Genetic polymorphisms in the genes coding for these enzymes are suspected to play a key role in the genetic predisposition to tobacco-induced head and neck cancers .[44]

Alcohol and oral Cancer:

The major metabolite of alcohol is acetaldehyde whose transformation is mainly carried out by the enzyme alcohol dehydrogenase . Acetaldehyde a metabolite of alcohol damage DNA . It interferes with the DNA synthesis and repair. It also induces sister chromatid exchanges and specific gene mutations .[45] Acetaldehyde inhibits the enzyme

6-methylguanitransferase which is responsible for repairing injuries caused by alkylating agents.

Alcohol increase the permeability of oral mucosa producing an alteration in morphology characterized by epithelial atrophy, which in turn leads to easier penetration of carcinogens into the oral mucosa.[23] The role of alcohol as an independent factor in oral carcinogenesis is still unclear.

HPV and oral cancer:

Virions released from the stratum corneum and granulosum directly infect the basal layer through capsid synthesis and promoter activation. Three major replication mechanisms occur after virion penetration: plasmid, vegetative, and productive replication. HPV genes and gene products are capable of disturbing the cell cycle machinery.

HPV encodes two major oncoproteins namely, E6 and E7. The E6 and E7 proteins phosphorylates and destroy p53 and Rb tumor suppressor genes, respectively, thereby disrupting the cell cycle with loss of control on DNA replication, DNA repair, and apoptosis.[11] But in India incidence of HPV induced cancer is less. p16 protein is a surrogate tumor marker.

Genetics and oral cancer

Certain individuals inherit the susceptibility of inability to metabolize carcinogens or procarcinogens and/or an impaired ability to repair the DNA damage. Also the metabolism of tobacco carcinogens, genetic polymorphisms in the genes coding for the enzymes (P450

enzymes and XMEs) responsible for tobacco carcinogen metabolism are suspected to play major role in the genetic predisposition to tobacco-induced head and neck cancers .[46]

Cytogenetics of oral cancer

Extensive research has been conducted to identify genetic alterations in oncogenes or tumour suppressor genes, role of genomic instability ,epigenetic modifications and to generate a gene expression profile in oral oncogenesis .[46]

Several studies have identified specific genetic alterations in oral carcinomas and in premalignant lesions of the oral cavity. Recently, using comparative genomic hybridisation on 50 primary head and neck carcinomas, Bockmuhl and colleagues reported deletions of chromosome 3p, 5q, and 9p with 3q gain in well differentiated tumours, whereas in poorly differentiated tumours deletions of 4q, 8p, 11q, 13q, 18q, and 21q and gains in 1p, 11q, 13, 19, and 22q were identified, thus suggesting an association with tumour progression .[47]

Loss of chromosome 17p is also frequent in most human cancer including oral cancer. It is seen in approximately 60% of invasive lesions. Loss of chromosome arm 10 and 13q are also noted in primary tumors. [48]

Role of oncogenes and proto-oncogenes:

Several oncogenes have also been implicated in oral carcinogenesis.[46] Overexpression or mutation of epidermal growth factor receptor (EGFR), K-ras, c-myc, int-2, Parathyroid adenomatosis 1 and B-cell lymphoma like oncogenes have been reported in oral cancer development. [47]Transforming growth factor-alpha (TGF-α) is known to promote

neovascularization and mitogenesis. It has been shown to be aberrantly expressed in human OC.[49]

Tumor Suppressor Genes

More than 50% of all primary head and neck SCC, harbour p53 mutation . The loss of chromosome 9p21 occurs in the majority of invasive tumors in head and neck cancer.[50]p16 (CDKN2) present in this deleted region, is a potent inhibitor of cyclin D1. [51]Loss of p16 protein has been observed in most advanced pre-malignant lesions also.[49] Mayo along with his colleagues identified an alternative RNA transcript for p16 termed as Alternative Rating Frame.[53] Introduction of p16 or p16ARF into head and neck cell lines result in potent growth suppression.[54]

MicroRNA and oral cancer :

MicroRNAs (miRNAs) are small non-coding RNAs that mediate gene expression at the post-transcriptional level by degrading or repressing target messenger RNAs . Dysregulated expression of miRNAs is known to affect cell growth and can function as tumor suppressors or oncogenes in various cancers .In oral cancer, miRNAs have been shown to affect cell proliferation, apoptosis, and even chemotherapy resistance in Oral SCC patients. miRNAs can be potentially used as biomarkers to detect early-stage diagnosis of oral cancer and lead to the development of miRNA-based cancer-treatment and therapies.[55]

Clinical presentation : [56]

Oral cancer may manifest as the following:

- A red lesion (erythroplakia)
- A ulcerated area with everted edges which doesn't heal
- A white or mixed white and red lesion
- An indurated lump/ulcer (ie, a firm infiltration beneath the mucosa)
- A lesion fixed to deeper tissues or to overlying skin or mucosa
- Cervical lymph node enlargement: Cervical lymphnode can be first detected clinically and later the primary in oral cavity can be found especially in tongue cancer.
- Loosening of one or more teeth for no known reason, not connected with periodontal disease
- Persistent foreign body sensation
- Reduced mobility of the tongue
- Numbness of the tongue, teeth, or lips
- Bleeding of unknown origin
- Halitosis
- Altered dental occlusion
- Trismus

Site :

- All over India the most common site to be involved is buccal mucosa .
- In a review of oral cancer cases in Regional Cancer Center Trivandrum ,it was found that the highest prevalence of cancer of buccal mucosa (49.9%) outnumber that of tongue (23.97%) .[57]
- It was reported from a study in western Uttar Pradesh that the most common site was buccal mucosa, followed by the retro molar area, floor of mouth, lateral border of tongue, labial mucosa, and palate.[58]
- In Maharashtra it has been reported that the most common site is the buccal mucosa and lower alveolus region .[59]
- In a retrospective study performed in Kerala tongue was found to be the commonest site to be affected .[60]

TNM staging [61]

Physical examination is the basic procedure in checking cervical lymph nodes .However USG/CT imaging supplement the clinical examination To facilitate treatment planning and to provide a sense of outcome, clinical staging is generally used. The TNM clinical stage system has been adopted worldwide which includes:

TNM STAGING OF ORAL CANCER:

X	Primary tumour cannot be assessed
T0	No evidence of primary tumour
Tis	Carcinoma in situ
T1	Tumour 2 cm or less in greatest dimension

T2	Tumour more than 2 cm but not more than 4 cm in greatest dimension
T3	Tumour more than 4 cm in greatest dimension
T4a (lip)	Tumour invades through cortical bone, inferior alveolar nerve, floor of mouth, or skin (chin or nose)
T4a	Tumour invades through cortical bone, into deep/extrinsic muscle of tongue (genioglossus, hyoglossus, palatoglossus, and styloglossus), maxillary sinus, or skin of face
T4b	Tumour invades masticator space, pterygoid plates, or skull base; or encases internal carotid artery

N - Regional Lymph Node

NX	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	Metastasis in a single ipsilateral lymph node, 3 cm or less in greatest dimension
N2	Metastasis as specified in N2a, 2b, 2c below
N2a	Metastasis in a single ipsilateral lymph node, more than 3 cm but not more than 6 cm in greatest dimension
N2b	Metastasis in multiple ipsilateral lymph nodes, none more than 6 cm in greatest dimension
N2c	Metastasis in bilateral or contralateral lymph nodes, none more than 6 cm in greatest dimension
N3	Metastasis in a lymph node more than 6 cm in greatest dimension

M – Distant metastasis

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

Stage grouping

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

Eight edition Head and Neck AJCC Cancer Staging [61]

It incorporates significant changes based including changes to the tumor T categories in the oral cavity and the addition of tumor ENE(extra nodal extension) to the lymph node category .this

T Category for Oral Cavity Cancer

TX	Primary tumor cannot be assessed
Tis	Carcinoma in situ
T1	Tumor ≤2 cm, ≤5 mm depth of invasion DOI(depth of invasion)
T2	Tumor ≥2 cm, DOI ≥ 5 mm and ≤ 10 mm or tumor>2cm but ≤ 4 cm, and ≤10 mm DOI
T3	Tumor >4cm or any tumor>10 mm DOI
T4	Moderately advanced or very advanced local disease
T4a	Moderately advanced local disease: (lip) tumor invades through cortical bone or involves the inferior alveolar nerve, floor of mouth, or skin of face (ie, chin or nose); (oral cavity)tumor invades adjacent structures only (example through cortical bone of the mandible or maxilla, or involves the maxillary sinus or skin of the face); note that superficial erosion of bone/tooth socket (alone) by a gingival primary is not sufficient to classify a tumor as T4
T4b	Very advanced local disease; tumor invades masticator space, pterygoid plates, or skull base and/or encases the internal carotid artery

Regional Lymph Nodes Pathologic Category Criteria

NX	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	Metastasis in a single ipsilateral lymph node, 3 cm or less in greatest dimension and ENE-negative
N2a	Metastasis in a single ipsilateral or contralateral lymph node 3 cm or less in greatest dimension and ENE- positive; or metastasis in a single ipsilateral lymph node more than 3 cm but not more than 6 cm in greatest dimension and ENE-negative
N2b	Metastasis in multiple ipsilateral lymph nodes, none more than 6 cm in greatest dimension and ENE-negative
N2c	Metastasis in bilateral or contralateral lymph nodes, none more than 6 cm in greatest dimension and ENE-negative
N3a	Metastasis in a lymph node more than 6 cm in greatest dimension and ENE-negative
N3b	Metastasis in a single ipsilateral node more than 3 cm in greatest dimension and ENE-positive; or metastasis in multiple ipsilateral, contralateral, or bilateral lymph nodes, with any ENE-positive

Oral cavity malignancy detection:

Biopsy:

Punch or wedge biopsy is the gold standard for detecting and grading oral cancer, In punch/wedge biopsy a small piece of tissue is cut from an abnormal-looking area. If the abnormal region is easily accessed, the sample may be taken in the out patient department. If the tumour is deeper inside the mouth or throat, the biopsy may need to be done in an operating room, with general anaesthesia administered to prevent any pain.

Scrape cytology :

It acts as an adjunct and not a substitute for biopsy. A negative scrape cytology with strong clinical suspicion warrants biopsy. This is usually due to inadequate sampling of a representative area from the lesion. The biopsy needs to be repeated in such cases.

Supra-vital staining in assisting site of biopsy:

Toluidine blue is an acidophilic dye that stain acidic cellular components such as DNA and RNA. Its use in the detection of precancerous/cancerous tissue is based on the fact that dysplastic tissue contains quantitatively more DNA and RNA than non dysplastic tissue. Supra vital staining can aid in taking the biopsy from the representative site.

Newer tools like contact endoscopy and narrow band imaging can aid the diagnosis and can guide in taking biopsy from representative site. Imaging techniques complements clinical examination in assessing the extent of the primary lesion and also indicates nodal involvement

Metastasis:

Metastasis consists of sequential and selective steps including proliferation, stimulation of angiogenesis, detachment, motility, invasion into bloodstream and cross-talk with components of the new microenvironment, including parenchymal, stromal and inflammatory cells . Minority of malignant cells undertake the metastatic route, due to an interplay between host factors and intrinsic characteristics of cancer cells; thus metastasis may represent an escape of these cells from the hostile environment they themselves created, such as shortage of oxygen and nutrients, inflammation and immune system attacks .[62,63]

Metastasis of oral cancer is a complex process that involve detachment of cells from the tumor tissue, regulation of cell motility and invasion, proliferation and evasion through the lymphatic system or blood vessels. This process is due to reduced intercellular adhesion of tumor cells as they progress to malignancy because of loss of E-cadherin; they thereby begin to express proteins such as mesenchymal vimentin and N-cadherin, promoting cell elongation and interfering with cell polarity. This morphological transition, called epithelial-mesenchymal transition leads to molecular alterations interfering the behavior of these cells.[64]

Oral cancer and lymphnode metastasis:

Lymph node metastatic tumors occur in about 40% of patients with oral cancer. Occult metastasis varies according to tumor stage .It is seen <10% in T1, 10-30% in T2 ,30-40% in T3 and >40% in T4 tumors.[65] The presence of metastatic lymph nodes greatly affects the outcome (5-year survival). [5] The 5-year survival for a patient is halved if a single ipsilateral neck node is involved and is reduced by three-quarters if bilateral nodes are present. Spread of the disease outside the capsule reduces survival by a further 50%. [6] Aggressiveness of tumor is reflected in the ratio of metastasis to lymph nodes and is important for assessing prognosis and as well as to decide upon how extensive treatment patient has to undergo.

Predictor of lymph node metastasis:

To predict the biological behavior of lymph node metastasis ,histological grading system has been proposed . Multiple histological features have been tested to predict lymph node metastasis that includes nuclear pleomorphism, mitotic index, lympho-plasmacytic response, pattern of invasion , tumor nest, tumor thickness, tumor depth, lympho-vascular invasion, perineural invasion and grade of tumor. IHC markers like p53, Ki-67, cyclinD1, epidermal

growth factor receptor, CD31, cyclooxygenase 2, mucin 1, laminin 5g2, E-cadherin and β-catenin are useful markers to predict lymph node metastasis .[66,67,68]

Need for preoperative investigation

General policy of elective neck dissection based on clinical TNM staging exposes many Oral SCC patients to aggressive neck dissection that may not be necessary .[69] Many patients receive unnecessary treatment of the neck, causing morbidity, including shoulder dysfunction, pain, lymphedema, contour changes, and lower lip paresis, even in more conservative types of selective neck dissection.

To confirm metastasis in lymph nodes prior to neck dissection ,there is no reliable preoperative assessment .In a study by Koch and his colleagues , a concordance rate of 52% was found for the cT- and pT-classification. Overstaging of the extent of the primary tumor(59%) was found more frequently than understaging of the size. Because of the impact of nodal status on treatment and survival in Oral SCC, accurate staging of cervical lymphnode is critical. [70]

Investigations for metastasis:

Detection of cervical metastases accurately in patients with oral malignancies is of utmost important for all clinicians.

Palpation has been used for years as usual method of neck staging. Clinical palpation is the routine method in evaluating metastatic cervical lymphadenopathy. Clinically, lymph nodes are assessed for location, number, size, shape, consistency, and fixation. If their size is greater than 1 cm, hard and fixed ,they are considered to be malignant.[71]However, the

accuracy, sensitivity and specificity of palpation is usually reported to be low and is subjective.

Assessment of lymph node metastasis in oral carcinoma patients is difficult. Therefore various imaging methods such as computed tomography (CT), magnetic resonance imaging (MRI), positron emission tomography , lymphoscintigraphy , ultrasonography (USG),FNAC ,sentinel lymph node biopsy and USG-guided fine-needle aspiration cytology are used in assessing the lymph node status.

In enlarged lymph node ,fine needle aspiration cytology has found to play an important role in diagnosis. In it, a small needle attached to a syringe is inserted into the questionable mass and cells are aspirated, or pulled out into the syringe . FNAC is a quick, relatively easy, safe, and repeatable method of obtaining tissue for microscopic examination and allows rapid interpretation of the specimen. This procedure can be performed as an out patient procedure and it permits an early and efficient treatment planning process . FNAC is a useful method in comparison to more expensive surgical excision biopsies and also it allows rapid onset of therapy .But main drawback of FNAC is taking the representative sample. Deep-seated LNs may be difficult to access using FNAC and can be confused with complex anatomical structures in the head and neck because of the lack of accuracy in needle tip localization and possible risk of injury to surrounding vital tissues. USG guided FNAC can be performed to improve outcomes.[72]

USG has many advantages over CT scan such as absence of radiation and contrast medium. US is better than CT and MRI in many aspects including the affordability for the patients. US has been studied adequately and has proved its efficiency, sensitivity, specificity and accuracy for the detection of lymph node metastasis. On USG features such as round

shape, absence of echogenic hilum, sharp nodal borders, presence of intranodal necrosis and peripheral vascularity are suggestive of metastatic cervical lymph nodes.[73]

The main advantage of CT and MRI is less inter-observer variation and these are relatively standardized techniques that can be performed in most institutions and can be interpreted by radiologists without specific knowledge of images of the head and neck. The detection of nodes in the submental and submandibular regions is found to be superior with USG whereas CT and MRI studies have occasionally been impaired by artefacts from bones and dental amalgam restorations.[74] Being less invasive than CT, USG is particularly indicated for follow-up studies aimed at assessing the efficacy of chemotherapy or radiotherapy.

Though the sensitivity, specificity and accuracy of detecting metastatic lymph nodes by USG is better, it is not yet proved to be 100% accurate. Lymph node necrosis in ultrasonography is a highly specific sign of oral SCC metastasis but this finding is thought to ensue relatively late in the course of the disease and it is rare for lymph nodes less than 10 mm to show this sign . Normal and reactive nodes have an echogenic hilum due to the interfaces between multiple lymphatic sinuses as they join on the medulla. [75] Malignant nodes have no visible hilum and effacement of the hilum is considered as a diagnostic criteria of malignancy. However, loss of fatty hilum is not a definite indicator of malignancy and may be seen in as many as 9% of reactive lymph nodes . Numerous studies have tried to determine the optimal size criteria; however, wide variations in measurement techniques make the decision difficult . As small size lymph nodes may harbor metastatic foci, reducing cut-off size will increase the sensitivity at the cost of lowering the specificity. Because of the limitations of US, combining a highly specific test with US is considered helpful in the assessment of metastasis.[76]

With USGFNAC it is possible to sample suspicious cervical lymph nodes to complete radiologic findings and differentiate benign from malignant nodes These drawbacks can be overcome with the application of US-fine needle aspiration cytology (FNAC). In the literature, the accuracy of USG-FNAC varies from 89-97%.[77]

False-negative results is the main drawback of USGFNAC. USGFNAC inaccuracy could have been due to small metastasis missed by the needle, a single tumor cell overlooked by the pathologist or aspiration of a wrong lymph node .Moreover, lymph nodes near the mandible are difficult to visualize or aspirate due to the shadow of the mandible.

A systematic review of studies comparing palpation with computed tomography (CT), found a sensitivity of 75% and specificity of 83% for palpation and a sensitivity of 81% and specificity of 83% for CT.[78]

In the literature USG shows a sensitivity ranging from 78-97%. In a study, USG yielded a sensitivity, specificity, PPV (positive predictive value), NPV(negative predictive value) and accuracy of 85.7%, 90%, 92.3%, 81.8% and 87.5% whereas clinical palpation yielded a sensitivity, specificity, PPV, NPV and accuracy of 68.7%, 87.5%, 91.6%, 58.3% and 75%.[79]

In another study it was found a sensitivity of 64% and specificity of 85% for palpation, sensitivity of 72% and specificity of 96% for ultrasound and a sensitivity of 81% and specificity of 96% for CT .[80]

Imaging methods like USG,CT,MRI can also guide to take the representative sample for FNAC. It was found that the accuracy of US-guided aspiration cytology was superior to other technique.81

In a recent study it has been found that USG guided FNAC have the highest positive predictive value (100%)followed by MRI (75%) and then by USG(57%).USG guided FNAC was also found to have the highest specificity(100%) but sensitivity of MRI(83%) was found to be slightly more than USG guided FNAC(80%) .[82]

USG combined with FNAC is followed only in European countries to stage patients with head and neck malignancy preoperatively. However, in majority of cancer institutes of India ,ultrasound guided FNAC is not practised as standard protocol .Study of cervical node metastasis by ultrasound guided FNAC help oncosurgeon to plan the surgery.

Treatment:

Accurate staging is required for the treatment of head and neck cancer to determine the type and extent of therapy and to predict the clinical outcome.

Previously, the gold standard for addressing metastatic cervical lymph node was Radical Neck Dissection. This safe oncological surgical procedure involves not only resection of level I to V lymph nodes of the neck but also the tail of the parotid, submandibular gland, sternocleidomastoid muscle, internal jugular vein and spinal accessory nerve. It reduces the risk of regional recurrences, but produces significant post-operative morbidity, mainly shoulder dysfunction. In 1960 radical neck dissection was modified which had control rates equal to previous radical neck dissection and non-

lymphatic structures like sternocleidomastoid muscle, internal jugular vein and spinal accessory nerve.

Later on the concept of selective neck dissection was introduced, where the most likely level of lymphatic drainage and one level beyond that were dissected in N0 neck staging(by few authors in N1 neck) again to further reduce morbidity. Elective supra omohyoid neck dissection (Level I-III) is now an acceptable method of elective neck dissection in T1-T2 cancer of buccal mucosa with clinically N0 neck, it has produced good results with minimal morbidity. A wait-and-see policy of the N0 neck after transoral tumour excision only seems feasible if a strict and accurate follow-up regimen can be provided . [83] In a landmark study done in Tata memorial hospital ,Mumbai where elective neck dissection(END) versus wait and watch policy followed by salvage neck dissection was evaluated in early oral cancer with N0 neck ,clear benefit of END was found for these cases. This study showed that with the help of USG-FNAC during follow-up, it is possible to detect a significant percentage of neck recurrences in an early stage.[84]

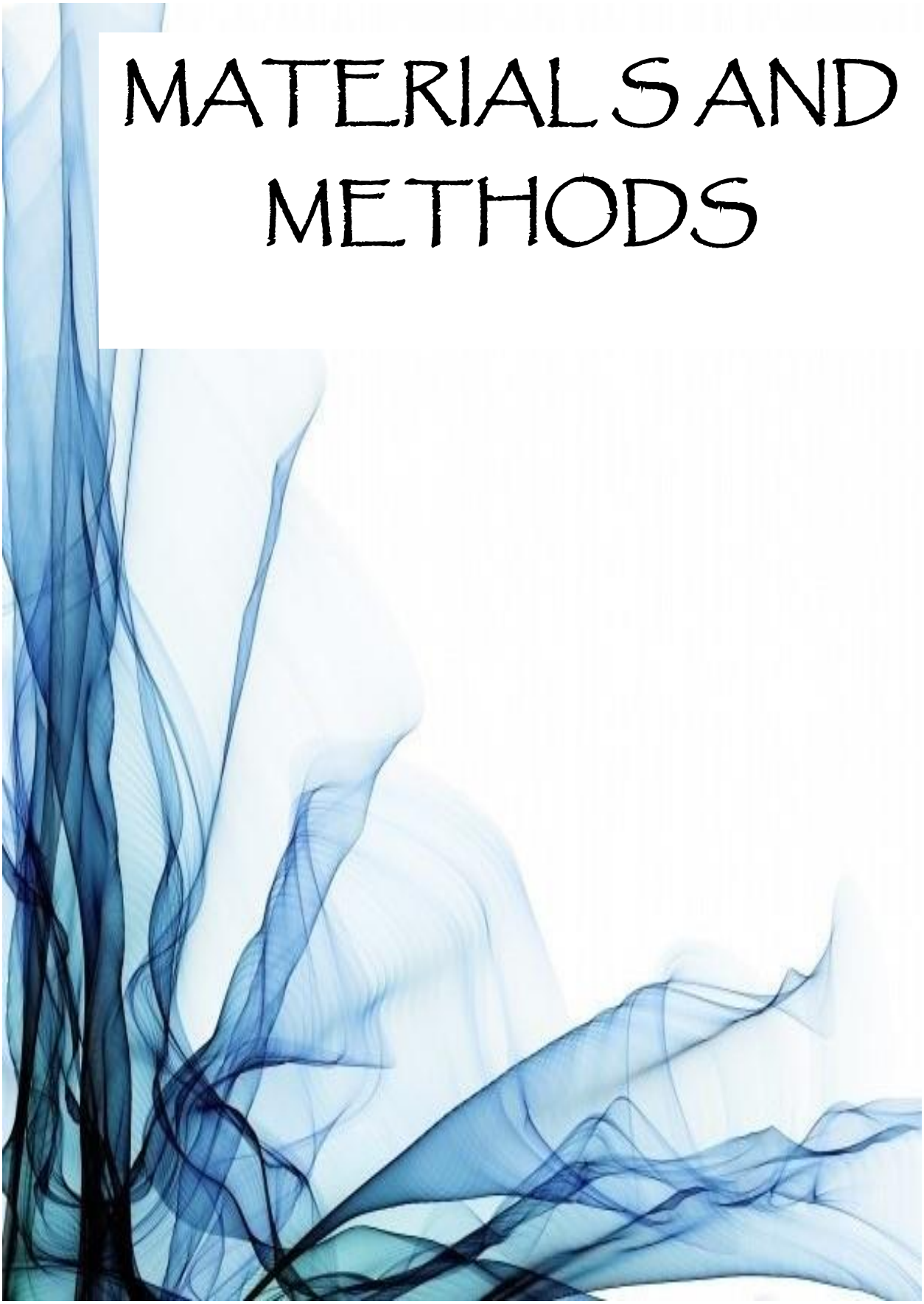
Adjuvant treatment (re-resection, radiotherapy, or chemotherapy) may be indicated based on adverse tumor features diagnosed at histopathological examination. Adjuvant radiotherapy is indicated for T3/T4 cancers, presence of high risk features lymphovascular emboli ,extranodal extension, perineural invasion , poorly differentiated node ,close margins. Adjuvant chemoradiation is indicated for positive margins and extranodal disease[85]

Oral cancer prognosis [86]

Prognosis of oral cancer depends on specific oral locations. For example, cancer of the lip, have a much better prognosis compared to cancer at the base of tongue or on the gingiva . Prognosis of intra-oral cancer is generally poor, with a 5-year survival less than

50%. Particularly in post-transplant oral tumours, outcomes are worse compared with the normal population. Local recurrences occur in a significant percentage of patients, whereas distant metastases are less frequent. Prognosis is related mainly with the size of the lesion and the nodal status at the time of diagnosis. HPV positivity is correlated with better cancer outcomes. A significant association of p16 [INK4a] overexpression with improved survival in young patients with squamous cell cancers of the oral tongue has also been demonstrated. Of course, p16 [INK4a] overexpression was not a reliable predictor of HPV positivity.

MATERIALS AND METHODS



MATERIAL AND METHODS

SOURCE OF DATA:

- All cases of oral squamous cell carcinoma with palpable cervical lymph node presenting to the department of ENT and Head and Neck Surgery RL Jalappa hospital and research center and who were planned for neck dissection during the period of Jan 2016 to August 2017 were included in the study.
- Study design: Laboratory based diagnostic study.

Sample size:

Sample size was estimated by using ultrasound guided FNAC sensitivity as 89.2% for diagnosis of cervical metastasis of oral squamous cell carcinoma from the study done by Knappe et al. [72]

With gold standard sensitivity of 100% ,At 80% power, α error of 5% , the minimum sample size of 68 was obtained

Sample size was calculated using nMaster2.0 software.

SELECTION CRITERIA

INCLUSION CRITERIA:

All patients with clinically palpable cervical lymph node with biopsy proven Oral squamous cell carcinoma and were planned for neck dissection.

EXCLUSION CRITERIA:

1.Patients who had undergone preoperative radiotherapy .

2.Patient who had undergone neoadjuvant chemotherapy.

3.Patient with recurrent or second primary cancers.

METHOD OF COLLECTION OF DATA:

- All biopsy proven oral squamous cell carcinoma patients with palpable lymph nodes were included in the study.
- Clinically, lymph nodes were assessed for location, number, size, shape, consistency,
- The patient was positioned supine with the neck hyper-extended. USG was done using Siemens Acuson X 300 using a linear high-resolution transducer and cervical lymph node were scanned for ultrasonographic features suggestive of metastasis such as round shape, absence of echogenic hilum, irregular nodal borders, presence of intranodal necrosis , increased vascularity and clustering of lymphnodes.[87] The cervical lymph node showing one or more above features was selected for aspiration

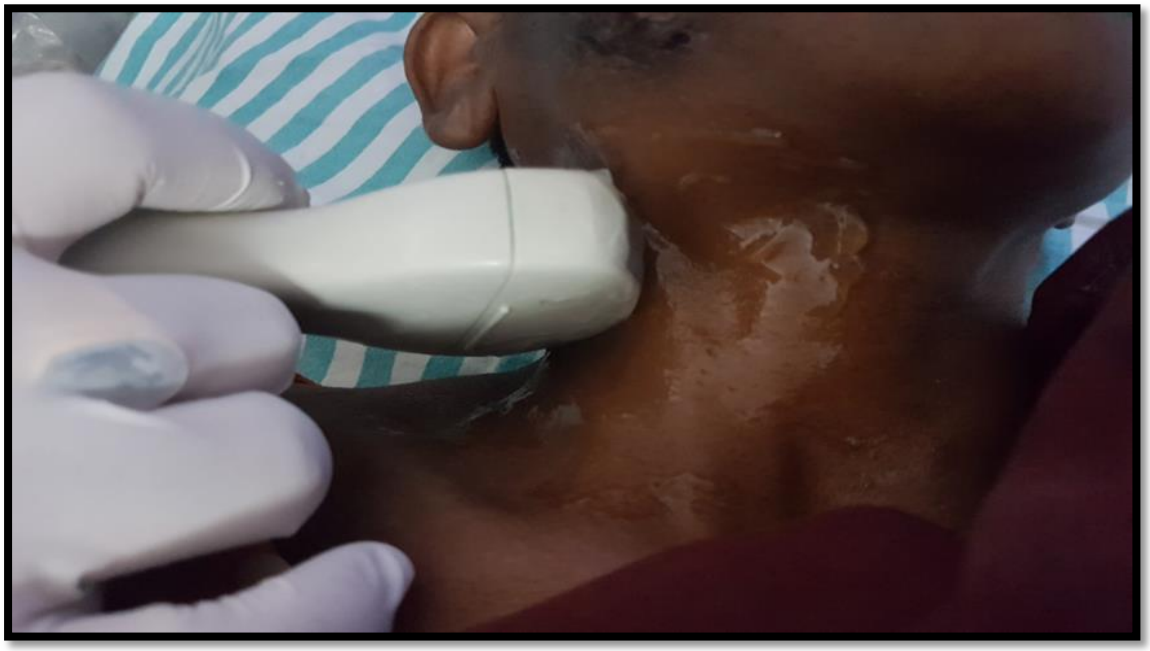


Figure 4: USG of neck using linear transducer

- In cases where there is no definite USG evidence of metastasis largest lymphnode was selected for FNAC.
- After sterile preparation 23 gauge needle using a 5ml syringe was introduced into the skin 0.5-1cm from the transducer at the middle of long axis of the transducer. After confirmation of presence of needle tip within the node multiple passes were made in

different directions without withdrawing the needle completely out of node and aspiration was done.

- Minimum of three slides was prepared from the aspirated material. One slide was air dried and remaining two slides were fixed in alcohol. Air dried slide was stained with Giemsa.
- The two alcohol fixed slides were stained first with methylene blue and if it shows enough cellular material it was considered satisfactory. Then it was stained with hematoxylin and eosin and PAP stain.
- The FNAC diagnosis was correlated with the histopathological findings.
- Clinical and histopathological features were correlated with status of cervical lymphnode.

Stains were done according to the Standard of protocol (SOP) followed by our laboratory which is modified from Bancroft's Theory and Practice of Histological Techniques . [88]

METHYLENE BLUE STAIN

1. Smear was fixed for at least 15 secs in 70% to 95% ethanol or methanol
2. Slides were removed from fixative and placed on paper towels
3. 1 or 2 drops of methylene blue was applied
4. After 10-15 secs excess stain was removed by washing in tap water
5. The slides were examined for adequacy of specimen while it is still wet
6. The slides were reimerged in fixative and submitted for further preparation for PAP stain or H & E stain.

HEMATOXYLIN AND EOSIN STAIN

1. The alcohol fixed smears were washed in distilled water

2. It was dipped in Harris Hematoxylin for 8-10 mins
3. Then it was washed in running tap water until excess stain was removed
4. The slides were dipped in 1% acid alcohol 2-3 times
5. It was washed in running tap water
6. Counterstaining with Eosin for approximately 20 secs was done
7. Again it was washed in running tap water until excess stain is removed
8. Then it was dipped in Xylene for 20 sec, dried and mounted with cover glass using a drop of D.P.X

PAP STAIN

1.The fixed smear was dipped for a minute in tap water and excess water from slide was blotted out

2.It was dipped for 45 secs in RAPID-PAP(Nuclear stain)

3.Washed in Scotts's tap water buffer for 30 seconds and excess water from the slide was blotted out

4.It was dipped for 30 secs in RAPID-PAP(dehydrate)

5.It was dipped 45 secs in working cytoplasmic stain

6.Washed in Scotts's tap water buffer for 20 seconds and excess water from the slide was blotted out

7.Dehydration in a second bath of RAPID-PAP(Dehydrate) for 30 secs was done and it was air dried

8.It was dipped in Xylene for 20 sec, dried and mounted with cover glass using a drop of D.P.X

Giemsa stain

1. 4-5 drops of working Giemsa stain was put on air dried smear for 30seconds to 1 min

2.Double the amount of buffer solution was flooded on the slide for 15min

3.The slide was washed in running tap water until excess stain was removed

4.The slide was air dried and was dipped in Xylene for 20 sec, dried and mounted with cover glass using a drop of D.P.X

STATISTICAL ANALYSIS:

Data was entered into Microsoft excel data sheet and was analyzed using SPSS 22 version software. Categorical data was represented in the form of Frequencies and proportions. **Chi-square test** was used as test of significance for qualitative data. Continuous data was represented as mean and standard deviation.

Graphical representation of data: MS Excel and MS word was used to obtain various types of graphs such as bar diagram and Pie diagram.

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 analyze data.

True positive results are metastasis carrying (positive) nodes detected by USG guided FNAC.

True negative results are unaffected by (negative) nodes demonstrated by USG guided FNAC.

False positive results are unaffected nodes demonstrated as carrying metastasis by USG guided FNAC.

False negative results are metastasis carrying nodes demonstrated as unaffected by USG guided FNAC.

Sensitivity determines how well positive lymph nodes are diagnosed by USG guided FNAC.

$$\text{Sensitivity} = \frac{\text{True positive}}{\text{True positive} + \text{False negative}}$$

Specificity determines how well positive nodes are distinguished from non-affected nodes by USG guided FNAC

$$\text{Specificity} = \frac{\text{True negative}}{\text{True negative + False positive}}$$

Accuracy determines how well this method of USG guided FNAC functions

$$\text{Accuracy} = \frac{\text{True positive + True negative}}{\text{Total}}$$

Positive predictive value determines the probability of a node with a positive diagnostic result being actually positive

$$\text{Positive predictive value} = \frac{\text{True positive}}{\text{True positive + False positive}}$$

Negative predictive value determines the probability of a node with a negative diagnostic result being actually unaffected

$$\text{Negative predictive value} = \frac{\text{True negative}}{\text{True negative + False negative}}$$

RESULTS

RESULTS:

The present study includes 70 USG guided FNAC of cervical lymph nodes from squamous cell carcinoma of oral cavity.

Age:

Mean age of subjects was 52.94 ± 12.02 years. Patient age ranged from 27 to 80 years .Most of our cases belonged to the age group of 40 to 70 years (74.3%) followed by 30 to 40years (11.5%)

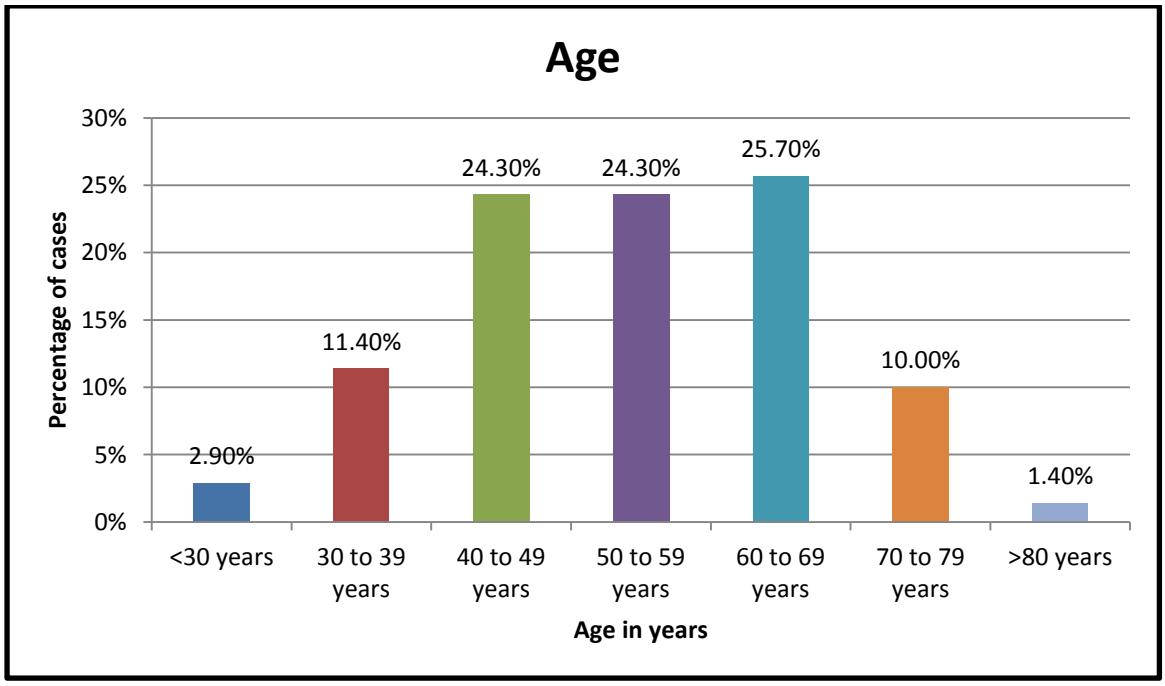


Chart 1: Bar diagram showing Age distribution of subjects in the study

Gender distribution:

Female predominance was seen and Male to female ratio of 1:2.1 was observed

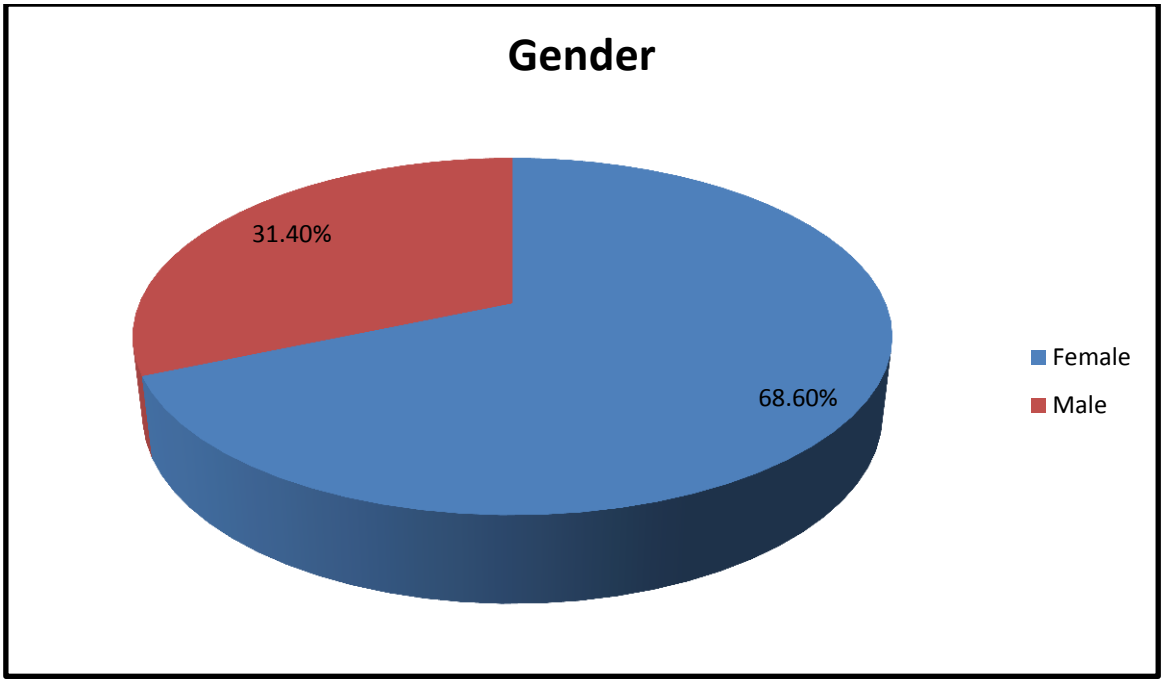


Chart 2: Pie diagram showing Gender distribution of subjects

Presenting complaints:

In our study most common complaint was ulcer in the mouth seen in 75.70% cases

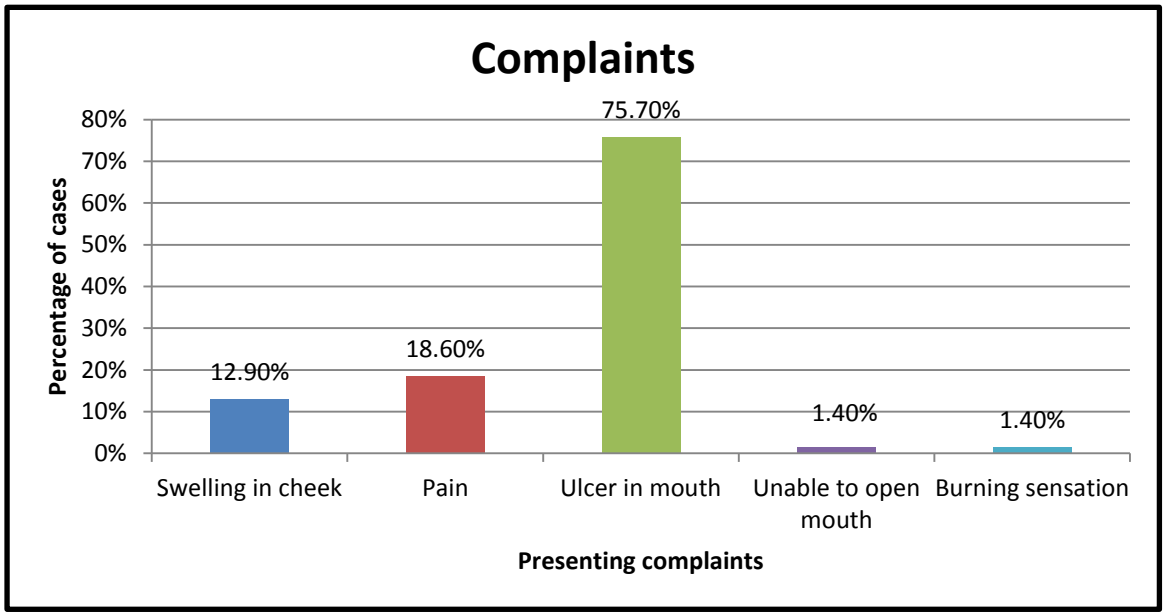


Chart 3: Bar diagram showing Complaints on presentation among subjects

Duration of symptoms:

Most of the cases ie. 40 cases(57.1%) in our study presented within 3months

Table 1: Duration of symptoms

Duration of symptoms	No. of cases	Percentage
2 weeks	4	5.8%
1 month	14	20%
2months	17	24.3%
3months	5	7.1%
4 months	8	11.4%
5 months	7	10%
6months	14	20%
1 year	1	1.4%

Predisposing factors:

Betel quid chewing was the most common predisposing factor leading to oral cancer

Table 2: Predisposing factors among subjects

Predisposing factor	No. of cases	Percentage (%)
Betel quid chewing	69	98.6%
Smoking	22	31.4%
Both smoking and betel quid chewing	20	28.6%
Poor Oral hygiene	45	64.3%
Caries tooth	38	54.3%
Both smoking and alcohol	21	30%

Betel quid consumption:

Most of the cases consumed betel quid for 20 years following which they developed cancer of oral cavity.

Table 3: Years of betel quid consumption:

Years of consumption	**No. of cases**	**Percentage**
0-10 years	9	12.8%
11-20 years	30	42.8%
21-30years	24	34.3%
31-40years	4	5.7%
41-50years	3	4.2%

Site involved:

The common site of carcinoma was Buccal mucosa (71.4%) followed by lower alveolus(11.3%).Tongue carcinoma was seen in 10% of cases

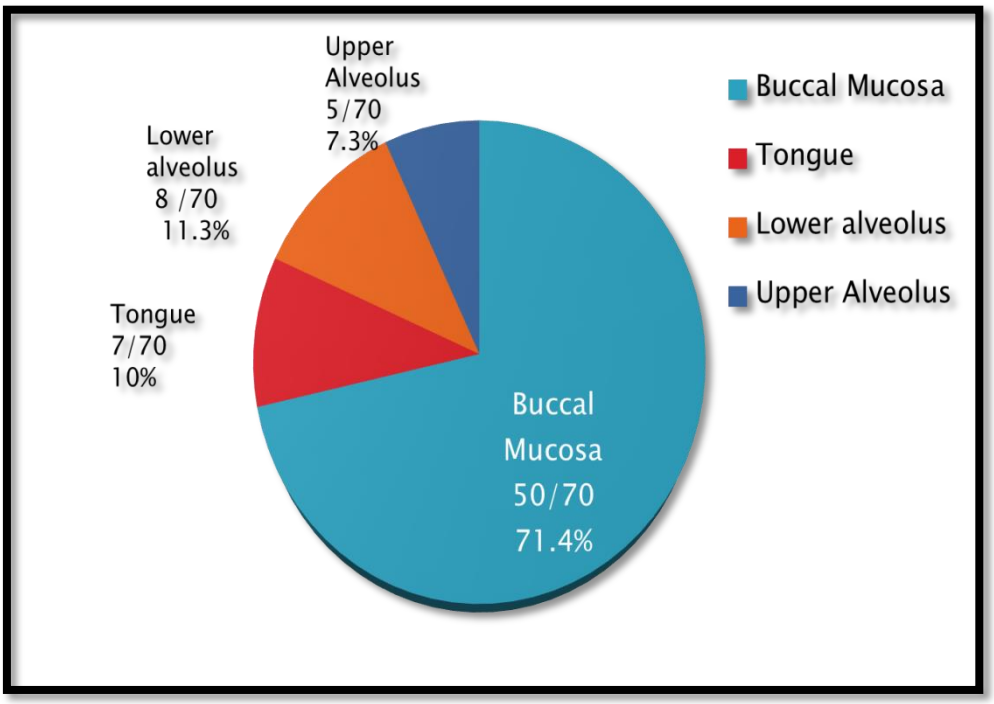


Chart 4: Pie diagram showing site involved by tumor

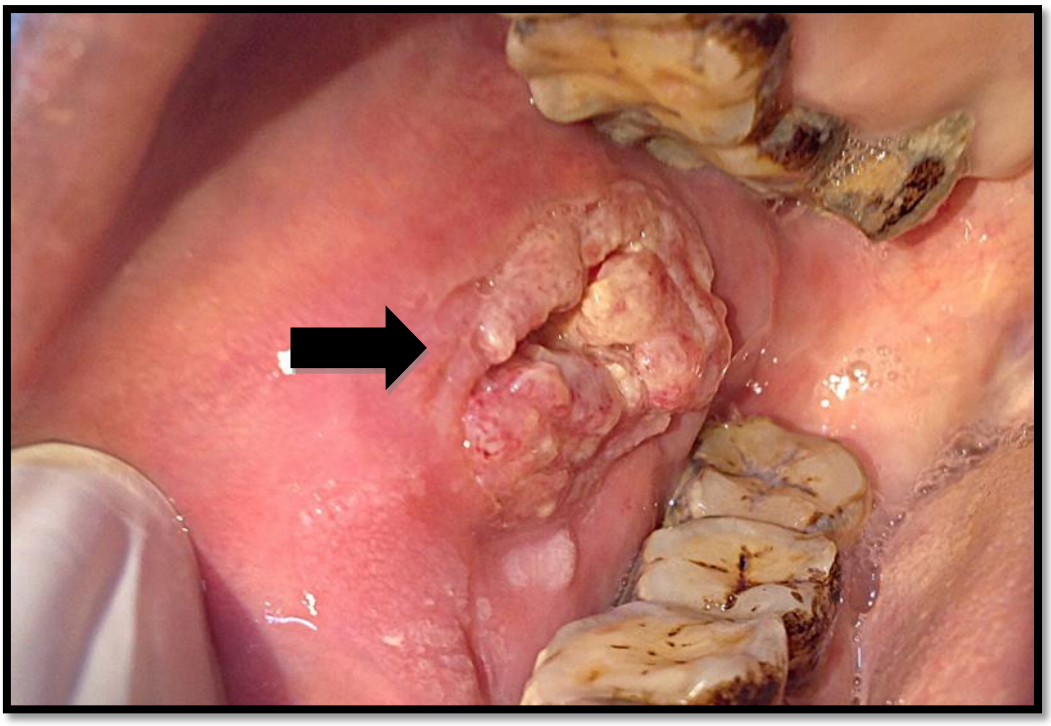


Figure 5: Microphotograph showing involvement of buccal mucosa by ulceroexophytic growth

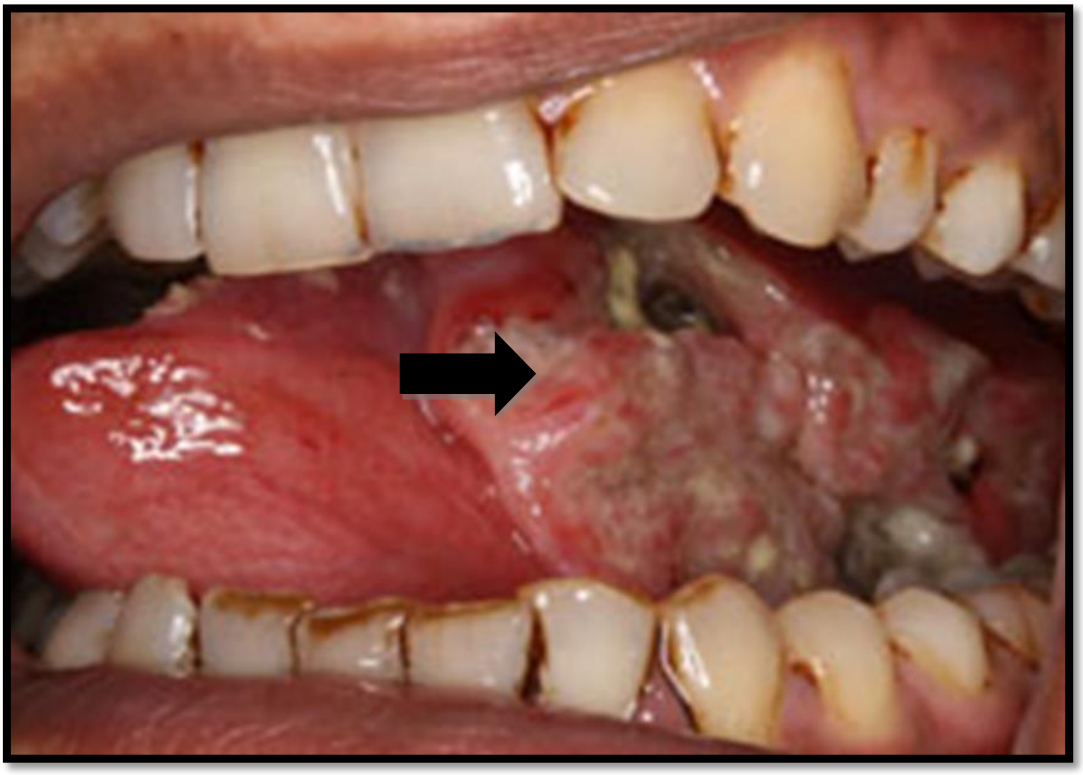


Figure 6: Microphotograph showing involvement of lateral border of tongue

Gross appearance of tumor:

Most of our cases showed Ulceroexophytic tumor which constituted up to 70.0% followed by ulcerative (21.4%).The least common tumor appearance was flat type constituting 2.9%.

Table 4: Gross appearance of tumor among subjects

		Count	%
Gross appearance	Ulceroexophytic	49	70.0%
	Ulcerative	15	21.4%
	Infiltrative	4	5.7%
	Flat	2	2.9%

Tumor size:

In most of our oral cancer cases tumor size was >2 to 4cm comprising of 55.7% followed by up to 2cm in 25.7% cases.

Table 5: Tumour size among subjects

		Count	%
Tumour size	Up to 2cm	18	25.7%
	>2 to 4 cm	39	55.7%
	>4 cm	13	18.6%

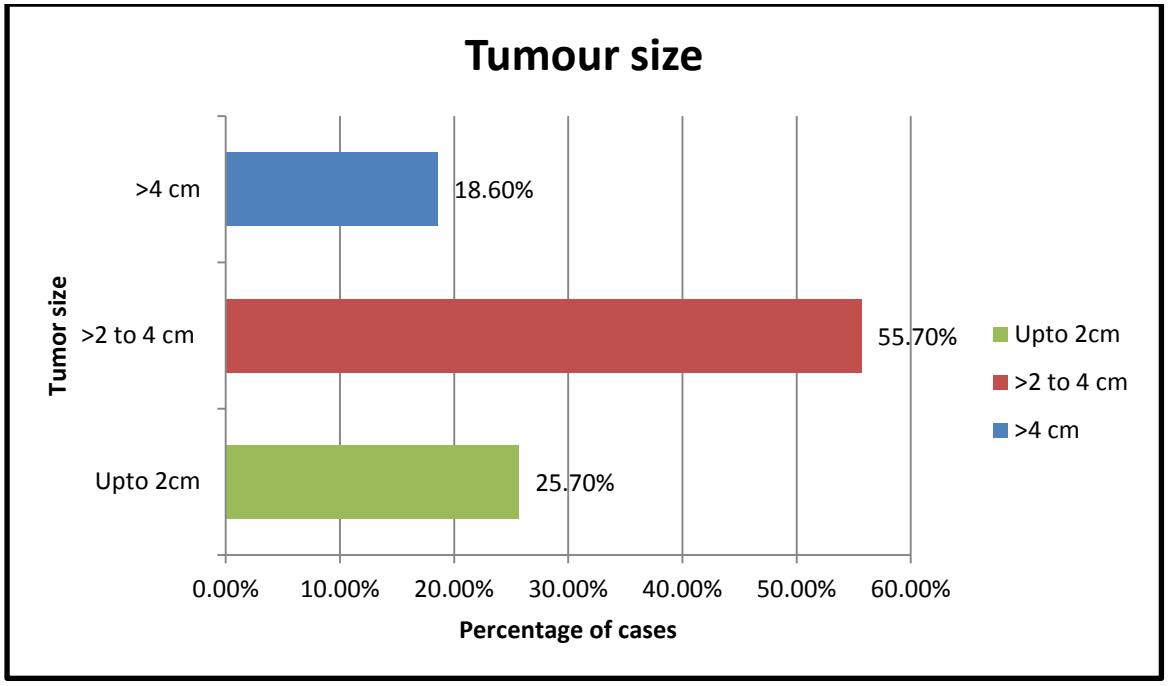


Chart 5: Bar diagram showing Tumour size among subjects

Level of enlarged lymph node:

In this study most common level of lymph node to be involved was Level I in 65 cases followed by level II in 53 cases.

Table 6: Level of enlarged Lymph node

		No. of Cases
Level of enlarged lymph node	I	65
	II	53
	III	6
	V	8

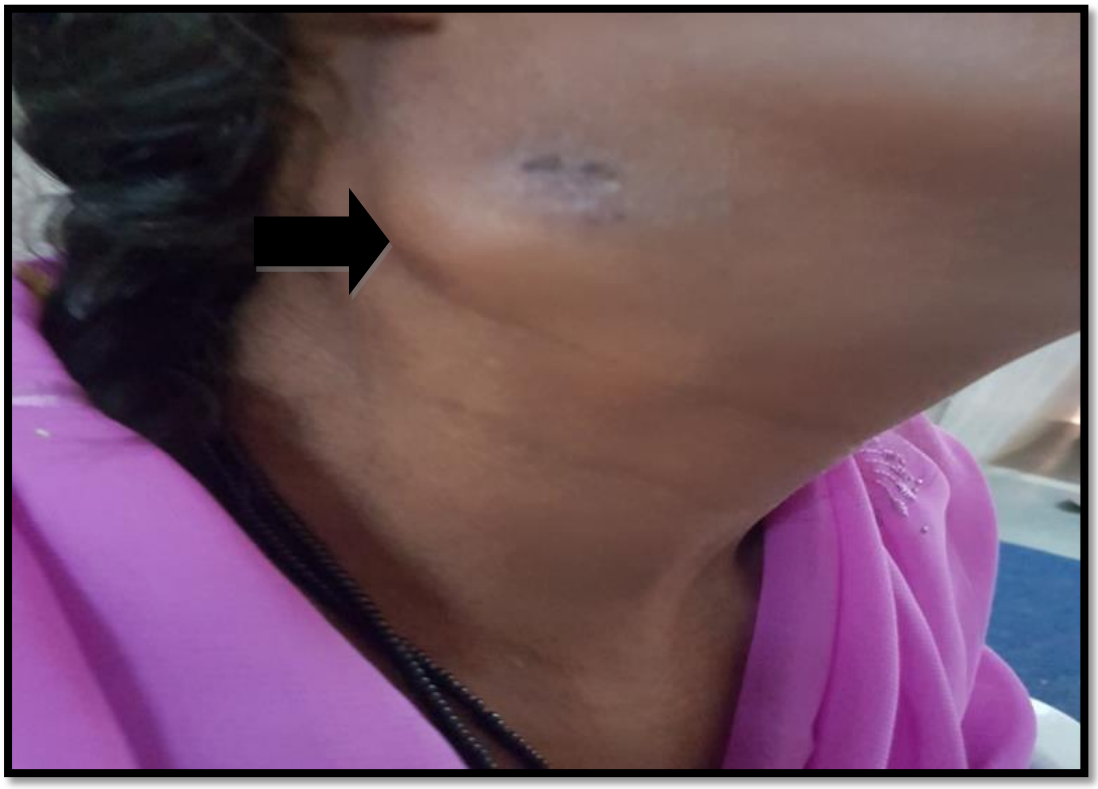


Figure 7: Enlargement of level II lymph node

Lymph node size:

In our study majority of the cases had lymph node size up to 3cm(94.2%)

Table 7: Lymph node size distribution among subjects

N stage	Number of cases	Percentage
Up to 3cm	66	94.2%
3-6cm	4	5.8%
>6cm	0	0%

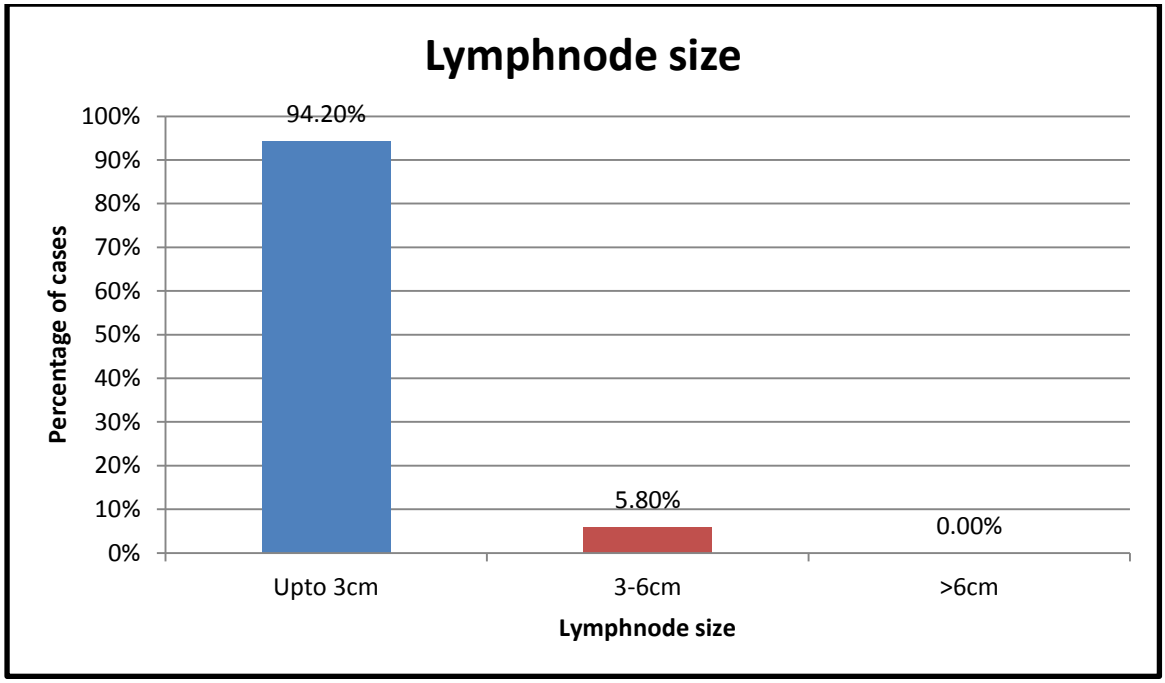


Chart 6: Bar diagram showing Regional lymph node size

Clinical TNM staging:

Most of our cases presented clinically at a late stage .Most were in Stage IV (65.8%) followed by Stage III (34.2%).

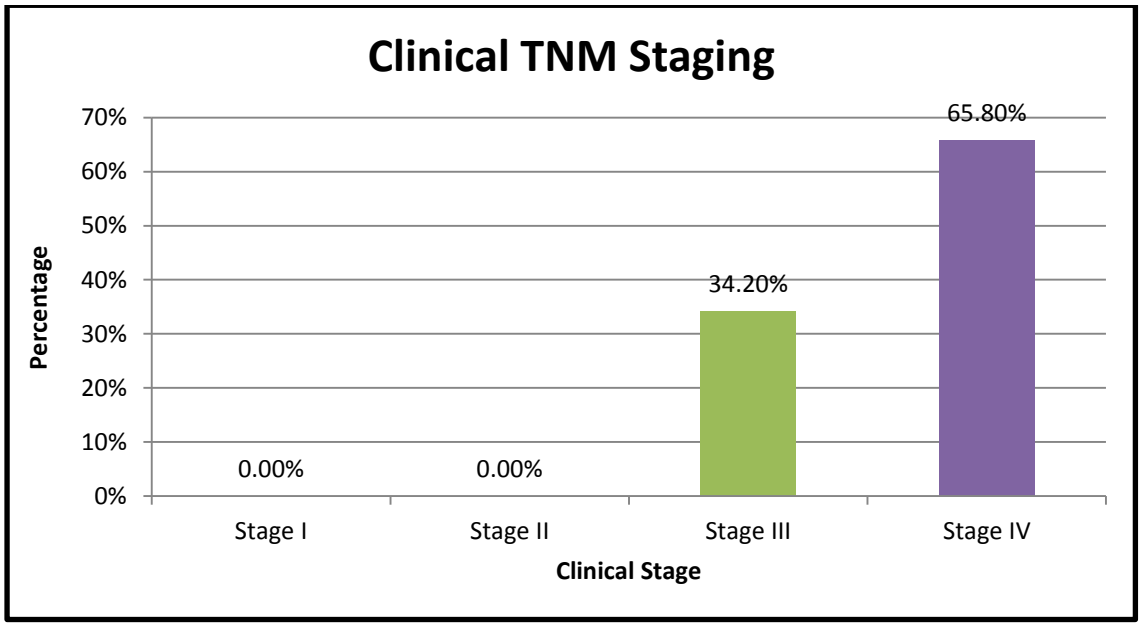


Chart 7 :Bar diagram showing clinical TNM stage

Ultrasound findings:

Ultrasound parameters like round shape,absence of echogenic hilum ,presence of necrosis ,increased vascularity and irregular borders suggestive of malignancy was noted. 45.7% cases showed round nodes, 42.9% cases showed absence of hilum ,37.10% showed increased vascularity and 25.7% cases showed necrosis. Cystic changes were seen in 15.7% of cases and irregular borders were seen in 11.4%.

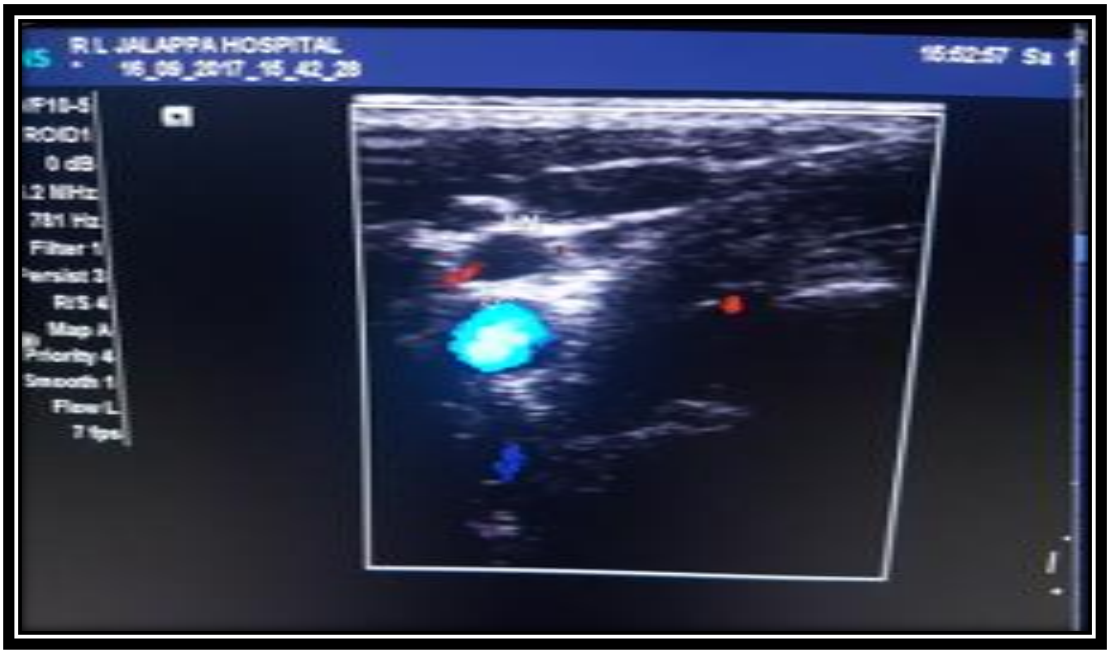


Figure 8: Ultrasound of metastatic lymph node showing increased vascularity

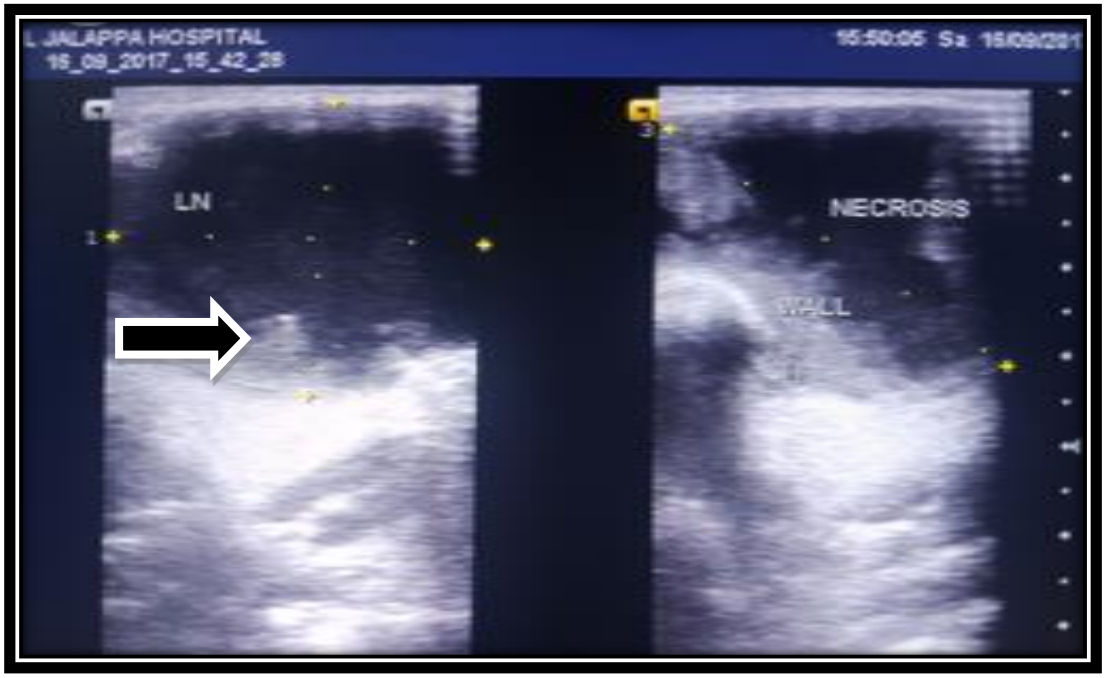


Figure 9: Ultrasound of metastatic lymph node with necrosis and irregular border

Table 8: Ultrasound findings of lymph node

		Count	%
Shape	Oval	38	54.3%
	Round	32	45.7%
Fatty Hilum	Absent	30	42.9%
	Present	40	57.1%
Necrosis	Absent	52	74.3%
	Present	18	25.7%
Cystic	Absent	59	84.3%
	Present	11	15.7%
Irregular borders	Absent	62	88.6%
	Present	8	11.4%
Increased vascularity	Absent	44	62.9%
	Present	26	37.1%

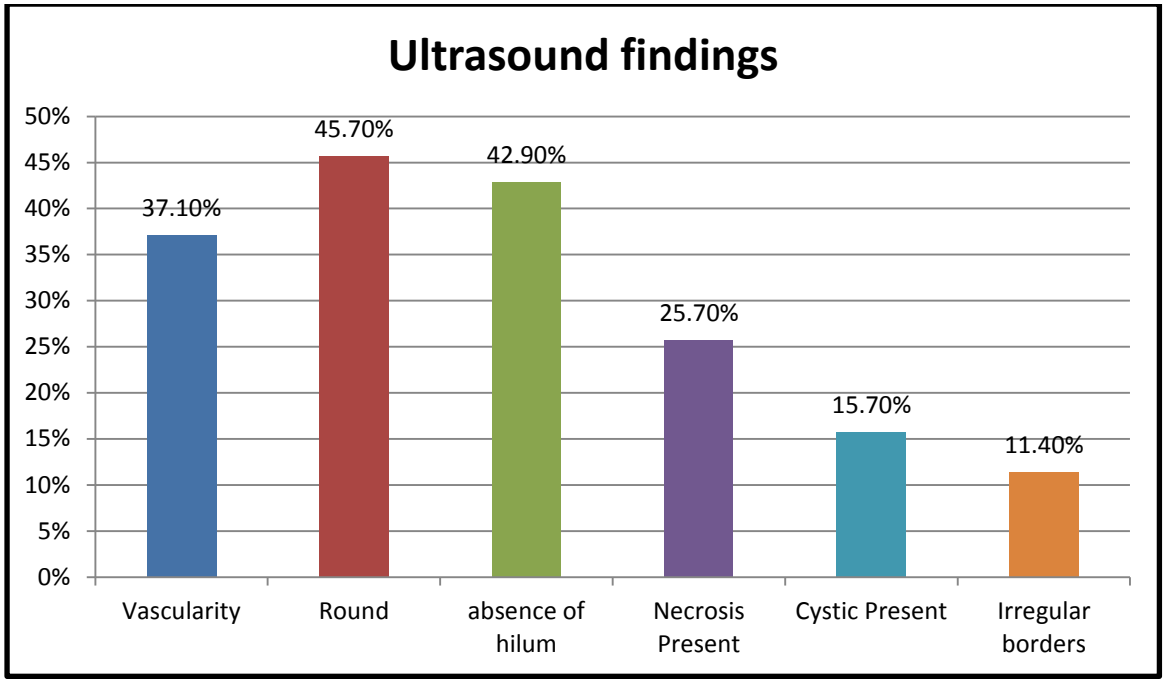


Chart 8: Bar diagram showing features of metastatic lymph node

Level of lymph node aspirated in USG guided FNAC:

With ultrasound guidance the most common level to be aspirated was level I comprising of 65.8% cases followed by Level II comprising of 33.2% cases

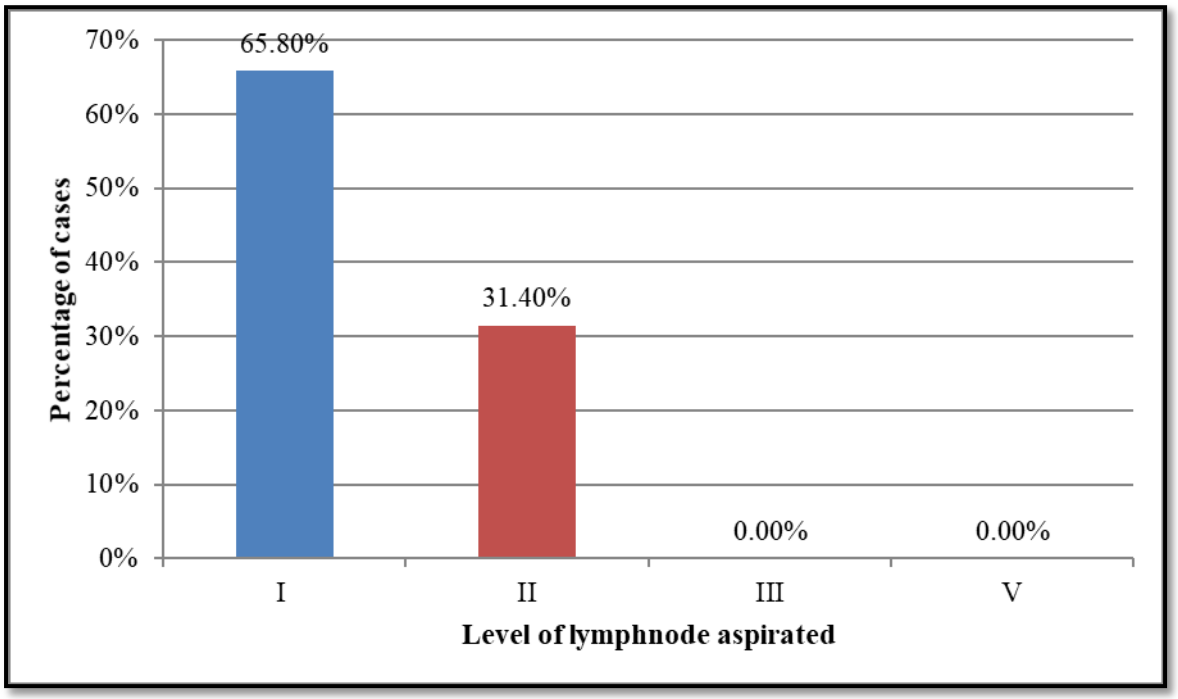


Chart 9: Bar diagram showing level of lymph node aspirated among subjects

Cytological findings:

On cervical lymph node aspiration 29 cases (41.4%) showed positivity for malignant squamous cells while 41 cases (58.6%) were negative for metastasis.

Cytological cellularity:

Most of the metastatic cases showed high cellularity while most of the cases which were negative for malignant cells showed moderate cellularity.

Table 9: Association between Cellularity and lymph node status

		Reactive(n=41)		Malignant(n=29)	
		Count	%	Count	%
Cellularity	Mild	8	19.6%	0	0%
	Moderate	28	68.2%	4	13.8%
	High	5	12.2%	25	86.2%

$\chi 2 = 38.4$, df = 2, p <0.001*

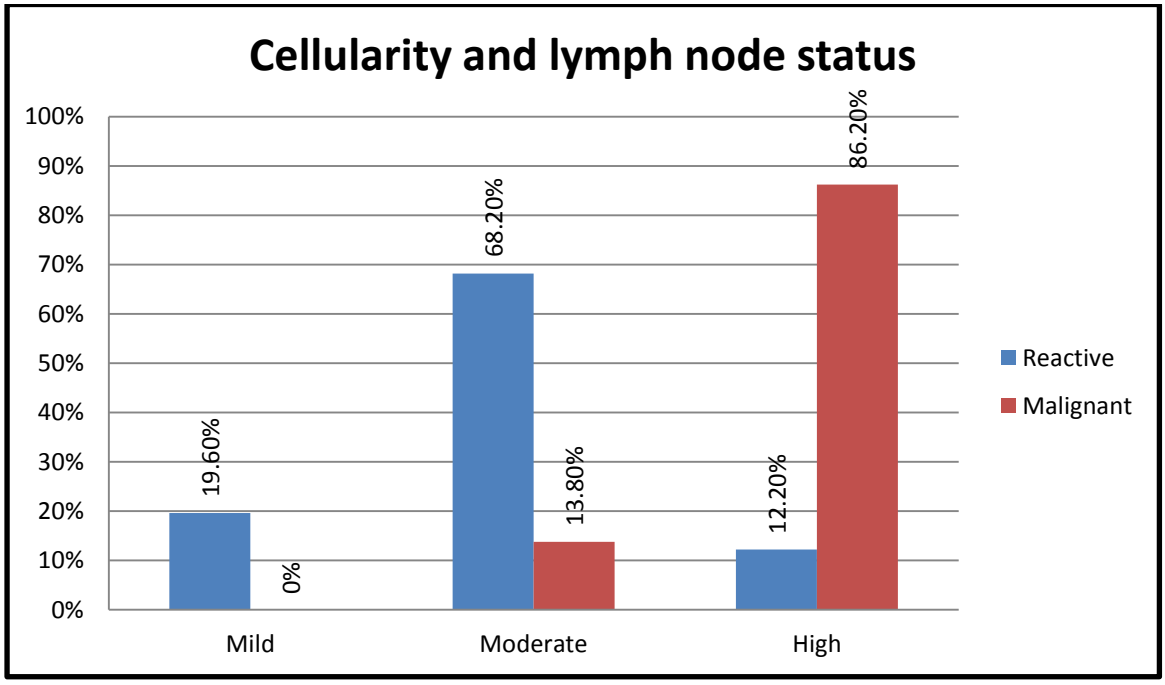


Chart 10: Bar diagram showing Association between Cellularity and lymph node status

Other cytological findings:

Among the metastatic cases dyskeratosis was seen in 51.7% cases, necrosis was seen in 31% cases, acute suppurative inflammation was seen in 20.6% cases and cystic change was seen in 13.8% cases. None of the metastatic cases showed granuloma on cytology.

Table 10: Cytological findings in metastatic and non metastatic group

	Positive for Malignancy (n = 29)		Negative for Malignancy (n = 41)	
	Count	**%**	**Count**	**%**
Dyskeratosis	15	51.7%	0	0%
Necrosis	9	31%	1	2.4%
Acute suppuration	6	20.6%	0	0%
Cystic	4	13.8%	0	0%
Granuloma	0	0%	5	12.2%

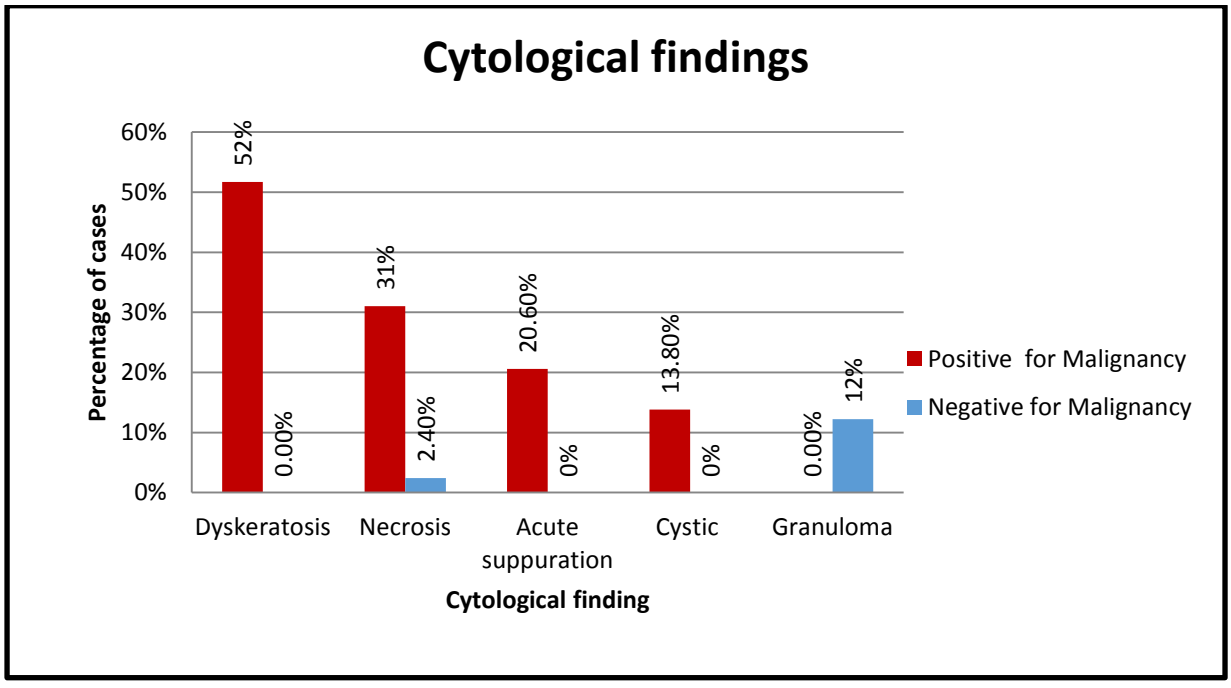


Chart 11 : Bar diagram showing cytological findings in metastatic and non metastatic group

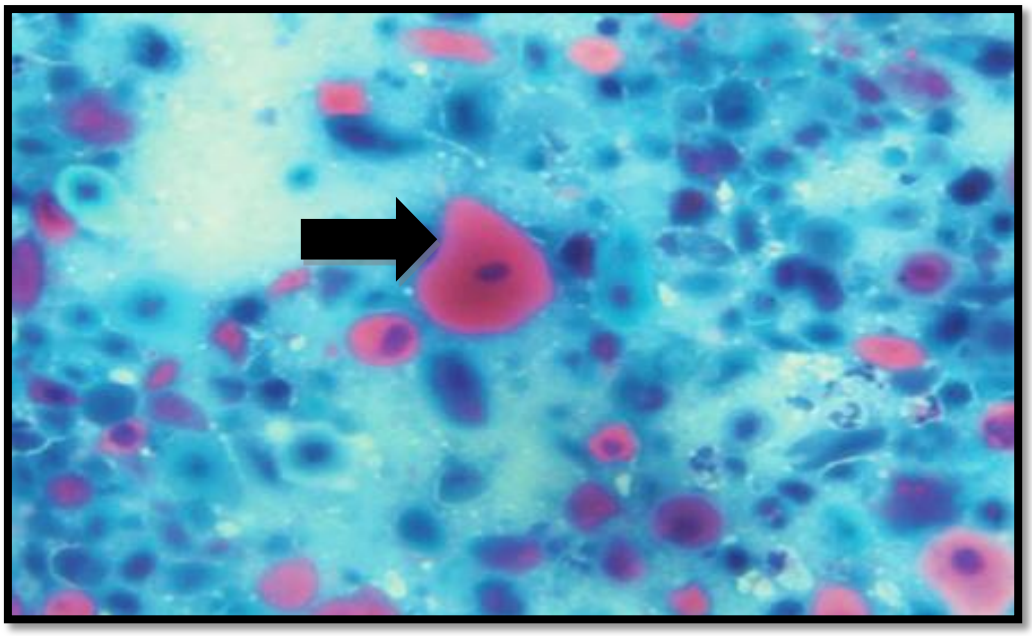


Figure 10: Microphotograph showing dyskeratotic squamous cells on cytology smear. 40X PAP stain

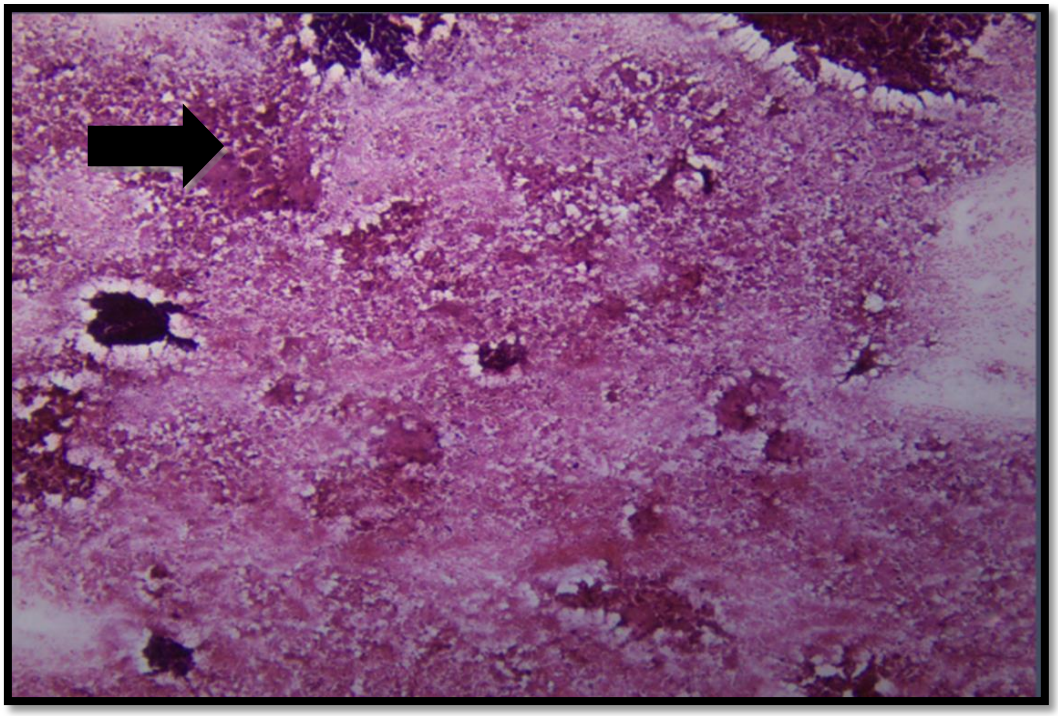


Figure 11: Microphotograph showing necrosis on cytology smear.10X H&E stain

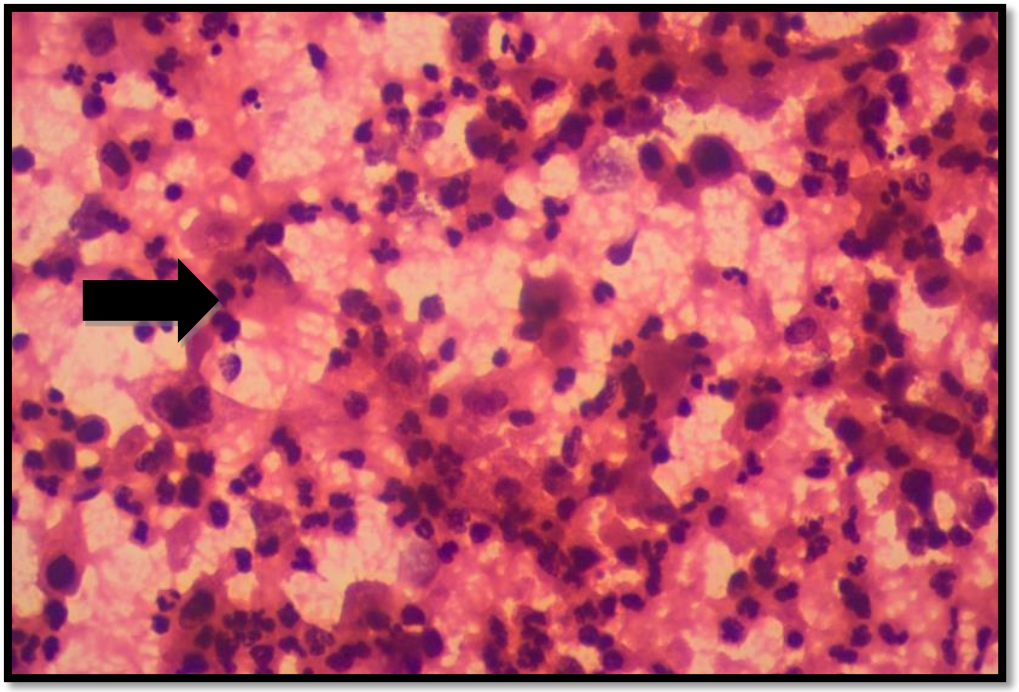


Figure 12: Microphotograph showing acute suppuration along with malignant squamous cells on cytology smear. 40X H&E stain

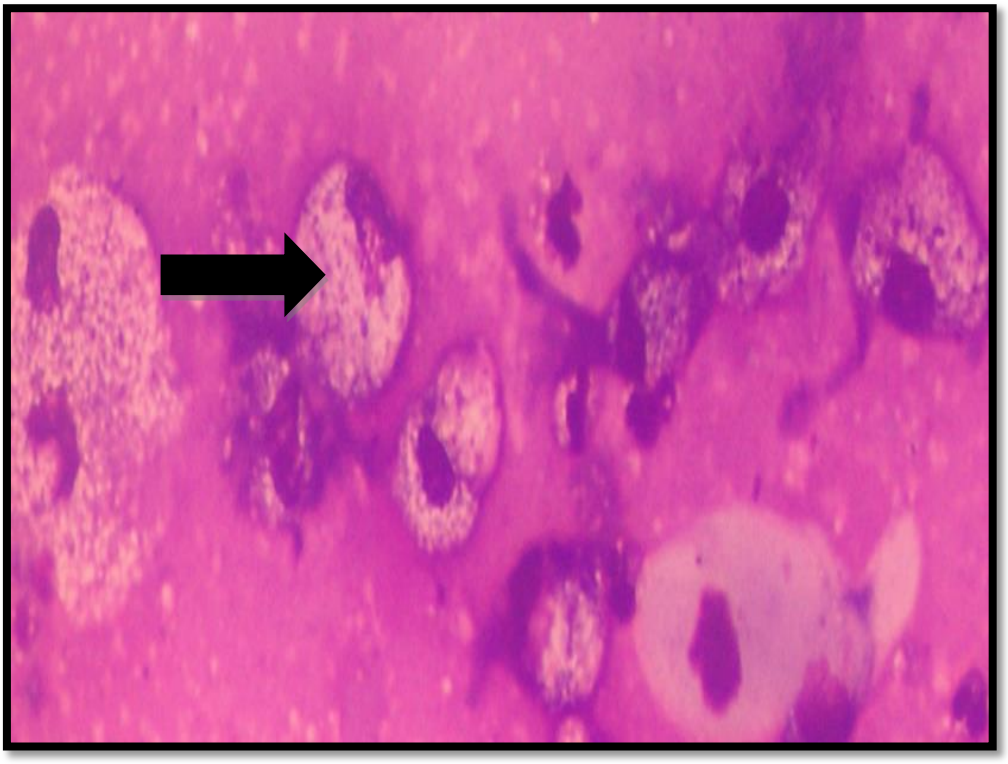


Figure 13: Microphotograph showing cyst macrophages on cytology smear .40X H&E stain

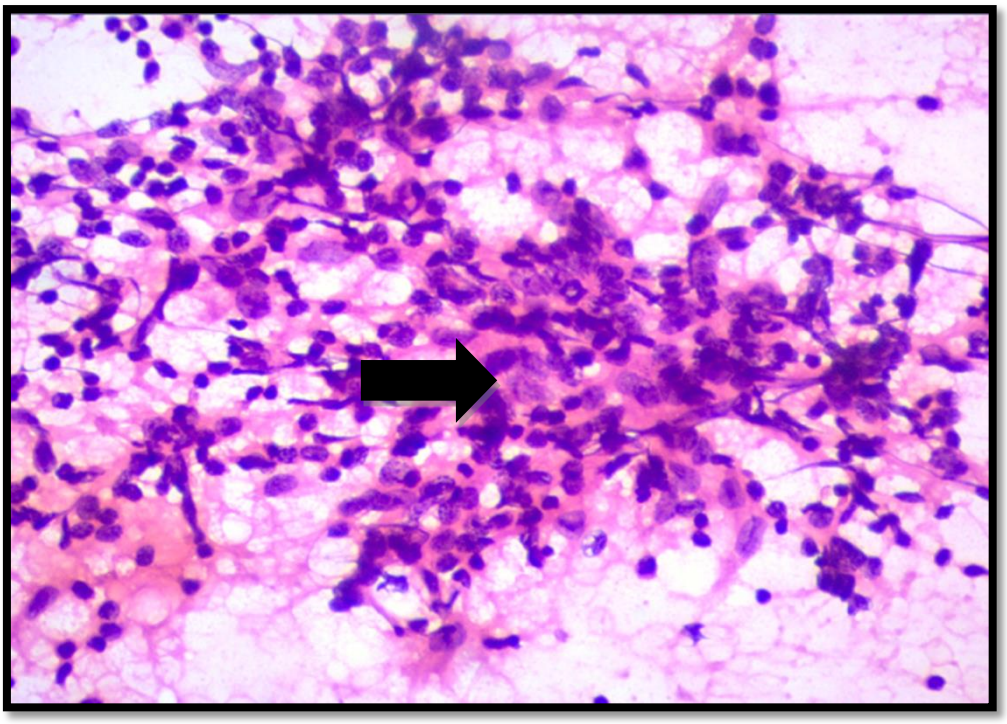


Figure 14: Microphotograph showing features of granulomatous lymphadenitis on cytology smear.40X H&E stain

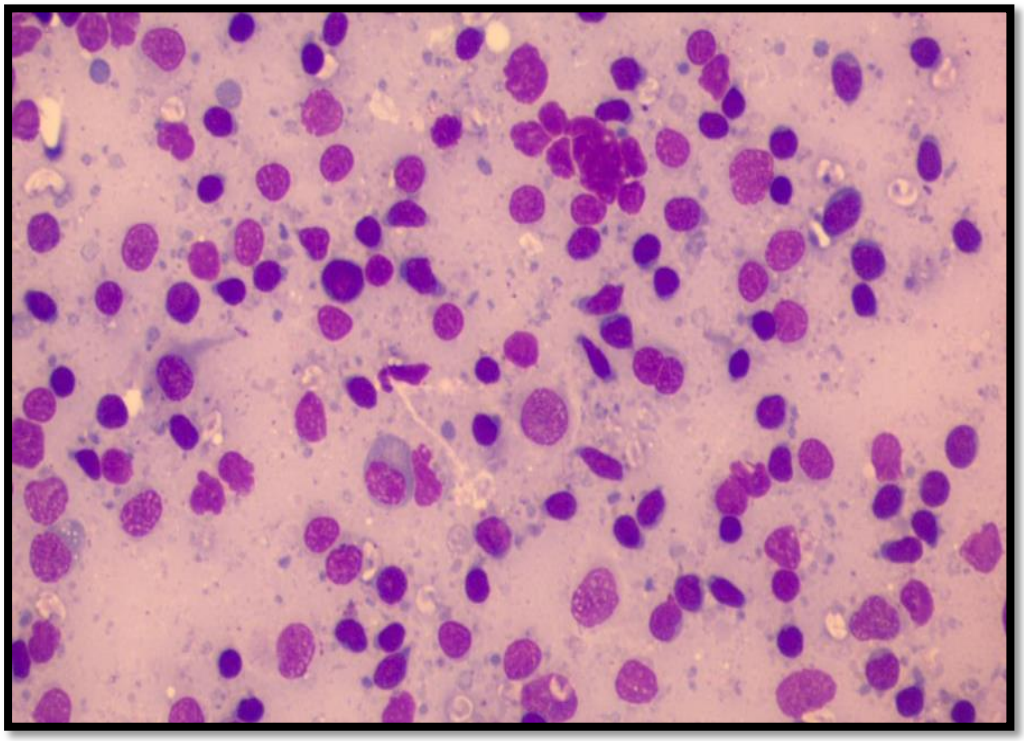


Figure 15: Microphotograph showing features of Reactive lymphadenitis on cytology smear.40X PAP stain

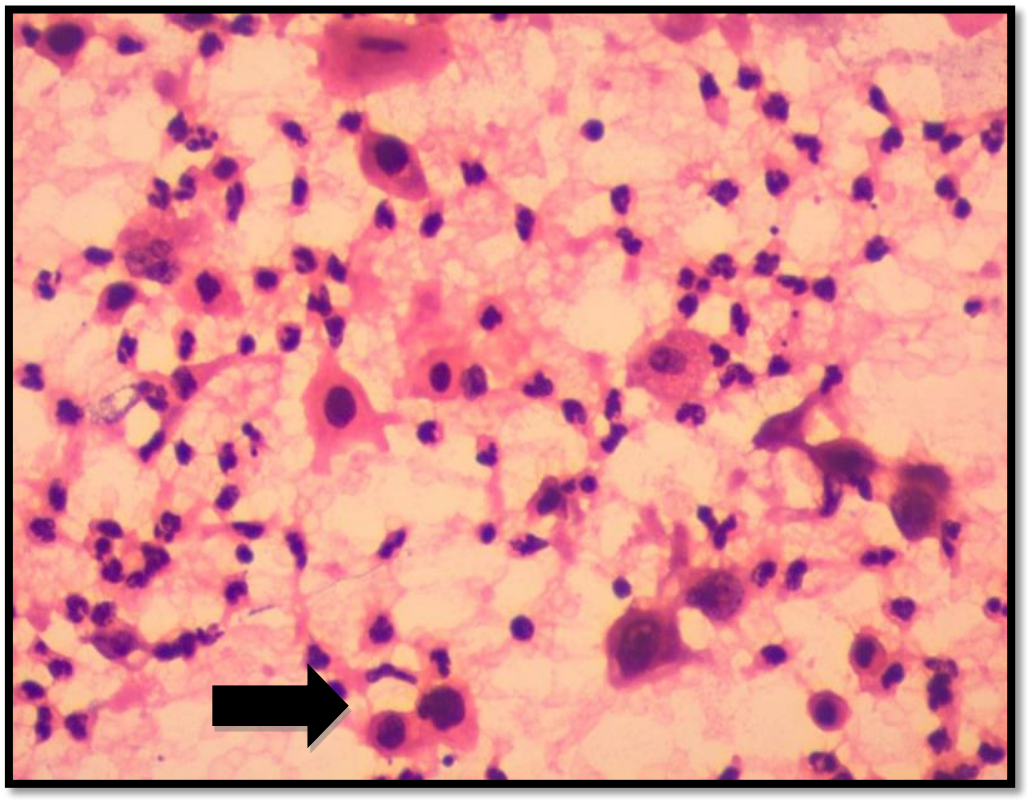


Figure 16: Microphotograph showing squamous cell carcinoma deposit in lymph node on cytology smear.40X H&E stain.

Histopathological diagnosis:

Lymph nodes retrieved from neck dissection specimen were subjected to detailed microscopic examination.

Total no. of lymph nodes retrieved were 1460

The average no. of lymph nodes examined per case were 21

31 cases (44.3%) showed cervical lymph node metastasis on histopathology.

39 cases (55.7%)were negative for tumor deposit in lymph node.

Table 11: Histopathological findings of lymph node

Cervical lymph node metastasis	No. of cases	Percentage
Present	31	44.3%
Absent	39	55.7%

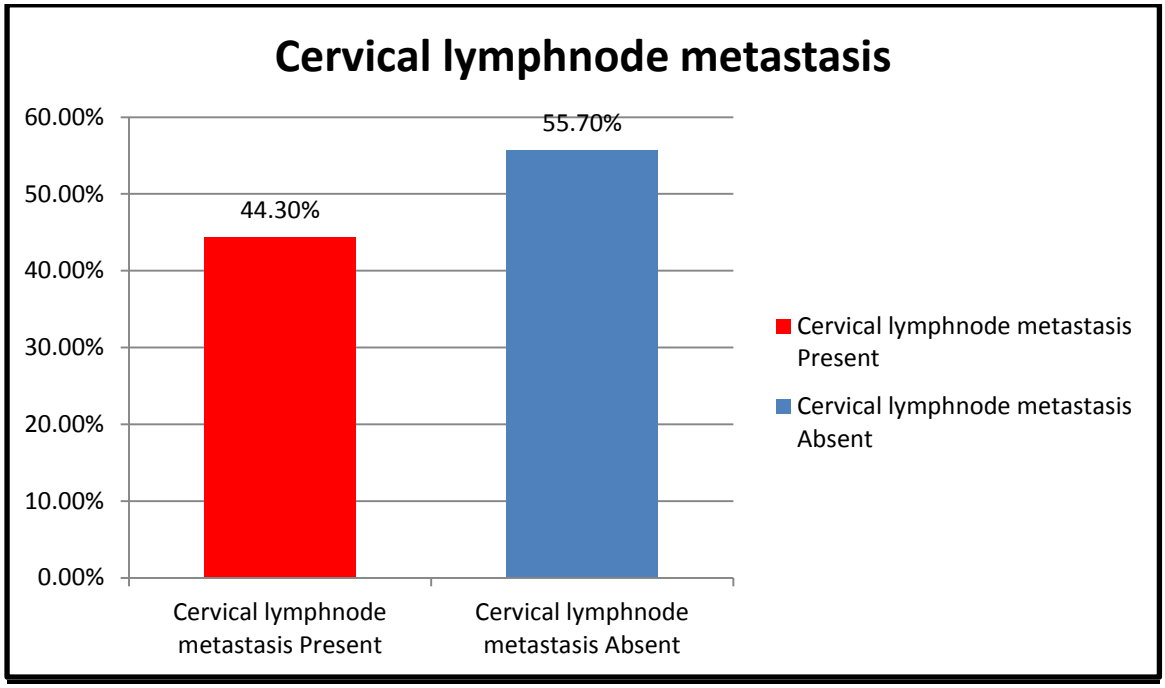


Chart 12: Bar diagram showing Histopathological findings of lymph node

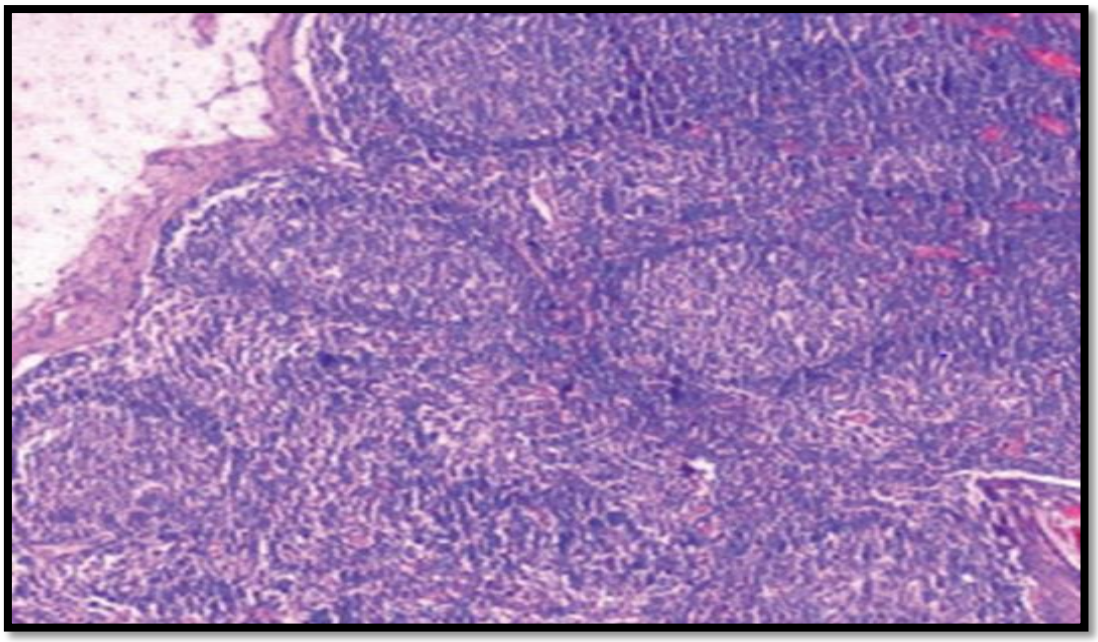


Figure 17: Microphotograph showing Reactive lymphadenitis on histopathology smear 10X H&E stain

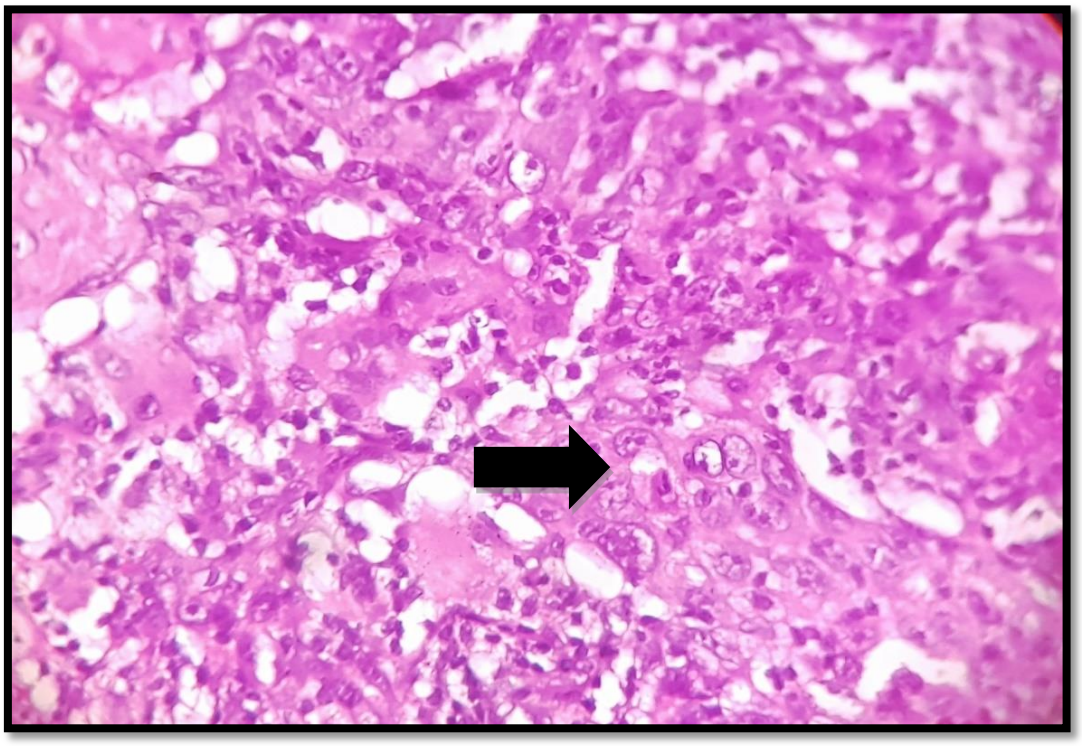


Figure 18: Microphotograph showing squamous cell carcinoma metastatic deposit in lymph node on histopathology smear 40X H&E stain

Association between Tumour site and cervical lymph node metastasis :

In the study among those with lymph node positivity, 67.7% had lesion on Buccal mucosa, 19.4% had lesion on tongue and 6.5% had lesion on GBS and Alveolus respectively. Among those with HPE negativity, 74.4% had lesion on Buccal mucosa, 2.6% had lesion on tongue, 15.4% had lesion on GBS and 7.7% had lesion on Alveolus. **There was no significant difference in site between cervical lymph node metastasis positive and negative subjects.**

Table 12: Association between Tumour site and cervical lymph node metastasis

		Cervical lymph node			
		Positive		Negative	
		Count	%	Count	%
Site	Buccal Mucosa	21	67.7%	29	74.4%
	Anterior 2/3rd Tongue	6	19.4%	1	2.6%
	Lower alveolus	2	6.5%	6	15.4%
	Upper Alveolus	2	6.5%	3	7.7%

$\chi 2 = 6.218$, df = 3, p = 0.101

Association between gross features of tumor and cervical lymph node metastasis:

In the study among those with cervical lymph node metastasis positivity, 22.6% had ulcerative type, 74.2% had Ulceroexophytic, 3.2% had infiltrative type. Among those with HPE negativity, 20.5% had Ulcerative type, 66.7% had Ulceroexophytic, 7.7% had infiltrative type and 5.1% had flat type. **There was no significant difference in gross appearance of tumor between cervical lymph node positive and negative subjects.**

Table 13: Association between gross appearance of tumor and cervical lymph node metastasis

		Cervical lymph node			
		Positive		Negative	
		Count	%	Count	%
Tumour appearance	Ulcerative	7	22.6%	8	20.5%
	Ulceroexophytic	23	74.2%	26	66.7%
	Infiltrative	1	3.2%	3	7.7%
	Flat	0	0.0%	2	5.1%

χ 2 = 2.367, df = 3, p = 0.500

Association between tumour size and cervical lymph node metastasis:

In the study among those with lymph node positivity, 22.6% had tumour size upto 2cm, 51.6% had tumour size of >2 to 4 cm and 25.8% had tumour size of >4 cm. Among those with HPE negativity, 28.2% had tumour size upto 2cm, 59% had tumour size of >2 to 4 cm and 12.8% had tumour size of >4 cm .**There was no significant difference in Tumour size between cervical lymph node metastasis positive and negative subjects.**

Table 14: Association between Tumour size and cervical lymph node metastasis

		Cervical lymph node metastasis			
		Positive		Negative	
		Count	%	Count	%
Tumour size	Up to 2cm	7	22.6%	11	28.2%
	>2-4cm	16	51.6%	23	59.0%
	>4cm	8	25.8%	5	12.8%

χ 2 = 1.949, df = 2, p = 0.377

Association between level of Lymph node and cervical lymph node metastasis:

In the study among those with cervical lymph node metastasis positivity, 58.1% had lymph node at level I and 41.9% had lymph node at level II. Among those with HPE negativity, 75.7% had lymph node at level I and 24.3% had at level II. There was no significant difference in level of lymph node between cervical lymph node metastasis positive and negative subjects.

Table 15: Association between Level of Lymph node and cervical lymph node metastasis

		Cervical lymph node			
		Positive		Negative	
		Count	%	Count	%
Level of Lymph node	I	18	58.1%	28	75.7%
	II	13	41.9%	9	24.3%

$\chi 2 = 2.39$, df = 1, p = 0.122

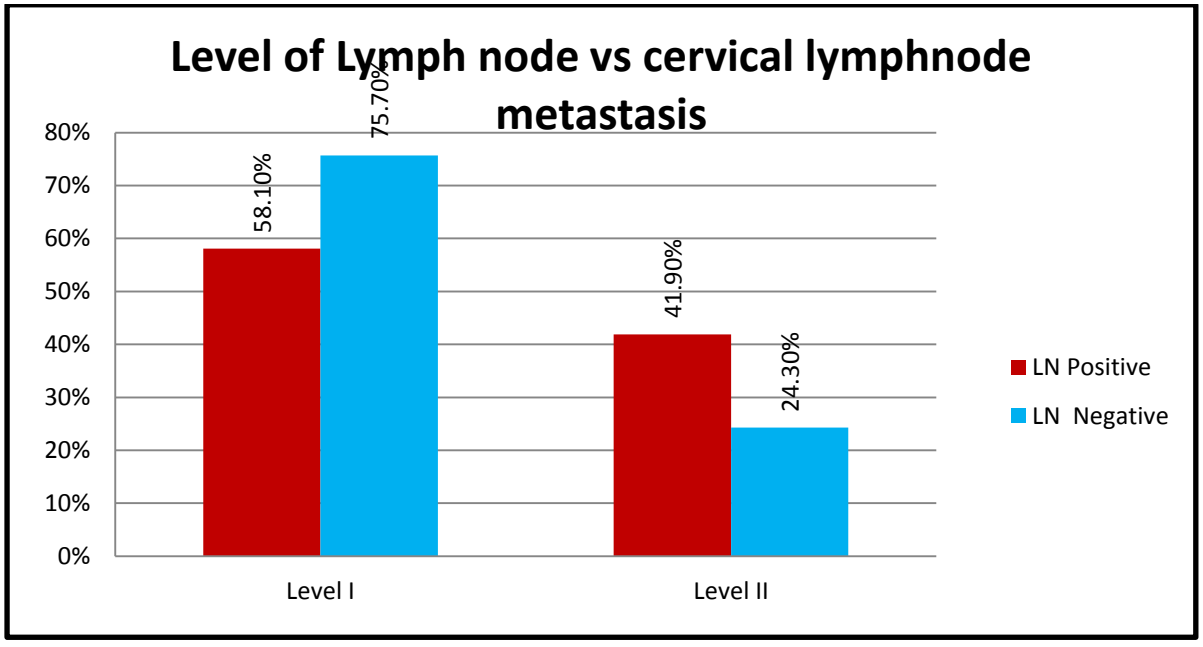


Chart 13 :Bar diagram showing Association between Level of Lymph node and cervical lymph node metastasis

Association between Lymph node size and cervical lymph node metastasis:

In the study among those with cervical lymph node metastasis positivity, 58.1% had lymph node size of 11 to 20mm,29% had lymph node size of 21 to 30 mm and 12.9% had lymph node size of >30 mm. Among those with HPE negativity,12.8% had lymph node size of <10 mm, 66.7% had lymph node size of 11 to 20mm, 20.5% had lymph node size of 21 to 30 mm and 0% had lymph node size of >30 mm. **There was significant difference in size of lymph node between cervical lymph node metastasis positive and negative subjects.**

Table 16: Association between largest lymph node size and cervical lymph node metastasis

		Cervical lymph node			
		Positive		Negative	
		Count	%	Count	%
Lymph node Size classification	<10 mm	0	0.0%	5	12.8%
	11 to 20 mm	18	58.1%	26	66.7%
	21 to 30 mm	9	29.0%	8	20.5%
	>30 mm	4	12.9%	0	0.0%

$\chi 2 = 9.726$, df = 3, p = 0.021*

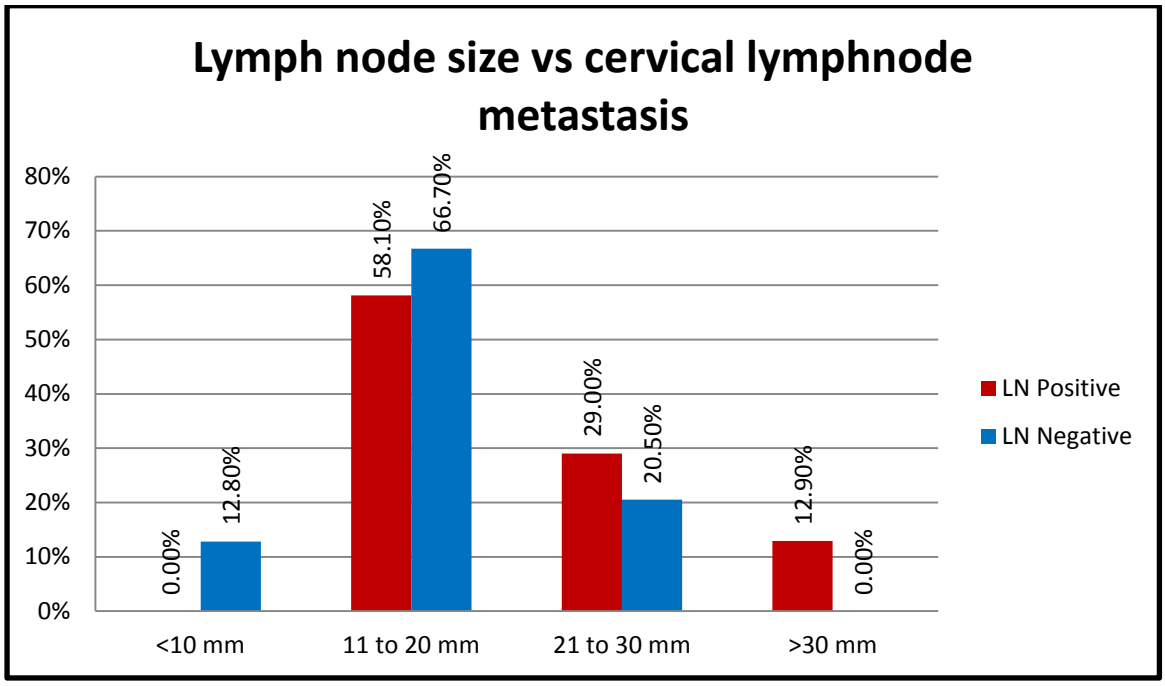


Chart 14: Bar diagram showing Association between Lymph node size and cervical lymph node metastasis

Tumour Differentiation and cervical lymph node metastasis:

In the study among those with metastatic lymph node, 58.1% of tumours were well differentiated, 38.7% were moderately differentiated and 3.2% were poorly differentiated. Among those with negative lymph node, 76.9% of tumours were well differentiated, 23.1% were moderately differentiated and 0% was poorly differentiated. There was no statistical correlation between tumor differentiation and cervical lymph node metastasis.

Table 17: Association between Tumour Differentiation and cervical lymph node metastasis

		HPE			
		Positive		Negative	
		Count	%	Count	%
Differentiation	Well differentiated	18	58.1%	30	76.9%
	Moderately differentiated	12	38.7%	9	23.1%
	Poorly differentiated	1	3.2%	0	0.0%

$\chi 2 = 3.561$, df = 2, p = 0.169

Cytology and histopathology correlation:

Out of 31 cases with HPE positivity, 93.5% were also positive on cytological examination and 6.5% were negative (False negative) on cytology .Remaining 39 cases were negative for metastasis both on cytology and histopathology. There was significant correlation between cytological and histopathological findings.

Table 18: Correlation between Cytology and Histopathological diagnosis

		HPE			
		Positive		Negative	
		Count	%	Count	%
Cytology	Positive	29	93.5%	0	0.0%
	Negative	2	6.5%	39	100.0%

$\chi 2 = 62.29$, df = 1, p <0.001*

In our study 6.5% showed false negative results on USG guided FNAC. Rescreening of cytology smears in these 2 cases showed no evidence of metastasis. In first case out of 34 lymph nodes retrieved only one lymph node showed metastatic deposit and in the second case out of 26 lymph nodes retrieved only one showed metastatic deposit. The reason for false negativity could be due to aspiration from non metastatic node.

Diagnostic value of cytology in comparison to histopathology:

Ultrasound guided FNAC in comparison with HPE had sensitivity of 93.55%,specificity of 100%,positive predictive value of 100%,negative predictive value of 95.12% and diagnostic accuracy of 97.14%

Table 19: Diagnostic value of Ultrasound guided FNAC in comparison with Histopathological diagnosis

Parameter	Estimate	Lower - Upper 95% Cis
Sensitivity	93.55%	79.28 - 98.21
Specificity	100%	91.03 – 100
Positive Predictive Value	100%	88.3 – 100
Negative Predictive Value	95.12%	83.86 - 98.65
Diagnostic Accuracy	97.14%	90.17 - 99.21

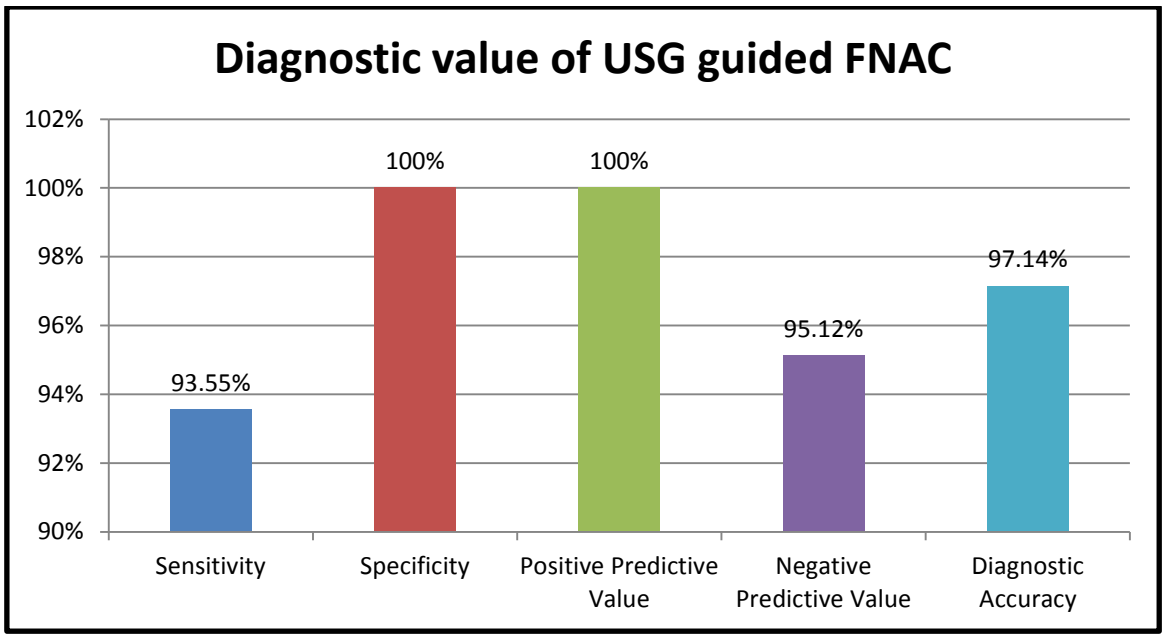


Chart 15: Bar diagram showing diagnostic value of USG guided FNAC in comparison with Histopathological diagnosis

DISCUSSION

DISCUSSION

Metastasis plays a critical role in the management and prognosis of oral cancer patients. Cervical lymph node status is an important predictor for survival of patients with oral squamous cell carcinoma. The presence of metastatic lymph nodes greatly affects the outcome (5-year survival).[5] The 5-year survival is halved if a single ipsilateral neck node is involved, and is reduced by three-quarters if bilateral nodes are involved in oral carcinoma patient.[6] Most of the patients with oral cancer present at late stages. Early diagnosis may reduce morbidity and may decrease the mortality rate. Reported reasons for such delay can be due to limited accessibility of primary health care for patients with a low social economic status and failure of primary health care providers to refer patient in time.

Various methods are used to evaluate lymph node metastasis which includes palpation, US (Ultrasonography), FNAC (Fine Needle Aspiration Cytology) ,USG guided FNAC,CT (Computed Tomography) and MRI (Magnetic Resonance Imaging). Clinical palpation is the routine method and lymph nodes are assessed for location, number, size, shape, consistency, and fixation but the accuracy, sensitivity and specificity of palpation is usually reported to be low. The sensitivity and specificity of palpation are in the range of 60-70% .Few studies reported low diagnostic accuracy in the range of 60% to 80% for palpation. [76,81,89,90] With such low accuracy it is apparent that clinical examination is a poor, unreliable, inaccurate method to determine the presence of cervical lymph node involvement.

CT and MRI has the advantage of less inter-observer variation and these are relatively standardized techniques but there is exposure to ionizing radiation. [81,91]

USG is an useful preoperative evaluation of the neck and is a reliable, inexpensive and easily available method which does not use ionizing radiation. CT and MRI uses size criteria

and calcification to detect malignant nodes but USG can detect shape, echogenic pattern, peripheral vascularity which is important to differentiate malignant nodes from benign nodes. But still all these criteria are not specific and many a times show false positivity. In the literature USG shows a sensitivity ranging 78% to 97%, specificity ranging from 71% to 83% and diagnostic accuracy ranging from 67% to 95% .[91,92,93] Limitations of USG is that it is highly operator dependent and nodes adjacent to mandible and sub centimetric nodes are likely to be missed.

FNAC is a highly specific test to identify metastatic deposits in lymph nodes but biggest challenge is non representative sample which can be improved by USG. USG guided FNAC has not yet become a routine part of preoperative evaluation in evaluation of cervical lymph node status in Oral Squamous Cell carcinoma

USG guided FNAC is used as a preoperative investigation in thyroid, breast and abdominal lesions. Preoperative US guided FNAC from radiologically suspicious axillary lymph node is highly efficient in detecting metastases in breast carcinoma and a preoperative, positive axillary lymph node FNAC helps to stratify the patients for axillary node dissection in breast carcinoma. [94] The present study was conducted to know diagnostic accuracy of USG guided FNAC in oral squamous cell carcinoma patients. In our study USG guided FNAC was done for 70 cases and 68 cases showed correlation between cytology and histopathological diagnosis.2 cases showed false negative results on cytology.

Age at presentation:

Most of our cases belonged to the age group of 40 to 70 years (74.3%) but highest incidence was seen in 6th decade.In another study highest incidence was seen in 5th decade.[95] Our study

correlated with similar studies done in North India where highest incidence was seen in the 6th decade. [96,97] The incidence below 40 years is relatively low but they are not completely spared. The incidence of oral cancer increases with age. This can be due to the fact that increasing age reflect the time accumulation for genetic changes, duration of exposure to the initiator and diminished immunity with age.

Table 20 : Comparison of age of presentation in present study with other studies.

Age(years)	**Present study**	Shenoi et al. 2012 [96]	Krishna A et al. 2014 [95]	Singh MP et al. 2015 [97]
20-30yrs	**2.8%**	6.10%	10.2%	10.2%
31-40yrs	**11.5%**	21.36%	25.5%	22.7%
41-50yrs	**24.3%**	25.76%	25.7%	24.8%
51-60yrs	**25.7%**	27.8%	24.8%	25.8%
61-70yrs	**24.3%**	14.91%	12.1%	14%
71-80yrs	**11.4%**	4.07%	1.7%	2.5%

Gender:

In our study male to female ratio was found as 1:2.1.This is in contrast to most other studies in India where a male preponderance is seen. This is probably because most of these studies were done in North and western India. In north and western India betel leaf combined with areca nut (known as pan masala)consumption is common among males. Increasing

trend of oral cancer is seen among women in many rural areas of South India because of betel quid chewing habits as they believe that tobacco has magical and medicinal properties.

Table 21: Comparison of sex ratio in present study with other studies

Authors	Total no. of cases	Males	Females	M:F
Present study 2017	**70**	**22**	**48**	**1:2.1**
Sapna M et al.[98] 2013	172	42	120	1:2.8
Vijay CR et al [99] 2017	300	135	165	1:1.2
Singh MP et al.[97] 2015	479	64	115	3.1:1
Sharma et al.[100] 2010	80	55	25	2.2:1

Risk factors:

In our study the most common predisposing factor was betel quid chewing seen in 98.6% cases which is in agreement with a similar study where 91.1% cases had a habit of betel quid chewing. [101] Paan masala is a dry complex mixture, with or without tobacco, areca nut, catechu, lime, cardamom, flavourings and sweetening agents and is more common in North and western India. The practice of chewing betel quid is a very common practice in our geographical area of Kolar, Karnataka. Majority of them had the habit of chewing tobacco stem (Kaddipudi) with lime and betel leaf. Tobacco chewing has emerged as a stronger risk factor of oral carcinoma than smoking, since there is a direct exposure of tobacco chewing on

the mucosa for longer period, while smoking has more contact with pharynx, larynx, and lungs.

Most of the patients in our study had been chewing betel quid for 20-30years(64.3%) .The longest duration of this habit was for 50 years which was seen in 4.2% cases. The shortest duration that has lead to the symptoms was 5 years comprising of 4.2% cases. In another study among 99 oral cancer patients, two thirds had a history of tobacco use for more than 5 years . [102] Tobacco contains N-nitroso compounds, well-known carcinogens, which play a key role in the malignant transformation of oral cancer .

In a study done in Boston it was found that production and release of Reactive Oxygen Species (ROS) occurs under alkaline conditions during the autooxidation of areca nut polyphenols in the tobacco chewer's saliva. The ROS can directly involve in tumour initiation process by inducing genotoxicity and gene mutation or it attacks the salivary proteins and oral mucosa, leading to structural change in the oral mucosa. [103]

In a study conducted in London it was found that genetic polymorphisms in the genes coding for the enzymes (P450 enzymes and xenobiotic metabolizing enzymes)were responsible for tobacco carcinogen metabolism and were suspected to play key role in the genetic predisposition to tobacco-induced oral cancers. [104]

Prolonged exposure to tobacco carcinogens in the oral mucosa causes genetic changes in the epithelial cells. Cumulative genetic changes lead to genomic instability, development of premalignant lesions, and eventually invasive carcinoma. So the longer the duration of exposure higher the chances of developing malignancy.

Poor oral hygiene was seen in 64.3% cases in our study. In a study it was found that infrequent tooth brushing was associated with cancer of the oral cavity, particularly of the tongue. [105] In another study it was found that daily tooth brushing is a preventive factor against oral cancer and the number of missing teeth and the amount of decay, was associated with the risk of oral cancer. [106]

Most of our cases belonged to low socioeconomic status. It may be a risk factor for oral cancer because of their poor oral hygiene and poor educational qualification. Hence, the patients are unaware about the consequences of deleterious habits. Similar findings was stated in a study where low socioeconomic status contributed towards the risk of oral cancer.[107]

Table 22 : Comparison of most common risk factor in present study with other studies

Most common risk factor	**Present study**	Subapriya R [108] 2007	Khandekar P et al.[109] 2006
Chewing tobacco	**98.6%**	66.7%	71.3%
Smoking	**31.4%**	30.9%	63.3%

Only 31.4% cases in our study were smokers and alcohol consumption was seen in 30% of cases. Maximum duration of smoking was 20 years in our study population. In a study done in Indonesia it was concluded that 82.2% of the cases who smoked tobacco for >10 years developed oral cancer**.** [110]

30% of the patients in our study were smokers ,alcoholic and had a habit of chewing tobacco. In another study it was found that both males and females who developed oral cancer were addicted to all the three habits (smoking, alcohol, and chewing tobacco). [111]

Clinical presentation:

In our study the most common presenting complaint was ulcer in the mouth (77.1%) followed by pain (21.4%) and swelling in the cheek (18.5%). Similar results were found in another study where the commonest presenting complaint was non healing ulcer.[112, 113]
In contrast in another study pain was the the most frequent presentation. [56]

Small lesions tend to be asymptomatic and are often noted incidentally on dental examination. Pain commonly occurs as the lesion enlarges and ulceration develops. Oral intake may worsen the pain and lead to malnutrition and dehydration. Ulcer is a common complaint because most of these patients had a poor oral hygiene superimposed with habits of chewing betel quid and smoking.

Duration of symptoms:

In our study most of the cases presented within 3 months(57.2%).The earliest presentation was at 2 weeks and the late presentation was at 1 year. This study is in agreement with another study where most of the cases presented within 3 months of symptoms. [114] In a study by Venukumar R [115] majority of patients (80%)had a history of duration of symptoms of 6 months. In another study duration of symptoms varied between 1-6 months in 68.14% of cases. [96] This delay in presentation can be due to possibly resorting to home remedies poverty and illiteracy. Also most of the patients earn their living by daily wages and the loss of a working day means a loss of wages. Most of our cases were females and and they have a tendency for delayed seeking for medical help due to family commitments.

Site of presentation:

In our study the most common site of carcinoma was buccal mucosa(71.4%) followed by lower alveolus (11.3%).Anterior 2/3rd tongue carcinoma was seen in 10% of cases.

Many a times it is difficult to pinpoint epicenter in case of buccal mucosa and lower GBS as they present in advanced stage so they are nicknamed together as "Indian Oral Cancer". The site of occurrence depends on the predominant risk factors in that particular geographical region. The high incidence of buccal mucosa malignancy is due to the fact that betel quid is directly compressed against it and there is direct access to carcinogens. Even smoked or chewed noxious agents get dissolved in the saliva and some saliva normally remains in the vestibule of the mouth, causing greater and prolonged contact with the buccal mucosa.

Table 23: Comparison of most common presenting site in present study with other studies:

Most common site	**Present study**	Sharma et al.[100] 2010 UP	Dhage DH [116] 2017 Nagpur	Singh et al.[97] 2015 Lucknow	Shenoi et al.[96] 2012 Nagpur	Narwal A.[117] 2014 Haryana	Selvi et al [118] 2017 Trichy
Buccal mucosa	**70%**	63.75%	51%	43.8%	23.7%	26.2%	22.08%
Lower Alveolus	**11.3%**	15%	21%	32.1%	45.7%	37.95%	9.58%
Tongue	**10%**	3.75%	23%	18.4%	18.31%	10.9%	44.16%

Tumor appearance:

Oral cancer has a varied clinical appearance.The most common tumor appearance was ulceroexophytic which constituted upto 71.4% followed by ulcerative(21.4%).Similar findings were found in other studies. [114 ,118]

Tumor appearance serves as a prognostic marker in oral cancer. In a study in Bristol,U.K. it was reported that tumors in young patients (<35 years) were predominantly endophytic lesions affecting the tongue with an early spread to lymph nodes and tumors in older patients (>60 years) presented as exophytic lesions of the buccal mucosa or gingiva and spread late to the lymph nodes. [119]

Table 24: Comparison of most common tumor appearance in present study with other studies

Tumor appearance	**Present study**	Bharadwaj et al [114] 2015	Selvi et al [118] 2017	Phookan et al [113] 2017
Ulceroexophytic	**71.4%**	67.19%	47.3%	19.18%
Ulcerative	**21.4%**	27.7%	41.3%	58.90%

Clinical TNM stage :

Most of our cases presented at a late stage on clinical examination .Most were in Stage IV (65.8%) followed by Stage III(34.2%). Primary tumour site is also associated with delayed diagnosis or diagnosis at advanced stages. Tongue, buccal mucosa and lip cancer are easily recognised and they favour early-stage diagnosis whereas the floor of the mouth and the retromolar trigone have been linked to diagnosis at advanced stages and locations like palate

or gingivae have shown contradictory results. Other causes of presenting at advanced stages can be due to lack of awareness ,poverty, lack of screening programmes and resorting to home remedies until they have severe symptoms.

Table 25: Comparison of clinical TNM stage in present study with other studies

Clinical Stage of tumor	**Present study**	Singhania et al[120] 2015	Bharadwaj et[114] al. 2015	Singh MP et al[97] 2015
Stage I	**0%**	1%	2%	2.7%
Stage II	**0%**	8.5%	9%	5%
Stage III	**34.2%**	18.5%	66%	28.2%
Stage IV	**65.8%**	72%	23%	64.1%

Ultrasound findings:

Multiple criteria have been suggested to improve the accuracy of ultrasonography in diagnosing metastatic adenopathy. Lymph node necrosis seemed to be a significant indicator of malignancy and play an essential role in the evaluation of neck node status. Though necrosis is a reliable criterion, it is unfortunately quite rare in small nodes. Irregular borders are present in many lymph nodes, it is very difficult to distinguish these small irregularities from artifacts or anatomic irregularities. It is believed that effacement of the hilum is considered as a diagnostic criteria of malignancy; however in a study it was found that loss of fatty hilum is not a definite indicator of malignancy and may be seen in as many as 9% of reactive lymph nodes. [121] Numerous studies have tried to determine the optimal size criteria;

however, wide variations in measurement techniques make the decision difficult. In another study it was found that lymph node shape is not a very specific criterion and round adenopathies may be seen in tuberculosis, lymphoma or reactive lymphadenopathy. [122]

In view of absence of gold standard ultrasound criteria for metastatic lymph node ,combination of ultrasound features were considered for selection of lymph node for FNAC .Nodes which showed round shape, absence of hilum, contour irregularity ,presence of necrosis were selected for FNAC. 45.7% cases showed round nodes ,42.9% cases showed absence of hilum and 25.7% cases showed necrosis. Cystic changes were seen in 15.7% of cases and irregular borders were seen in 11.4%.

Cytological findings

On cervical lymph node aspiration 29 cases(41.4%) showed positivity for metastatic squamous cells while 41 cases(58.6%) were negative for metastatic squamous cells.

In our study among the metastatic cases ,dyskeratosis was seen in 51.7% cases, necrosis in 31% cases, acute suppurative inflammation in 20.6% cases and cystic change in 13.8% cases. Out of 41 cases which were negative for metastasis granuloma was seen in 5 cases. ZN stain and PAS stain showed no organism.

An important clue to the diagnosis of metastatic SCC is the presence of necrosis and keratinization, which is better appreciated on Pap stain than on H & E stain. Keratinizing SCC are readily identified when cells with abundant sharply demarcated dense eosinophilic/orangeophilic cytoplasm and hyperchromatic nuclei are present in smears. Non keratinizing squamous cell carcinoma are represented by round, oval or polygonal cells with sharply demarcated pale cytoplasm and coarsely granular nuclear chromatin .[123]

Smears with large amounts of inflammatory cell infiltration and abscess formation should be carefully searched for metastatic squamous cells. Inflammatory infiltrate can obscure the metastatic squamous cells. The presence of keratinous debris and foreign body giant cell formation indicates the possibility of keratinising squamous cell carcinoma in such cases. In a study diagnostic dilemma's were described in a series of 12 patients with metastatic squamous cell carcinoma in regional lymph nodes associated with abscess formation.[124]

The aspiration from metastatic nodes with cystic change is often hypocellular .The aspirate should be centrifuged and the centrifuged deposit should be very carefully examined for any malignant squamous cells. USG guided FNAC from the solid area of the lymph node swelling can also be helpful. Cystic lesions can lead to false negative results . In a study of 42 cases 5 false negative cases were found all of which were cystic nodal metastasis in neck.[125]

Granulomatous reaction especially in a country like ours where tuberculosis is rampant is a very common finding in lymph nodes .Occasionally, lymph nodes containing metastatic Squamous cell carcinoma may show granuloma's. In a study it was found that granulomatous response along with necrosis and inflammation as a cause for false negative diagnosis in metastatic SCC. [126]

Histopathological findings

On histopathological study of neck dissection specimen ,31 cases (44.3%) showed cervical lymph node metastasis. 39 cases(55.7%)were negative for tumor deposit in lymph node.

Tumor site and lymph node positivity:

In our study there was no statistical correlation found between tumor site and cervical lymph node metastasis($p = 0.101$). In a study it was found that tumor site is a predictor of lymph node metastasis and tumor involving anterior 2/3rd of tongue showed higher number of cases of lymph node metastasis in comparison to other sites. [127] In another study squamous cell carcinoma of the tongue and floor of the mouth generally have poor prognosis due to the frequent presence of cervical metastases, inaccessibility, and late reporting by patients. [128] Tongue, because of its structure and function and complex lymphatic drainage is prone for early local and regional spread of cancer. Cross-drainage in the oral tongue is common, thereby placing both sides of the neck at risk for nodal metastases. However in our study only 7 cases of anterior 2/3rd tongue carcinoma were included as the prevalence of tongue carcinoma is less in compared to buccal mucosa in our geographic area.

Tumor size and lymph node positivity:

In our study there was no statistical correlation found between tumor size and cervical lymph node metastasis($p =0.377$).

In a study in Iraq it was found that tumor size is a predictor of lymph node metastasis ($P=0.004$). [127] In another study it was found that lymph node metastasis was more common in tumors with size >4cm. [129] In a study by Venukumar R [115] it was found that patients with cervical lymph node metastasis 56% of these patients had a tumor size of >4cm.

Level of lymph node and lymph node positivity:

In our study the most common lymph node to be involved was Level I comprising of 58.1% of the cases. In our study there was no statistical correlation found between level of lymph node and cervical lymph node metastasis (**$p = 0.122$).**

Level I lymph nodes are regarded as the primary basin for lymphatic drainage and are most commonly affected in oral cancer. Buccal mucosa, tongue, retromolar trigone, floor of mouth ,hard palate and upper alveolus has direct lymphatic drainage to Level I and they are most commonly involved. Level IV and V involvement is rare.

Level of nodal involvement decides the extent of surgical excision- radical, modified radical or selective neck dissection .

Table 26 : Comparison of level of lymph node involved in present study with other studies

Study	Most common level of lymph node involved	Percentage
Present	**Level I**	**58.1%**
Sureshkannan P et al [130]	Level I	70%
Essig et al [131]	Level I	66.7%
Nithya CS et al [132]	Level II	63.6%

Lymph node size and lymph node positivity:

In our study there was a statistical correlation found between size of lymph node and cervical lymph node metastasis (**p = 0.021**). In our study it was found that chances of lymph node metastasis are higher if size of lymph node is >30mm. Many studies have shown that histopathologic positive rates were higher for larger lymph nodes, which suggested that the size is an important diagnostic criterion .

In a study in Turkey the highest rates of metastasis and ECS were seen in lymph nodes measuring 31 to 60 mm. They evaluated 200 neck dissections and concluded that larger the lymph node size higher the chance of metastasis . [133]

In a study done in Wardha the size of the lymph nodes was >2 cms in majority of the cases (80%) of metastatic SCC . [134]

In a collaborative study positive rates of metastasis was observed in 86.5%, 93.3% and 100%, respectively, were observed in lymph nodes with a size of 1–2 cm, 2–3cm and lymph nodes of more than 3 cm. [135]

Differentiation of tumor and lymph node positivity:

In our study majority were well differentiated SCC (70%)followed by moderate differentiated SCC(26%) with only one case(4%) of poorly differentiated tumor. No statistical correlation was found between tumor differentiation and lymph node metastasis.(p = 0.169)

In a study in our institute a significant correlation was found between grades of differentiation and cervical LN metastasis and high occurrence of metastasis was seen in moderate-and poorly-differentiated SCC (55%).[136]

In a study in Japan a significant correlation was found between a poorly differentiated carcinoma and the presence of occult nodal metastases in the neck dissection specimen. [137] In another study similar results were found. [138]

In another study in Japan the prevalence of nodal metastasis in elective neck dissection was 32% for well and 75% for poorly-differentiated carcinoma. [144]

There are many histopathological parameters like depth of invasion, perineural invasion ,lymphovascular invasion, pattern of invasion, inflammatory response, tumor thickness which can predict lymph node metastasis but all these parameters are better appreciated in the resected specimens limiting their use in preoperative investigation for detecting lymph node metastasis.

Correlation between cytological and histopathological findings:

In our study out of 31 cases with HPE positivity, 29(93.5%)were positive on cytological examination and 2 (6.5%) were negative on cytology . All 39 cases showed reactive lymphadenitis features both on cytology and histopathology. There was significant correlation between cytology and HPE findings. **(p value <0.001)**

Table 26: Showing comparison of diagnostic value of USG guided FNAC in present study with other studies

Statistical parameters:	**Present study**	Azam SM et al [82] 2014	Dabirmoghaddam P.[139] 2014	Rottey S [140] 2014	Geeta NT[141] 2010
Sensitivity (%)	**93.55%**	92.31 %	87.5%	86.7 %	67 %
Specificity (%)	**100%**	90.91 %	100%	87.5 %,	100%
Positive predictive value (%)	**100%**	96.00 %	100%	81.3 %	100%
Negative predictive value (%)	**95.12%**	83.33%	95%	91.3 %	67%
Accuracy (%)	**97.14%**	91.89%	96%	87.2 %	80%

In a study it was found that the sensitivity of blind FNAC in correctly diagnosing malignant cervical adenopathy is 90%. [89] Ultrasound-guided FNAC is an accurate method for the evaluation of neck nodes with a sensitivity of 89–98%, specificity of 95–98% and overall accuracy of 95–97%. It has been reported that ultrasound-guided FNAC correctly stages the neck nodes in 93% of patients with head and neck malignancy .[72]

In our study 6.5% showed false negative results on USG guided FNAC. Rescreening of cytology smears in these 2 cases showed no evidence of metastasis. In first case out of 34 lymph nodes retrieved only one lymph node showed metastatic deposit on HPE and in the second case out of 26 lymph nodes retrieved only one showed metastatic deposit on HPE. The reason for false negativity was due to aspiration from non metastatic node. Aspiration from multiple lymph nodes may improve the sensitivity of USG guided FNAC.

In a study in Lucknow 4.7% of the cases were found false negative on USG guided FNAC.[142] In a study in Iran false negative results on USG guided FNAC was found to be 3.77% . [139] This false negative results could be been due to small metastasis missed by the needle, a single tumor cell overlooked by the pathologist or aspiration of a non metastatic lymph node . In addition, lymph nodes near the mandible are difficult to visualize or aspirate due to the shadow of the mandible . Cystic change in metastatic lymph nodes is an important cause of false negative errors. FNAC should be repeated in case of suspicious hypocellular cystic aspirations especially in patients with known malignancy.In a study by it was found that most of the micrometastasis are found in lymph nodes with a size less than 10 mm diameter. [72] The size of these lymph nodes is within the resolution of ultrasound; however, placement of the needle into the lesion during FNAC is problematic. So micrometastasis is easily missed .

Various challenges are experienced in cytological diagnosis of lymph node metastasis. The benign inclusions of salivary gland, squamous epithelium, and thyroid are most common to occur in lymph nodes of the head-neck area and can lead to diagnostic challenge. [139] Thyroid gland inclusions can be found within lymph nodes in 1–5 % from neck dissections secondary to head and neck cancer. Salivary gland ducts or acini are found in cervical lymph

nodes in ~10 % of adults and are detected in patients undergoing head and neck surgery. Even skin contaminant can lead to diagnostic challenge.

Regional lymphadenopathy in a known carcinoma patient is not always due to metastatic tumour, and not every nodule represents a lymph node. Lymph nodes are enlarged in infections, immune disorders, lymphoma. Especially when the primary tumour is ulcerated, as seen in advanced squamous carcinoma, enlargement of the regional nodes may signify a response to inflammation rather than metastasis. In our study more than half(55.7%)of patients didn't show metastatic deposit in cervical lymphnodes in a known patient with oral carcinoma. All these lymph nodes were reactive suggesting inflammatory/ infectious etiology for regional lymphadenopathy. Many patients with oral cancer have been found to have low immunity making them prone to get infected by various viruses ,bacteria and fungal infection. Poor orodental hygiene also leads to reactive adenopathy.

In another study similar results were found and metastasis in the cervical nodes was detected only in 43% of the cases among 90 neck dissection cases on histopathology and remaining 51% cases showed reactive nodes. [143] In a study in Japan 21 out of 38 cases (55.3%) were reactive on histopathology. [144] In another study in Iran in 53 neck dissection specimens metastases was seen only in 16 cases(30.1%) and remaining 69.9% were reactive on histopathology. [139]

Our study shows high sensitivity, specificity and diagnostic accuracy of USG guided FNAC in preoperative assessment of cervical lymph node status in squamous cell carcinoma of oral cavity. USG guided FNAC findings should be considered in the management of squamous cell carcinoma of oral cavity.

SUMMARY

SUMMARY

This study entitled "**Ultrasound guided Fine needle aspiration cytology in evaluation of cervical lymphnode metastasis in Oral Squamous Cell Carcinoma"** was carried out at R L Jalappa Hospital and Research Centre during the period of January 2016 to September 2017.It included 70 USG guided FNAC of cervical lymphnodes of squamous cell carcinoma of oral cavity .The FNAC diagnosis was correlated with the histological findings.

The salient features of the study were:

1. Patient age ranged from 27 to 80 years .Most of the cases belonged to the age group of 40 to 70 years with a male to female ratio of 1:2.1.
2. The most common presenting complaint was ulcer in the mouth seen in 75.70% cases and most cases had a symptoms < 3 months.
3. Most common predisposing factor was betel quid chewing seen in 98.6% cases.
4. The common site of carcinoma of oral cavity was Buccal mucosa (71.4%) followed by lower alveolus (11.3%).Tongue carcinoma was seen in 10% of cases.
5. Most of the cases showed Ulceroexophytic tumor which constituted up to 70.0% followed by ulcerative tumor (21.4%).
6. Most of our oral cancer cases tumor size was >2 to 4cm comprising of 55.7% followed by up to 2cm in 25.7% cases.
7. Most of the cases presented clinically at a late stage .Most were in Stage IV (65.8%) followed by Stage III(34.2%).
8. USG showing round nodes , absence of hilum ,necrosis, cystic changes or irregular borders were aspirated
9. On cervical lymph node aspiration 29 cases(41.4%) showed positivity for malignant squamous cells while 41 cases(58.6%) were negative for malignant cells .

10. On cytology among the metastatic cases dyskeratosis was seen in 51.7% cases, necrosis was seen in 31% cases, acute suppurative inflammation was seen in 20.6% cases and cystic change was seen in 13.8% cases. Out of 41 cases which were negative for metastasis 12.2% of cases showed epithelioid cell granuloma's.
11. On histopathological study of neck dissection specimen ,31 cases (44.3%) showed cervical lymph node metastasis. 39 cases(55.7%)were negative for tumor deposit in lymph node.
12. Out of 31 metastatic cases on HPE, 29(93.5%)were positive in cytology and 2 (6.5%) were negative .Remaining 39 cases were negative for metastasis both on HPE and cytology . There was significant correlation between cytology and HPE findings.

    (p value <0.001)
13. Sensitivity of USG guided FNAC was **93.55%,**specificity was **100%**

    positive predictive value was **100%** ,negative predictive value was **95.12%** and diagnostic accuracy was **97.14%.**
14. **6.5%** showed false negative results on USG guided FNAC. This is due to aspiration from non representative node.
15. There was no statistical correlation found between tumor site ,tumor size, level of lymph node, differentiation of tumor with cervical lymph node metastasis.

16. Statistical correlation found between size of lymph node and cervical lymph node metastasis (**p = 0.021**). Chances of lymph node metastasis were higher if size of lymph node is >30mm.

CONCLUSION

CONCLUSION

Oral cancer is one of the most common cancer. Metastatic spread to cervical lymph nodes greatly affects the patient outcome. Accurate preoperative diagnosis of cervical metastasis is important to reduce the unnecessary morbidity or mortality due to neck dissection or radiation .

High diagnostic accuracy of FNAC combined with advantage of ultrasound guidance helps in accurate diagnosis of cervical lymph node metastasis in oral cancers. Ultrasound guided FNAC should be recommended in the preoperative assessment of cervical lymph node metastasis in Oral squamous cell carcinoma.

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ANNEXURE

PROFORMA

NAME : CASE

HOSPITAL NO:

AGE/SEX:

HOSPITAL NO :

CHIEF COMPLAINT :

HISTORY OF PRESENTING ILLNESS :

PAST HISTORY:

DIABETES ☐ HYPERTENSION ☐ RADIOTHERAPY ☐

CHEMOTHERAPY ☐

PERSONAL HISTORY :

TOBACCO/BETEL QUID CHEWING ☐ SMOKIING ☐ ALCOHOL

CONSUMPTION ☐

LOCAL EXAMINATION:

CARRIES TOOTH ☐ ORAL HYGIENE ☐ WHITE PATCH ☐

TUMOUR SITE :

BUCCAL MUCOSA ☐ GINGIVA ☐ TONGUE ☐

FLOOR OF MOUTH ☐

TUMOUR SIZE :

TUMOUR TYPE:

ULCERATIVE ☐ ULCEROPROLIFERATIVE ☐ INFILTRATIVE ☐

LYMPHNODES:

SIZE :

NUMBER:

LEVELS INVOLVED: I ☐ II ☐ III ☐ VI ☐ V ☐ VI ☐

CLINICAL TNM STAGING:

ULTRASOUND FINDING:

CYTOLOGY NO :

CYTOLOGY FINDING:

PREVIOUS BIOPSY REPORT:

BIOPSY NO.

HISTOPATHOLOGICAL DIAGNOSIS :

GROSS :

MICROSCOPY:

LYMPHNODE FINDINGS:

TOTAL NUMBER:

LEVEL:

NO. OF RECURRENT LYMPH NODE:

PTNM STAGING:

IMPRESSION:

PATIENT CONSENT FORM

Ultrasound guided Fine needle aspiration cytology in evaluation of cervical lymph node status in Oral squamous cell carcinoma

I,the undersigned, agree to participate in this study and authorize the collection as outlined in this consent form.

I understand the purpose of this study, the risks and benefits of the technique(Ultrasound guided Fine needle Aspiration Cytology) and the confidential nature of the information that will be collected and disclosed during the study. The information collected will be used only for research.

I have had the opportunity to ask questions regarding the various aspects of this study and my questions have been answered to my satisfaction.

I understand that I remain free to withdraw from this study at any time and this will not change my future care.

Participation in this study does not involve any extra cost to me.

Subject's name and signature /thumb impression Date:

Name and signature of witness Date:

Name and signature of person obtaining consent Date:

ಮಾಹಿತಿ ಸಮ್ಮತಿಯ ನಮೂನೆ

Ultrasound guided fine needle aspiration cytology in evaluation of cervical lymph node metastasis in oral squamous cell carcinoma

ನಾನು ರುಜುಮಾಡಿರುವ ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಪಾಲ್ಗೊಳ್ಳಲು ಮತ್ತು ಈ ಸಮ್ಮತಿಯ ರೂಪದಲ್ಲಿ ವಿವರಿಸಿರುವಂತೆ ಸಂಗ್ರಹವನ್ನು ದೃಢೀಕರಿಸಲು ಒಪ್ಪುತ್ತೀರಿ.

ನಾನು ಈ ಅಧ್ಯಯನದ ಉದ್ದೇಶವನ್ನು ಅರ್ಥಮಾಡಿಕೊಂಡಿದ್ದೇನೆ. ಅಧ್ಯಯನದ ಅಪಾಯಗಳು ಮತ್ತು ಪ್ರಯೋಜನಗಳನ್ನು (ಅಲ್ಟ್ರಾಸೌಂಡ್ ಮಾರ್ಗದರ್ಶಿ ಸೂಕ್ಷ್ಮ ಸೂಜಿ ಆಕಾಂಕ್ಷೆ ಸೈಟೋಲಜಿ) ಮತ್ತು ಮಾಹಿತಿಯ ಗೌಪ್ಯತೆ ಯನ್ನು ನನಗೆ ವಿವರಿಸಲಾಗಿದೆ ಅಧ್ಯಯನದಲ್ಲಿ ಸಂಗ್ರಹಿಸಿ ಬಹಿರಂಗಪಡಿಸಲಾಗುವುದು ಸಂಗ್ರಹಿಸಿದ ಮಾಹಿತಿಯನ್ನು ಸಂಶೋಧನೆಗೆ ಮಾತ್ರ ಬಳಸಲಾಗುತ್ತದೆ.

ಈ ಅಧ್ಯಯನದ ವಿವಿಧ ಅಂಶಗಳ ಬಗ್ಗೆ ಪ್ರಶ್ನೆಗಳನ್ನು ಕೇಳಲು ನನಗೆ ಅವಕಾಶವಿದೆ ಮತ್ತು ನನ್ನ ಪ್ರಶ್ನೆಗಳಿಗೆ ನನ್ನ ತೃಪ್ತಿಕರ ಉತ್ತರಗಳನ್ನು ನೀಡಲಾಗಿದೆ ಎಂದು ಅರ್ಥ.

ಈ ಅಧ್ಯಯನದಿಂದ ಯಾವುದೇ ಸಮಯದಲ್ಲಿ ಹಿಂತೆಗೆದುಕೊಳ್ಳಲು ಮುಕ್ತವಾಗಿರುತ್ತೇನೆ ಮತ್ತು ಇದು ನನ್ನ ಮುಂದಿನ ಆರೈಕೆಯನ್ನು ಬದಲಾಸುವುದಿಲ್ಲ ಎಂದು ಅರ್ಥಮಾಡಿಕೊಂಡಿದ್ದೇನೆ.

ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸುವುದಕ್ಕೆ ನನಗೆ ಯಾವುದೇ ಹೆಚ್ಚಿನ ವೆಚ್ಚವನ್ನು ಒಳಗೊಂಡಿರುವುದಿಲ್ಲ.

ವಿಷಯದ ಹೆಸರು ಮತ್ತು ಅರ್ಜಿದಾರರ ಸಹಿ ದಿನಾಂಕ

ರೋಗಿಯ ಹೆಸರು ಮತ್ತು ಸಾಕ್ಷಿಯ ಸಹಿ ದಿನಾಂಕ

ಹೆಸರು ಮತ್ತು ವ್ಯಕ್ತಿ ಪಡೆಯುವ ಒಪ್ಪಿಗೆ ಸಹಿ ದಿನಾಂಕ

KEYS TO MASTER CHART:

COLUMN A: Serial Number

COLUMN B: Histopathology Number

COLUMN C: Cytology number

COLUMN D: Age

COLUMN E: Gender

1-Female 2-Male

COLUMN F: Side

1-Left 2-Right

COLUMN G: Site

1-Buccal mucosa, 2-Tongue, 3-Lower alveolus, 4-Upper alveolus ,5-Lip

COLUMN H: Clinical presentation

1-Swelling in the cheek ,2-Pain in the oral cavity,3-Ulcer in the mouth 4-Unable to open mouth 5-Burning sensation

COLUMN I: Duration of symptoms(months)

COLUMN J: Years of consumption of betel quid

COLUMN K: Tumor size(mm)

COLUMN L: Clinical N stage

COLUMN M: Clinical M stage

COLUMN N: Clinical Tumor stage

COLUMN M: Pathological T stage

COLUMN O: Pathological N stage

COLUMN P: Pathological M stage

COLUMN Q:Pathological tumor stage

COLUMN R:Tumor type

1-Ulcerative 2-Ulceroexophytic 3-Infiltrative 4-Flat

COLUMN S: Differentiation

1-Well differentiated 2-Moderately differentiated 3-Poorly differentiated

COLUMN T: Cytology positive lymphnodes

0-Negative 1-Positive

COLUMN U: Histopathology number of positive nodes

COLUMN V: Total nodes retrieved

COLUMN W: Largest lymphnode involved(mm)

COLUMN X: Level of lymphnode which was aspirated

S. No	B.No	cyto no.	age	gender	side	site	clinical presentation	Duration of symptoms(months)	Years of consumption of betel quid	TUMOR SIZE	CLIN N	CLINICALM	stage	T	N	M	STAGE	TUMOR TYPE	DIFFERENTIATION	CYTO	NODES +	EXAMINED	LARGEST LN	LEVEL aspirated
1	3251	3019	60	1	1	1	1	1	30	10	1	X	3	1	2	X	4	1	1	1	2	24	14	IB
2	2879	2669	80	1	2	1	2,1	1	40	45	1	X	3	3	1	X	3	2	2	1	1	11	16	IB
3	2812	2760	27	2	1	1	2,5	2	40	46	2	X	4	4	2	X	4	2	1	1	4	13	18	IB
4	3010	2991	38	2	2	2	3	6	5	43	2	X	4	3	2	X	4	1	1	1	8	37	28	II
5	3088	2811	40	1	2	3	3	2	10	55	2	X	4	3	2	X	4	1	1	1	8	24	41	IB
6	613	214	45	1	2	1	3	1	20	26	1	X	3	2	1	X	3	1	1	**0**	**1**	**34**	15	IB
7	542	382	39	1	1	1	2,3	6	20	20	1	x	3	2	2	x	4	1	2	1	2	32	14	II
8	53	11	65	1	1	1	3	1	20	25	2	x	4	2	2	x	4	2	2	1	7	10	30	II
9	289	118	50	1	1	1	4	6	30	24	1	x	4	2	0	x	2	2	1	0	0	20	28	II
10	3198	2670	50	1	2	4	2,3	6	30	30	2	x	4	2	0	x	2	3	2	0	0	14	12	IB
11	2752	2497	46	2	1	1	1,2	1	20	40	1	x	3	3	0	x	3	1	1	0	0	21	10	IB
12	2919	2733	65	1	1	1	3	2	40	35	1	x	3	4	0	x	4	2	1	0	0	29	14	IB
13	2425	2152	45	1	1	1	3	5	20	25	1	x	4	2	0	x	2	2	2	0	0	11	14	IB
14	829	672	48	1	1	1	2,3	1	15	22	2	x	4	4	2	x	4	2	1	1	3	27	12	II
15	2488	1983	65	2	2	1	1	5	30	35	1	x	3	4	0	x	4	1	1	0	0	15	10	IB
16	2682	2434	70	2	2	1	3	1	10	35	1	x	3	3	0	x	3	3	1	0	0	11	19	IB
17	2443	2108	65	1	1	3	1	2	20	5	2	x	4	4	0	x	3	2	1	0	0	15	15	IB
18	3238	2668	51	1	1	3	1	1	30	25	2	x	4	2	0	x	2	4	2	0	0	21	24	IB
19	2313	1976	65	1	1	1	4	6	20	42	1	x	4	3	0	x	3	2	1	0	0	26	11	V
20	3288	3133	62	1	2	1	3	1	50	5	1	x	3	1	0	x	1	1	2	0	0	10	14	II
21	3359	3266	65	1	1	1	3	2	30	24	1	x	4	2	0	x	2	2	1	0	0	8	20	IB
22	262	109	50	1	1	1	3	2	20	10	1	x	3	1	0	x	1	2	1	0	0	10	10	IB
23	389	313	65	1	2	1	3	3	30	13	1	x	3	1	0	x	1	2	1	0	0	19	13	III
24	3073	607	32	2	1	2	1	6	10	44	2	x	4	3	2	x	4	2	1	1	4	24	33	II
25	2588	2355	21	2	2	1	1	4	15	35	2	x	4	4	1	x	4	2	1	1	3	28	17	II
26	203	98	45	1	2	1	3	3	15	25	2	x	4	2	2	x	4	2	1	1	3	31	22	IB
27	1522	876	56	2	1	1	3	2	30	20	2	x	4	4	1	x	4	2	2	1	1	10	20	II
28	1736	1594	60	2	2	1	3	4	30	30	2	x	4	2	2	x	4	2	2	1	**2**	**28**	23	IB
29	1498	1374	50	1	1	1	2,3	1	20	24	2	x	4	2	2	x	4	2	2	1	3	9	17	II
30	1797	1034	70	2	1	1	3	2	30	32	2	x	4	4	0	x	4	2	1	0	0	15	13	IB
31	980	636	37	1	2	2	2	1	5	35	2	x	4	2	2	x	4	2	1	1	3	31	18	IB
32	1639	1488	45	2	2	1	3	2	20	30	2	x	4	2	1	x	3	2	2	1	2	11	20	IB
33	1764	1745	45	2	2	1	3	2	10	23	2	x	4	2	2	x	4	2	3	1	5	15	31	IB
34	1381	1316	38	2	2	2	2	2	5	30	2	x	3	2	0	x	2	2	1	0	0	18	24	II
35	1908	1677	60	1	1	1	3	4	30	30	1	x	4	2	1	x	3	1	2	1	2	28	25	IB
36	2162	1769	70	1	1	1	3	2	30	45	2	x	3	4	2	x	4	2	1	1	2	20	26	II
37	2022	1558	55	2	2	5	1	2	20	25	1	x	4	2	0	x	2	2	1	0	0	22	23	IB
38	1520	1326	49	2	1	1	3	3	40	30	2	x	3	4	2	x	4	2	2	0	0	11	21	II
39	1720	1489	65	1	1	4	3	6	30	20	1	x	4	3	0	x	2	2	1	1	**2**	**44**	25	II
40	2162	1593	40	1	1	1	3	1	40	40	3	x	3	4	2	x	4	2	1	1	2	20	28	IB
41	2425	1713	67	1	2	4	3,2	2	20	20	1	x	4	2	0	x	2	1	2	0	0	11	15	IB
42	2807	2087	50	1	2	3	4	1	20	30	1	x	4	2	0	x	2	2	1	0	0	14	18	IB
43	1419	768	50	2	2	1	3	6	30	40	1	x	4	4	2	x	4	2	1	1	2	6	15	IB
44	928	704	50	1	1	1	6	3	15	35	2	x	4	2	0	x	2	2	1	0	0	21	17	II
45	1090	688	51	1	1	1	3	2	20	45	2	x	4	3	0	x	3	1	1	0	0	24	17	II
46	876	567	70	1	1	3	3	6	40	10	1	x	4	3	0	x	3	4	1	0	0	20	17	IB
47	1544	1202	58	1	2	1	3	3	40	18	2	x	4	4	0	x	4	2	1	0	0	11	17	II
48	1054	589	60	1	2	1	3	1	40	24	1	x	3	2	0	x	2	2	1	0	0	6	26	IB
49	434	1342	48	1	2	1	3	5	15	41	2	x	4	3	2	x	4	2	1	1	11	28	14	IB

50	2191	1888	65	1	1	1	3	7	15	25	1	x	3	2	0	x	2	2	1	0	0	11	10	IB
51	957	1315	35	1	1	1	3	1	15	25	1	x	3	2	0	x	2	2	1	0	0	7	14	IB
52	1785	1567	60	1	2	3	2,3	6	30	25	1	x	3	4	0	x	4	2	1	0	0	17	22	IB
53	1970	1678	65	1	1	1	3	1	40	42	1	x	4	3	0	x	3	2	1	0	0	26	11	IB
54	1741	1588	45	1	2	1	3	3	20	5	1	x	4	2	0	x	2	5	1	0	0	29	20	IB
55	1782	1490	50	2	2	1	3	1	20	23	1	x	3	2	0	x	2	2	1	0	0	18	19	IB
56	1522	1343	45	2	1	1	3	2	20	40	1	x	3	4	1	x	4	2	2	1	1	10	18	IB
57	753	1344	48	1	1	1	2	5	40	10	2	x	4	1	1	x	2	3	2	1	1	13	19	IB
58	1254	1004	59	1	2	2	3	6	30	42	2	x	4	3	2	x	4	2	2	1	6	15	21	II
59	1969	1589	70	1	2	1	3	4	20	20	1	x	3	1	0	x	1	1	1	0	0	19	19	IB
60	1637	1387	35	1	1	1	2,3	4	10	12	1	x	3	1	0	x	1	1	1	0	0	12	26	IB
61	1886	1786	61	1	2	3	3	6	30	25	1	x	3	2	1	x	3	1	2	1	1	40	18	II
62	1860	1699	70	1	1	2	3	4	40	15	2	x	4	3	2	x	4	2	1	1	2	45	32	II
63	1520	1400	45	1	2	1	3	6	15	30	1	x	4	4	0	x	4	2	2	0	0	11	16	II
64	798	269	50	2	1	1	3	4	25	34	1	x	3	2	0	x	2	2	1	0	0	23	20	IB
65	1897	1509	70	2	1	1	3	5	30	32	1	x	4	4	0	x	4	2	1	0	0	15	18	IB
66	3019	2888	45	2	2	2	3	3	20	32	2	x	4	2	2	x	4	2	1	0	1	37	19	IB
67	3091	2982	45	1	1	4	3	4	15	17	1	x	4	4	1	x	4	2	1	0	1	26	18	IB
68	2716	2116	38	1	2	3	3	4	10	5	1	x	3	1	0	x	1	1	1	0	0	40	10	II
69	2032	1980	52	2	1	1	3	6	20	55	1	x	4	4	0	x	4	2	2	0	0	18	15	IB
70	2056	1678	55	1	1	1	3	5	20	42	1	x	4	4	0	x	4	2	2	0	0	13	18	IB