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DR. SNIGDHA, MBBS

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TAMAKA, KOLAR, KARNATAKA
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

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UNDER THE GUIDANCE OF

DR. SUBHASHISH DAS, MD

PROFESSOR

DEPARTMENT OF PATHOLOGY

DEPARTMENT OF PATHOLOGY

SRI DEVARAJ URS MEDICAL COLLEGE, KOLAR

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"EVALUATION OF CYCLIN D1 AND HER2 NEU PROTEIN EXPRESSION IN HEAD AND NECK SQUAMOUS CELL CARCINOMA"
IN SRI DEVARAJ URS MEDICAL COLLEGE, KOLAR

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The Institutional Ethics Committee of Sri Devaraj Urs Medical College, Tamaka, Kolar has examined and unanimously approved the synopsis entitled **"Synopsis for dissertation evaluation of cyclin D1 and HER2 neu protein expression in head and neck SQUAMOUS cell carcinoma"** being investigated by **DR.SNIGDHA**, Dr. Subhashish Das & Dr. S M Azeem Mohiyuddin1 in the Departments of Pathalogy & ENT1 at Sri Devaraj Urs Medical College, Tamaka, Kolar. **Permission is granted by the Ethics Committee to start the study.**

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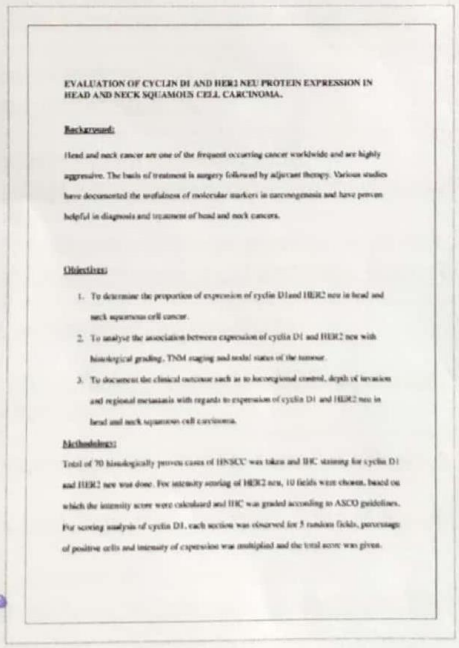

EVALUATION OF CYCLIN D1 AND HER2 NEU PROTEIN EXPRESSION IN HEAD AND NECK SQUAMOUS CELL CARCINOMA.

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Head and neck cancer are one of the frequent occurring cancer worldwide and are highly aggressive. The basis of treatment is surgery followed by adjuvant therapy. Various studies have documented the usefulness of molecular markers in carcinogenesis and have proven helpful in diagnosis and treatment of head and neck cancers.

Objectives:

1. To determine the proportion of expression of cyclin D1and HER2 neu in head and neck squamous cell cancer.
2. To analyse the association between expression of cyclin D1 and HER2 neu with histological grading, TNM staging and nodal status of the tumour.
3. To document the clinical outcome such as to locoregional control, depth of invasion and regional metastasis with regards to expression of cyclin D1 and HER2 neu in head and neck squamous cell carcinoma.

Methodology:

Total of 70 histologically proven cases of HNSCC was taken and IHC staining for cyclin D1 and HER2 neu was done. For intensity scoring of HER2 neu, 10 fields were chosen, based on which the intensity score were calculated and IHC was graded according to ASCO guidelines. For scoring analysis of cyclin D1, each section was observed for 5 random fields, percentage of positive cells and intensity of expression was multiplied and the total score was given.

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Date: Signature of the Candidate

Place: KOLAR **Dr. Snigdha**

LIST OF ABBREVIATIONS

HNSCC- Head and neck squamous cell carcinoma

OSCC- Oral squamous cell carcinoma

SCC- Squamous cell carcinoma

IHC – Immunohistochemistry

Her 2 - Human epidermal growth factor receptor 2

EGFR - Epidermal growth factor receptors

ROS - Reactive oxygen species

HPV – Human papilloma virus

CDK – Cyclin dependent kinase

AJCC – American joint cancer committee

WHO – World health organization

WDSCC – Well differentiated squamous cell carcinoma

MDSCC – Moderately differentiated squamous cell carcinoma

PDSCC – Poorly differentiated squamous cell carcinoma

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ABSTRACT

Background:

Head and neck cancer are one of the frequent occurring cancer worldwide and are highly aggressive. The basis of treatment is surgery followed by adjuvant therapy. Various studies have documented the usefulness of molecular markers in carcinogenesis and have proven helpful in diagnosis and treatment of head and neck cancers.

Objectives:

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Results:

52 cases out of 69 (75%) showed strong and moderate positivity for cyclin D1. P value was 0.017, 0.001 and 0.032 for DOI, TNM stage and lymph node metastases for cyclin D1 which was statistically significant. For HER2 neu, total of 5/70 cases were positive and P value was significant for depth of invasion which was 0.008.

Conclusion:

The expression of the above marker cyclin D1 increases with stage, DOI and positive lymph node status. Hence Cyclin D1 immuno-expression can be helpful in early assessment of behaviour of HNSCC and can serve as in independent prognostic marker.

HER2neu was significant with increase in depth of invasion of tumour which in AJCC 8th edition is consider an important factor in determining the stage of the tumour. Further research is needed to examine whether HER2neu can act as a prognostic factor for HNSCC and if it can be targeted for treatment options.

Keywords: Head and neck squamous cell carcinoma, cyclin D1, HER2 neu.

INTRODUCTION

Head and neck carcinomas are one among the most common carcinomas across the whole globe and is a widespread term used for carcinomas involving mainly oropharynx and upper respiratory tract. The most frequent group among head and the neck cancer is squamous cell carcinomas(SCC), comprising 9 out of 10 cases.[1] The prevalence of head and neck squamous cell carcinomas (HNSCC) is much less in west than in Asian and South Asian countries with the former accounting for only 1-4% of the total HNSCC malignancy.[2] On the other hand, a high incidence, that is above 57.5 % of entire HNSCC occur in Asia among which India contributes majority of the cases.[3] More than 2,00,000 case of HNSCC occur in India every year, which alone accounts for more than 30% of entire cancer in the country, making it one among the most encountered malignancies and also one of the principal cause of deaths in men due to cancer. Among this 2,00,000, more than 80,000 cases are alone found in oral cavity, especially in lower GBS, giving it a term 'Indian oral cancer'.[4]

The carcinoma starts with chief complains of an ulcer which can either be exophytic or endophytic or a growth which with increase in size leads to other symptoms such as trismus, obstruction, bleeding or pain.[5] HNSCC are also overall linked with bad prognosis and 5-year survival-rate is estimated less than 55%.[6]

Multifactorial risk factors are found when it comes to HNSCC, tobacco being the most common of all. With coming of age and recent discoveries, it is now well established that molecular events play a central role in tumour development and progression.[7] Hence, these days more emphasis is given to molecular markers to predict the prognosis and aggressiveness of the tumour.

One such molecular marker is Cyclin D1, which helps in progression of cell cycle from G1 to S phase. Overexpression of this marker leads to accelerated entrance of cells into S phase of cell cycle, in turn accelerating the entire process and uncontrolled proliferation.[8]

Human epidermal growth factor receptor 2 neu (HER2neu), a member of epidermal growth factor receptor (EGFR) family is an important signal transduction protein, which when overexpressed promotes the cell proliferation and inhibits apoptosis as a result leading to malignancy.[9]

These markers when associated and correlated with other features of carcinoma such as lympho-vascular invasion, perineural invasion, staging and other features can be of great help in knowing the biological behaviour of the tumour.

NEED FOR STUDY

An aggressive epithelial malignancy, oral squamous cell carcinoma (OSCC), poses a noteworthy threat to public health worldwide. There stands a high mortality rate and morbidity associated with oral cancer.

The survival rate for affected people with cancer has not substantially become better in past 40 years, despite breakthrough advances in treatment. The 5-year survival rate remains approximately the 55%.[10] Moreover, the TNM system is not adequate to accurately categorise affected population in terms of their betterment related to disease. Therefore, with the advent of coming up molecular tools, the study of distribution of molecular markers is of particular interest.

This will aid in identifying a subset of patients with a bad prognosis who require aggressive treatment strategies, and will further determine the course of action for their treatment and betterment, thus giving a better survival for those patients.

AIMS AND OBJECTIVES

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REVIEW OF LITERATURE

Anatomy of head and neck

Head and neck are a complex region of body which consist of many important structures compressed in a small space. In our study, we have mainly focused on SCC of mouth, palate, pharynx, larynx, nose and paranasal sinuses.

Oral cavity

Majority of carcinogens enter the body through oral cavity, hence making it the communal site for carcinoma among head and neck structures.

Embryology:

The structure in oral cavity is derived from both endoderm and ectoderm. The pharyngeal arches give rise to different part of tongue. Anterior two third of tongue arise from first pharyngeal arch which is derived from lingual swellings. The second and third part of fourth pharyngeal arch leads to development of posterior part of the tongue. The palate develops between six to twelve weeks of intra-uterine life and consists of primary and secondary embryological origins. Neural crest give rise to facial prominence consisting of lateral nasal, medial nasal and maxillary process which later fuse together to form facial structures and also leads to development of upper lip. The lower lip develops from fusion of mandibular process.[11]

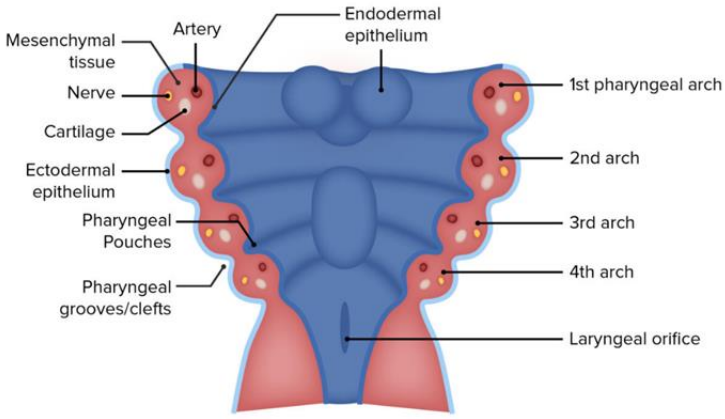


Figure 1: Embryology of oral cavity (Image adopted from Inderbir singh's Human Embryology 11 th edition)

Anatomy:

Oral cavity is distributed into two areas that is vestibule and oral cavity proper. Tongue fills most of the oral cavity proper and this is bounded by alveolar process containing teeth on the sides and anteriorly. The isthmus of the fauces marks the posterior boundary. The roof is formed by anteriorly by hard palate and posteriorly by soft palate. Mylohyoid muscles form the floor of the oral cavity proper.[12]

The oral cavity's mucosa and inner lining of mouth is composed of stratified squamous epithelium.[12]

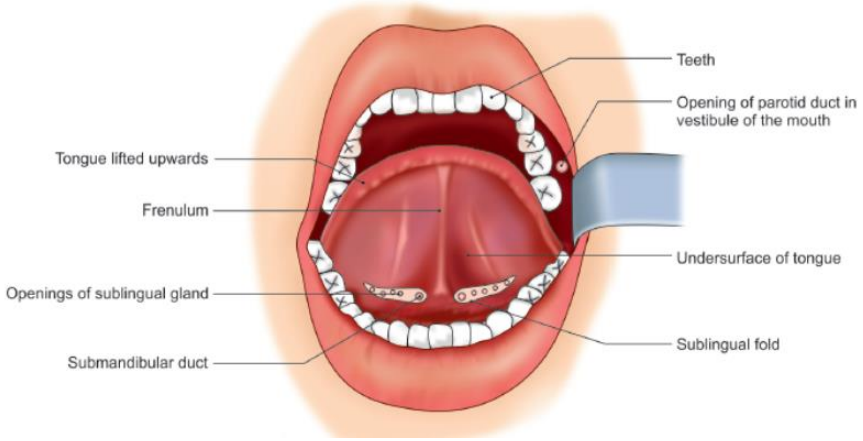


Figure 2: Anatomy of oral cavity (image adopted from B D Chaurasia human anatomy volume 3, 8th edition)[12]

Blood supply and lymphatics:

External carotid artery contributes mainly to the arterial supply of oral cavity. Oral tongue along with base of the tongue is supplied by lingual artery and greater palatine artery. superior alveolar artery supplies the hard palate.[12]

The venous drainage is predominantly to pterygoid plexus which in turn drains to internal jugular vein.[12]

The lymphatic drainage of tongue is mainly to submental nodes and upper cervical lymphatics. Level I and II cervical lymph nodes can be skipped in case of metastases of lateral tongue lesion which can directly spread to level III and IV cervical nodal group.[12]

Oropharynx:

The soft palate and hyoid bone along with valleculate forms the superior and inferior border of oropharynx while adaxial border is created by the base of the tongue ending at circumvallate papillae. The tonsillar fossae and lateral along with dorsal pharyngeal wall form the lateral and dorsal borders respectively.[12]

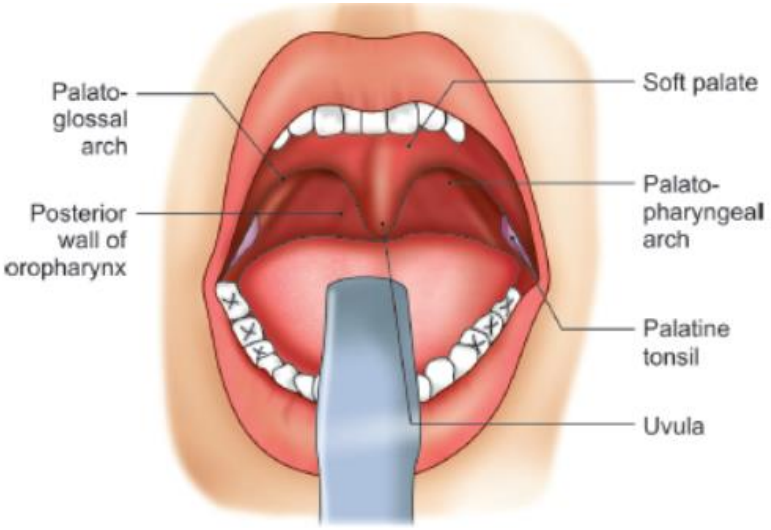


Figure 3: Anatomy of oropharynx (image adopted from B D Chaurasia human anatomy volume 3, 8th edition)[12]

Soft palate:

Nasopharynx and oral cavity are separated from oropharynx by soft palate which is derived from palatopharyngeal arch and uvula. The soft palate mainly contains palatopharyngeal and constrictor muscle on the lateral sides. The other muscle found are levator and tensor muscles of palate and uvular muscle.[12]

Soft palate is chiefly supplied by ascending branch of facial artery.[12]

Pharyngeal branch of vagus nerve caters motor innervation of soft palate and glossopharyngeal along with lesser palatine deals with sensory supply.[12]

Tonsillar fossae:

The tonsil is placed on the lateral part of pharyngeal wall in the tonsillar region. Anterior faucial pillar is formed by palatoglossal muscle the and palatopharyngeal muscle forms the posterior faucial pillar with palatine tonsils embedded between them.

Anterior pharyngeal artery, posterior branchof lingual artery, branches of facial artery and palatine branches of internal maxillary artery constitute the blood supply of tonsils.

Neural innervation is through lesser palatine branchof maxillary nerve and glossopharyngeal nerve.[12]

Hypopharynx:

Hyoid bone forms the superior border of hypopharynx merging it anteriorly with oropharynx and inferior border is formed by cricopharynx muscle which further transitions into the cervical oesophagus.

There are three subsites of hypopharynx

1. Pyriform sinus: the pyriform sinus is of inverted pyramid shape formed by anterior, medial and lateral wall. Pharyngo-epiglottic fold acts as a base and the apex reaches

down to cricoid cartilage. It is lateral in nature to aryepiglottic fold and thyroid lamina constitute the medial wall. The posterior wall of proglottic space is shaped by medial pyriform mucosa and aryepiglottic folds along with lateral cricoarytenoid muscle separates it from endo-larynx. Hence, the medial extension of hypopharyngeal tumours can be seen in the larynx.

2. Posterior cricoid region: Anterior wall of hypopharynx is formed by posterior cricoid. Cricoid cartilage along with posterior cricoarytenoid muscle are invaded by posterior cricoid tumours.
3. Posterior hypopharyngeal wall: potential retropharyngeal space separates posterior hypopharyngeal wall from vertebral and paravertebral space making it easy for the tumour to cross this space leading to extension into paravertebral tissue.

Superior thyroid artery forms blood supply to hypopharynx.[12]

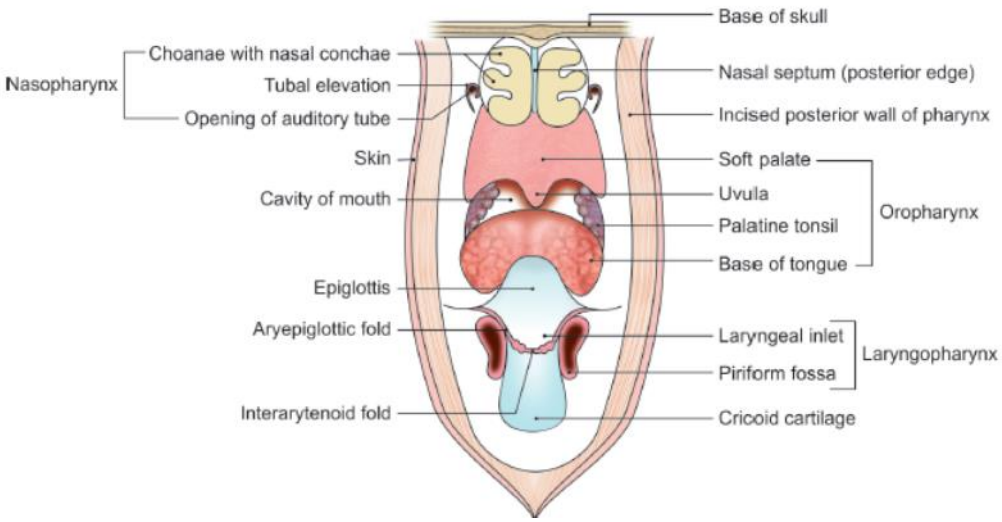


Figure 4: Anatomy of pharynx (image adopted from B D Chaurasia human anatomy volume 3, 8th edition)[12]

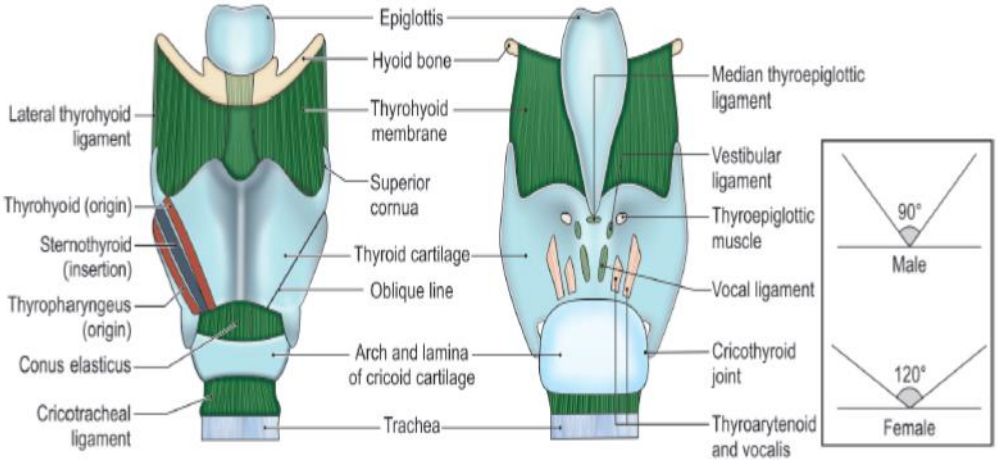
Larynx:

Larynx consist of six cartilage, 3 paired and 3 unpaired. Arytenoid, corniculate and cuneiform are the paired cartilage and the unpaired cartilages include epiglottis, thyroid and cricoid.

The lateral margin of epiglottis along the superior edge of folds of arytenoid forms the superior boundary. Thyroid cartilage, alone forms the anterior boundary at the level of glottis and anterior arch of cricoid cartilage along with cricothyroid membrane forms the anterior boundary at the level of subglottis. The inferior boundary is formed by horizontal plate, inferior edge of which passes through the cricoid cartilage.

The larynx is further divided into-

1. Supraglottic: formed by suprahyoid epiglottis, arytenoids laryngeal surface of aryepiglottic folds, infrahyoid epiglottis and false vocal cords. The epithelium of supraglottic is histologically composed of pseudostratified columnar type of epithelium and mucosa contains a large number of mucus glands and lymphatic vessels. The lymphatic drainage is composed of upper middle and lower jugular chain.
2. Glottis: it contains anterior and posterior commissure and true vocal cords. The inferior boundary is formed by a horizontal plate which lies subordinate to the inferior limit of epiglottis. The tumours of this region metastasize unilaterally and tend to have a less regional spread when compared to supraglottic tumour.
3. Subglottis: the space between inferior part of cricoid cartilage and the inferior part of glottis form subglottis. This lies in close contact to the cricothyroid membrane and cricoid cartilage and lining is of pseudostratified columnar epithelium. The tumour in this region tends to metastasize to paratracheal, lower and middle jugular lymph nodes.[12]



a b c

Figure 5: Anatomy of the larynx: (a)Anterior view; (b)Posterior view; (c)Angle of thyroid laminae in male and female (image adopted from B D Chaurasia human anatomy volume 3, 8th edition)[12]

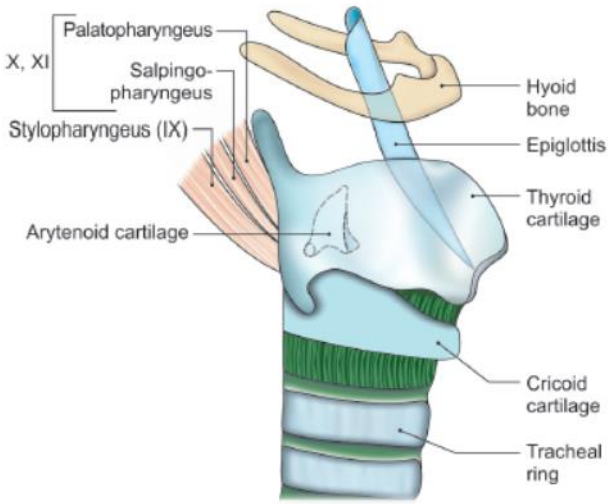


Figure 6: Cartilage of the larynx (image adopted from B D Chaurasia human anatomy volume 3, 8th edition)[12]

Histology of head and neck:

The lining epithelium of oral cavity comprises of non-keratinised stratified squamous epithelium with exceptions of few places where the epithelium is keratinised. The mucosa constitutes of stratum Basale, stratum spinosum and superficial layer starting from basement membrane to the surface.

Stratum granulosum along with stratum corneum lie above stratum granulosum in keratinised epithelium. The only layer that express proliferation of allied antigen along with Ribonucleic acid component of telomerase is stratum Basale.

The basal cells help in turnover of epithelium thus fulfilling the role of stem cells. They do so by continuously undergoing differentiation in the epithelium's superficial layer.

The variation in quantity of keratinization depends on the amount of mechanical stress the tissue is exposed to which further depends on the resilient of tissue in the exposed area.

Extended mucosal ridges are found in the buccal mucosa which helps in anchoring to the heavily collagenised lamina propria. The floor of the mouth has thin and shallow rete ridges with comparatively less collagenised stroma.

The tongue contains lymphoid tissue in its posterior one third.

The colour of the mucosa also depends on the extent of vascularisation, thickness, presence of pigments and level of keratinisation of lamina propria.[13]

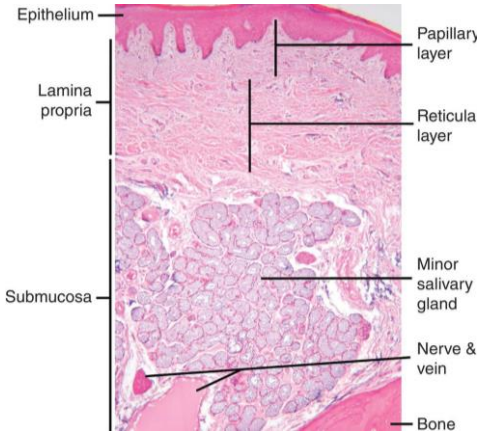


Figure 7: Histology of oral cavity (image adopted from Inderbir Singh textbook of human histology 8th edition)[13]

Aetiology of head and neck squamous cell carcinoma

Alcohol with tobacco independently account for total of 75-90% risk factors for carcinoma. The other risk factors which cause carcinogenesis include chronic irritation from ill fitted dentures, improper oral hygiene, infections, ultraviolet light, immunosuppression, dietary deficiencies, previous exposure to dentures and also genetic susceptibility.

Tobacco:

Tobacco uses alone is an independent risk factor for carcinoma. There exists a dose dependent relationship with overall duration and daily frequency of use in years. The age for starting of tobacco is inversely proportional to the risk of developing of cancer.. The relative risk of smoking decreases rapidly after quitting, and the relative risk compared with non-smokers drops to nearly 1 after 10 years or more.[14]

Betel quid chewing associated, a habit which is predominantly prevalent in southern India, is a sturdy cause for oral cancers. Also, recently the practice of chewing areca nut is proven carcinogenic for human.[15] The alternative chewing products like pan masala, guthka are strongly related to the sudden increase in cases of oral submucosal fibrosis and oral cancers. Various enzymes such as glutathione S transferase, N-acetyl transferase and cytochrome p450 helps in activation of these products and hence the genetic polymorphisms in the level of these enzymes play an important role.[16]

Alcohol:

Like tobacco, consumption of alcohol is also one of the important risk factors for carcinoma.[17] Three mechanisms are postulated for carcinoma caused due to alcohol use. 1)Disruption of systemic nutrient metabolism. During ethanol metabolism in the liver, systemic metabolism of zinc, iron, retinoids, and methyl groups is altered and are associated with the development of carcinoma. (2) Disruption of redox metabolism in squamous cells: When ethanol is metabolized in oral oesophageal squamous cells, reactive oxygen species

(ROS) are generated causing oxidative damage. (3) disruption of signalling pathways in squamous cells: Due to its physicochemical properties, ethanol can alter the fluidity and shape of cell membranes and influence several signalling pathways.[18] Consumption of 50 gm of alcohol on a daily basis increases the threat of oral, pharyngeal, oesophageal and laryngeal cancer by 2-5 times compared with non-drinkers.[19]

HPV infection:

The prevalence of HPV is well known HNSCC. Human tumour viruses are chief cause in about one-fifth of all cancers across the globe and are the primary cause. The link between HPV and HNSCC has been highlighted in 60s.[20] High risk HPV virus type 16 and 18 are associated with oral and laryngeal carcinoma. Sexual transmission is one of the most common routes for transmission of infection as HPV DNA is more frequently seen in patients who practice oral sex. [21]

Dietary factors:

A calculable defensive effect of a diet high in fruits and vegetables has been demonstrated in several studies. A meta-analysis showed 28% decreased risk of carcinoma per 50 g of non-starchy vegetables and 28% decreased risk when consumed 50g of citrus fruit per day proving a dose dependent relationship.[22]

Oral hygiene:

Studies have recognised the role of deprived quality of oral hygiene in oral carcinogenesis, establishing a reverse relationship between good hygiene and the prevalence of oral carcinoma.[23]

Immunosuppression:

The incidence of carcinoma is seen more in immunocompromised patients. Literature has demonstrated that association of immunosuppression with poor outcomes in oral SCC, with roughly 2-fold elevated morbidity and fatality.[24]

Pathogenesis:

Cancer progression models describe several phases that occur during development of a tumour. Oncogenes are activated and tumour suppressor genes go on deactivated, and a mixture of these shift are required for carcinogenesis. At the oral mucosal level, this genetic progression is histologically reflected by the transformation concerning normal mucosa transforming towards the dysplastic epithelium and finally into frank invasive squamous cell carcinoma. Data supporting this model are from studies showing genetic alterations in histologically native tissues and in precancerous lesions, including deletion of chromosomal heterozygosity 3p14 and 9p21, telomerase activation and cyclin D1 overexpression.[25] Tobacco can cause carcinogenesis in mouth, in part through its effects on the p53 and 3p chromosomal regions. Altered expression of p53 is associated with increased instability of genome in oral intraepithelial neoplasia and lead to accelerated rates of genetic change during oral tumorigenesis.[16]

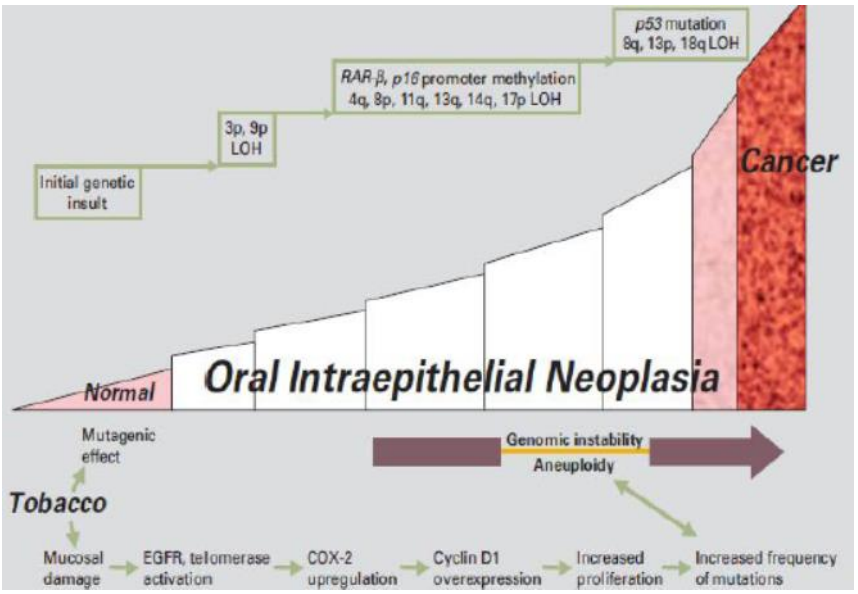


Figure 8: Molecular (genetic along with epigenetic) progression of multistep process of oral carcinogenesis.[26]

The central white stages in this diagram represents the forward progression of neoplasia of the oral epithelium from leucoplakia to erythroplasia, which leads to cancer. This process includes the initiation of action of the epidermal growth factor receptor (EGFR) and the related downstream events that lead to disordered proliferation, increasing the regularity of mutations that cause alteration in genomic stability and invasion.

Many studies have revealed the central role of tumour suppressor genes and proto-oncogenes regulation of cell cycle and apoptosis, suggesting their aberrant expression during transformation of various human cancers. The Rb pathway and the p53 pathway are two. Once activated, cyclin D1, CDK4 or CDK6 can phosphorylate the Rb gene protein, leading to its inactivation and the release of transcription factors required for progression into the S phase and cell cycle.[26]

Dysregulation of cell cycle in cancer:

Dysregulation of the cell cycle is one of the important aspects of cancer progression. Lot of these molecular changes that cause abnormal biological behaviour of cancer cells depends on deviation in cell cycle regulation. For example, escaping mitotic addiction or inducing faecal antigenic resistance, resistance to apoptosis, and growth of cells with oncogenes is demonstrated. activating and/or tumour suppressor genes are inactivated to pass multiple checkpoints leading to increased genomic instability.[27]

The core of the cell cycle is formed by cyclin and cyclin dependent kinase. The expression of cyclin is dependent on the stage of the cell cycle and is regulated during the phase of transcription, post-transcriptional, and translation/post-translation. Cyclin family interact with Cyclin dependent kinase (CDKs), and these complexes undergo particular phases in cell cycle.[28] D-type cyclins interact with CDK4 and CDK6 and are required for G0/G1 transition. Cyclin E binds to CDK2 and mediates entry into the S phase. The cyclin A/CDK2 complex regulates S phase. Cyclin B1 and CDC2 trigger mitosis-related molecular events.[29]

Pre malignant lesions of oral cavity:[30]

1. Leucoplakia
    a. Homogenous leucoplakia
    b. Non homogenous leucoplakia
    c. Ulcerated leucoplakia
    d. Nodular leucoplakia
    e. Verrucous leucoplakia
    f. Proliferative verrucous leucoplakia
    g. Syphilitic leucoplakia
2. Erythroplakia
3. Sublingual keratosis
4. Actinic cheilitis
5. Submucous fibrosis
6. Lichen planus
7. Chronic candidiasis
8. Dyskeratosis congenita
9. Patterson Kelly syndrome
10. Xeroderma pigmentosum

WHO classification of head and neck tumours:[31]

1) Carcinoma
    a) Keratinizing squamous cell carcinoma
    b) Non keratinizing squamous cell carcinoma
    c) Spindle cell squamous cell carcinoma
    d) Lymphoepithelial carcinoma
    e) Sino nasal undifferentiated carcinoma
    f) NUT carcinoma
    g) Neuroendocrine carcinoma
        i) Small cell neuroendocrine carcinoma
        ii) Large cell neuroendocrine carcinoma
    h) Adenocarcinoma
        i) Intestinal type adenocarcinoma
        ii) Non intestinal type adenocarcinoma
2) Teratocarcinosarcoma
3) Sino nasal papilloma
    a) Sino nasal papilloma, inverted type
    b) Sino nasal papilloma, oncocytic type
    c) Sino nasal papilloma, exophytic type
4) Respiratory epithelial adenomatoid hamartoma
5) Seromucinous hamartoma
6) Salivary gland tumours
7) Malignant soft tissue tumours
    a) Fibrosarcoma
    b) Undifferentiated pleomorphic sarcoma

c) Leiomyosarcoma

d) Rhabdomyosarcoma, NOS

e) Embryonal rhabdomyosarcoma

f) Alveolar rhabdomyosarcoma

g) Pleomorphic rhabdomyosarcoma, adult type

h) Spindle cell rhabdomyosarcoma

i) Angiosarcoma

j) Malignant peripheral nerve sheath tumour

k) Biphenotypic sinonasal sarcoma

l) Synovial sarcoma

8) Borderline or low grade malignant soft tissue tumour

a) Desmoid type fibromatosis

b) Sino nasal glomangiopericytoma

c) Solitary fibrous tumour

d) Epithelioid haemangioendothelioma

9) Benign soft tissue tumour

a) Leiomyoma

b) Haemangioma

c) Schwannoma

d) Neurofibroma

10) Other tumours

a) Meningioma

b) Sino nasal ameloblastoma

c) Chondromesenchymal hamartoma

11) Haematolymphoid tumours

a) Extra nodal NK/T cell lymphoma

b) Extra osseous plasmacytoma

12) Neuroectodermal / melanocytic tumours

a) Ewing sarcoma/ primitive neuroectodermal tumour

b) Olfactory neuroblastoma

c) Mucosal melanoma

Squamous cell carcinoma:[30,32]

SCC accounts for most of the head and neck malignancies. It is an invasive epithelial neoplasm which shows congeable degree of squamous differentiation. This squamous differentiation is seen in the form of keratinisation showing formation of keratin pearls. Invasiveness is a must criterion for diagnosis of SCC. This invasion is seen as tumour cells invading the basement membrane and their presence in the stroma. Furthermore, according to Broder's classification, this is classified as well differentiated (WDSCC), moderately differentiated (MDSCC) and poorly differentiated (PDSCC) based on the amount of keratinisation present.

Variants of squamous cell carcinoma:

1. **Verrucous carcinoma:** also called as Ackerman tumour. Grossly, they present as large fungating and papillary growth. These are difficult to diagnosed on small biopsy as a full thickness section is needed to differentiate it on the invasive front. Adequate biopsy of the tissue shows a well extended rete ridges with complex architecture extending deeper in the tissue. And pushing the underlying stroma. These tumours, rarely metastasize and rate of recurrence is high.
2. **Adenoid SCC:** actinic radiation is the most frequent underlying cause for this kind of carcinomas and lip is the most frequent site. They appear to be of pseudo-glandular or alveolar architecture.
3. **Adeno squamous carcinoma:** they are rare variants in head and neck with squamous differentiation intermixed with glandular differentiation.
4. **Basaloid SCC:** these tumours are the most aggressive ones. The common site for this variant is oral cavity, oropharynx, oesophagus and larynx. These tumours present with peripheral palisading with areas showing squamous differentiation intermixed with

solid tumours. Presence of basal lamina is the most characteristic feature of this tumour. Microscopically, nests of basaloid cells are seen invading the stroma.

5. **Papillary squamous cell carcinoma:** these are associated with HPV infections and presents as an exophytic growth. The presence of cytological atypia differentiates it from verrucous carcinoma.
6. **Spindle cell carcinoma:** they are infiltrated and polypoid mass presenting as an ulcerated growth. These tumours present with sarcoma like features intermixed with squamous differentiation and present as a recurrence of squamous cell carcinoma.

	Seventh edition	Eight edition
Lip and oral cavity		
T1	Tumor <2 cm	Tumor ≤2 cm, ≤5 mm depth of invasion
T2	Tumor 2–4 cm	Tumor ≤2 cm, >5 mm, and ≥10 mm depth of invasion or tumor >2 cm but ≤4 cm and depth of invasion ≤10 mm
T3	Tumor >4 cm	Tumor >4 cm or depth of invasion >10 mm , but ≤20 mm
T4a	Moderately advanced local disease: (Lip) tumor invades through cortical bone or involves inferior alveolar nerve, floor of mouth, or skin of face (oral cavity) tumor involves adjacent structures such as cortical bone of maxilla or mandible, maxillary sinus or skin of face, or extrinsic muscles of tongue	Extrinsic muscles of tongue removed, Included extensive tumors with bilatarel tongue involvement and / or DOI >20 mm
T4b	Very advanced local disease; tumor invades masticator space, pterygoid plates, skull base, and/or encases the internal carotid artery	No change
N1	Metastases to single lymph node, 3 cm or less in greatest diameter	Same, except node must be extranodal extension negative
N2a	Metastases to single ipsilateral node >3 cm but not >6 cm	Same, except node must be extranodal extension negative or single ipsilateral or node 3 cm or smaller with extranodal extension
N2b	Metastases to multiple ipsilateral nodes >3 cm but not >6 cm	Same, except nodes must be extranodal extension negative
N2c	Metastases to bilateral nodes or contralateral nodes none >6 cm	Same, except nodes must be extranodal extension negative
N3	Metastases to nodes >6 cm	Subdivided into 3a: Same as N3 before, but extranodal extension negative 3b: Single ipsilateral node >3 cm in greatest dimension with extranodal extension Or multiple ipsilateral, contralateral, or bilateral nodes, any with extranodal extension Or single contralateral node 3 cm or smaller and with extranodal extension
Nasopharynx		
T4a	Moderately advanced local disease: Involves larynx, extrinsic muscles of tongue, medial pterygoid, hard palate, or mandible	Medial pterygoid, lateral pterygoid, and prevertebral muscles downstaged to T2
T4b	Very advanced local disease: Involves lateral or medial pterygoid plates, lateral nasopharynx, or skull base or encases internal carotid artery	Unchanged
N3a	Metastasis to lymph nodes >6 cm	Combined to single designation of "N3"
N3b	Metastasis extending to supraclavicular fossa	

Fig 9: AJCC 8th edition staging of lip, oral cavity and nasopharyngeal carcinoma. [33]

(published in October 2016)

Table1: AJCC 8th edition staging for oral cavity carcinoma[34] (published in October 2016)

I	T1 or T2 without nodal metastases
II	T1toT3 and N0to N1
III	T1 to T4 and N1 to N2
IV	Any T, any N, and M1

Figure 1. Tumor Staging
Tumor staging is based on local extent and presence/absence of vocal cord fixation, according to AJCC 8th Edition. Pathologic T staging is identical to clinical staging.

Glottis

Clinical T Stage	Local Extent	Vocal Cord Function
1	Limited to vocal cord a – limited to one vocal cord b – involving both vocal cords	Normal
2	Other laryngeal subsite (supraglottis, subglottis)	+/- impaired mobility
3	Limited to the larynx. Invasion of paraglottic space or inner cortex of thyroid cartilage.	+/- Fixation
4a	Outer cortex of thyroid cartilage and/or tissues beyond larynx (trachea, soft tissues of neck, thyroid, esophagus)	Any
4b	Inoperable disease - prevertebral space invasion, carotid artery encasement, mediastinal invasion	Any

Supraglottis

Clinical T Stage	Local Extent	Vocal Cord Function
1	Limited to one supraglottic subsite	Normal
2	More than one supraglottic subsite or glottis or adjacent extraglottic site	No fixation
3	Limited to the larynx. Invasion of pre-epiglottic space or postcricoid area or inner cortex of thyroid cartilage	+/- Fixation
4a	Outer cortex of thyroid cartilage and/or tissues beyond larynx (trachea, soft tissues of neck, thyroid, esophagus)	Any
4b	Inoperable disease - prevertebral space invasion, carotid artery encasement, mediastinal invasion	Any

Subglottis

Clinical T Stage	Local Extent	Vocal Cord Function
1	Limited to subglottis	Normal
2	Extends to vocal cords	+/- impaired mobility
3	Limited to the larynx. Invasion of inner cortex of thyroid cartilage	+/- Fixation
4a	Outer cortex of thyroid cartilage and/or tissues beyond larynx (trachea, soft tissues of neck, thyroid, esophagus)	Any
4b	Inoperable disease - prevertebral space invasion, carotid artery encasement, mediastinal invasion	Any

Figure 2. ***Nodal Staging***
Clinical and pathologic nodal staging, based on size, number, laterality and presence/absence of extranodal extension (ENE), according to AJCC 8th Edition.

Clinical Nodal Stage	Involvement
X	Not assessed
0	No clinically-positive nodes
1	Single ipsilateral positive node, ≤ 3 cm
2	Single positive node, >3 but ≤6 cm
2a	Single positive node, >3 but ≤6 cm
2b	Multiple ipsilateral nodes, >3 but ≤6 cm
2c	Contralateral or bilateral nodes, >3 but ≤6 cm
3	Any node >6 cm
3a	>6 cm, ENE(-)
3b	Any lymph node with clinical ENE (+)

Pathologic Nodal Stage	Involvement
X	Not assessed
0	No regional lymph node metastasis
1	Single ipsilateral positive node, ≤ 3 cm AND ENE(-)
2a	Single ipslateral positive node, >3 but ≤6 cm and ENE(-) OR ≤ 3 cm and ENE(+)
2b	Multiple ipsilateral nodes, ≤6 cm AND ENE(-)
2c	Contralateral or bilateral nodes, ≤6 cm AND ENE(-)
3a	>6 cm, ENE(-)
3b	Single ipsilateral node >3 cm AND ENE(+), OR multiple lymph nodes (any laterality) with ENE(+), OR single contralateral lymph node any size and ENE(+)

Figure 3. Stage groupings according to T, N, and M staging, according to AJCC 8th edition. For all subsites, T3-4 disease or any nodal involvement are considered locally advanced

Grouping	TNM Staging
Stage I	T1N0M0
Stage II	T2N0M0
Stage III	T3N0M0 T1-3N1
Stage IVA	T4aN0-1M0 T1-4aN2M0
Stage IVB	T4bN0-3M0 T1-4bN3M0

Figure 10: AJCC 8th edition staging for laryngeal carcinomas[35] (published in October 2016)

Cyclin D1 in head and neck squamous cell carcinoma

D type cyclins signifies a link between upstream mitotic stimuli and the regulation of pRB function.[36] Cyclins D 2 and D 3 have been identified in tissues. There is an average 57% identity among each of the three human D-type cyclin genes, and 78% identity in the cyclin box, which interacts with CDKs. Cyclin D1 is strongly associated with the progression of G1 phase. Overexpression of cyclin D1 expedites the G1 phase and reduces mitogen requirements. Several studies have shown that D-type cyclins, together with their catalytic partners CDK4 and CDK6, are essential for the passage through G1 phase and modulate the response to extracellular stimuli.[37]

Microinjecting cyclin D1 antibody prevents cells from entering the S phase. When cyclin D1 is overexpressed, the G1 phase is accelerated and mitogen requirements are reduced. Several studies have shown that D-type cyclins, along with their catalytic partners CDK4 and CDK6, are necessary for passage through the G1 phase of the cell cycle and regulate responses to extracellular stimuli.[38] The role of cyclin D1 as an oncogene was established by its ability to cooperate with RAS in cell transformation assays or to complement the defective adenoviral E1a oncogene.[39] Expression of cyclin D1 has been reported in various human tumours of oral, laryngeal, oesophageal and other sites, including breast cancer, mantle cell lymphoma and squamous cell carcinoma.

Xinsheng Lin et al[40] in 2019 in a study on expression of cyclin D1 in laryngo-carcinoma tissue hypothesised that activation of EGFR can activate Ras/Raf pathway which further induces activation of cyclin D1 leading to progression of cell into S phase and causing uncontrolled proliferation. Further study by Vahid Zand et al[41] showed overexpression of cyclin D1 with respect to the stage of disease. The expression of the marker is also significantly upregulated in cancerous tissue than in pre-cancerous ones.[42] Pablo Ramos et al[43] in a meta-analysis stated that alteration of pathway which is involved in carcinogenesis

leads to overexpression of cyclin D1 causing development of larger carcinomas with increased risk of lymph node involvement.

Her2neu in head and neck squamous cell carcinoma

The HER2 protein also known as ErbB2, is a transmembrane tyrosine kinase receptor. It is part of a family of 4 receptors (ErbB1-4) and influences the cancer etiology.[44] in 1985, It was discovered that HER2 is amplified in breast carcinoma.[45] It was the first molecule to become the focus of targeted therapy in solid tunours. Trastuzumab, was approve by FDA in 1998 for metastatic breast carcinoma.[46] The Her2 protein is encoded by Her proto-oncogene located on long arm 39 from chromosome 17. All four ErbB family members are involved in cancer progression by forming homodimers with other similar molecules or heterodimers with other ErbB family members. With exception of Her2, all other dimerization is ligand induced. However, HER2 is preferred dimerization partner for other members of ErbB family. The Her2 plays an important role because it is active due to its specific confirmation. Overexpression of Her2 makes it a preferred binding partner for other family members triggering signals that that affects cell proliferation, apoptosis, metastasis, and angiogenesis in breast cancer. Due to its unique property, it is a major driver of tumour growth and cancer cell survival.[47]

A study by Xia W et al[48] hypothesised that overexpression of HER2 neu enhances the metastatic potential of the tumour by causing multiple adhesion and invasion in metastatic cascade. A positive association was found between the expression and positive nodal status of the tumour. Yumna Adnan et al[49] studied expression of HER2 neu in oral squamous cell carcinoma and correlated it with overall and disease-free survival in 2022. In this study, 21% of the total tumour showed positivity for HER2 expression. A positive correlation was found between the expression of the marker and the habit of betel nut chewing. There was also

significant association for the expression of HER2 neu with overall survival and disease-free survival. Also, this study concluded that HER2 positive patients have shorter 10-year survival. Warren EAK et al[50] studied HER2neu expression in head and neck squamous cell carcinoma in 2021 establishing a positive correlation with the expression of primary tumour site and nodal status. Furthermore, this study also concluded a positive association between the expression of Her2 neu with decrease overall survival and poor disease-free survival.

MATERIALS AND METHODS

STUDY DESIGN: Cross sectional analytical study.

STUDY TOOL: Immunohistochemical staining for cyclin D1 and HER2 neu in histopathological diagnosed cases of head and neck squamous cell cancer.

STUDY SETTING: This study was performed in Department of Pathology, Sri Devaraj Urs Medical College which included patients of HNSCC.

SOURCE OF DATA: All resected cases of head and neck squamous cell carcinoma, received in the Department of Pathology Sri Devaraj Urs Medical College from October 2020 to October 2022 will be included in this study.

STUDY DURATION: October 2020 to October 2022 (2 years)

STUDY POPULATION: All histologically proven cases of HNSCC.

INCLUSION CRITERIA:

All histologically proven HNSCC.

EXCLUSION CRITERIA:

Secondary metastasis to head and neck.

Recurrent lesion.

Second primary cancers.

Patients on neoadjuvant chemotherapy.

SAMPLE SIZE:

Considering p as 76.25% of cases of cyclin D1 positive as reported in study by Sunil D.[51]

Calculation: $Z: \frac{1-\alpha 2p\,(1-p)}{d2}$

Here,

Z1-α: Standard normal variant

P: Expected proportion in population based on previous studies.

D: Absolute error of 10%.

Sample size required for cross sectional study will be 69 for head and neck cancer with 95%

METHOD OF COLLECTION: Samples were collected from October 2020 to October 2022. All resected cases of HNSCC confirmed by histopathological examination were included in the study. Data regarding the clinical details were collected. H&E stained slides were reviewed for histopathological types of the tumour. Immunostaining for cyclin D1 and HER2 neu will be performed on all cases of head and neck cancer using appropriate positive and negative controls.

Methodology:

All the clinicopathological data of head and neck squamous cell carcinoma cases such as age, sex, occupation, habits, site, histological grading, lymph node status, staging were collected. H & E Slides of all cases were reviewed, then tumour tissue was selected and immunohistochemistry was performed against cyclin D1 and HER2 (mouse monoclonal antibody, prediluted, Biogenex) neu for all cases of head and neck squamous cell carcinoma

by following the peroxidase and anti-peroxidase method. For all cases positive and negative controls were performed. For cyclin D1, tonsillar tissue was taken as positive control and breast tissue was taken as positive control for HER2 neu.

Immunohistochemistry:

- Sections were cut at a thickness of 3-4 μm, then they are floated on positive charged slides, which was incubated at 37 degrees for 1 day and further at 58 degrees overnight.
- The slides were not allowed to dry at any stage.
- Steps of incubation were carried with antibody at 37 degrees.
- Deparaffinization using xylene-I And Xylene –II using both for 15 minutes each.
- Dexylenisation using Absolute alcohol-I and Absolute Alcohol –II using each for 1 minute.
- Dealcoholisation for 1 minute.
- Distilled water 5 min-Washing.
- Antigen Retrieval using microwave at power 10 for 6 minutes in EDTA tris buffer at pH of 9 for 3 cycles. The slides were then rinsed with distilled water for 5 minutes followed by 10-15 minutes of peroxidase block using 3% hydrogen peroxide. This was further washed thrice with TBS buffer for 5 minutes.
- Counterstain with haematoxylin was done followed by tap wash for 5 minutes to drain out the excess stain. Dehydration and clearing were done with alcohol: xylene for 2 minutes.
- The slides were then mounted with DPX.

Immunohistochemistry evaluation:

The immune-stained sections were examined using light microscopy.

For scoring analysis of cyclin D1, each section was observed for 5 random fields, expression score and intensity score were calculated and they were further multiplied to give a total score. This was done with reference to a study conducted by Dhingra et al.[52]

Table 2: Expression score criteria for cyclin D1[52]

1	1-25%
2	26-50%
3	51-75%
4	>76%

Table 3: Intensity score criteria for cyclin D1[52]

1	Mild
2	Moderate
3	strong

Table 4: Total score criteria for cyclin D1[52]

1-4	Mild
5-8	Moderate
9-12	strong

For intensity scoring of HER2 neu 10 fields were chosen, intensity of score was calculated and mean was taken out. IHC was scored according to the American Society of Clinical Oncologists/College of American Pathologists (ASCO/CAP) guidelines for Her2neu testing in breast cancer.

Table 5: Scoring criteria for HER2neu[53]

0:	No membrane staining or staining in <10% cells**.**
1	Weak and incomplete staining in >10% cells
2	Complete but moderate staining of >10% cells
3	Complete and intense membrane staining of >10%

Statistical analysis:

Data was entered into Microsoft excel data sheet and was analysed using SPSS 22 version software. Categorical data was represented in the form of Frequencies and proportions. Chi-square test or Fischer's exact test (for 2x2 tables only) was used as test of significance for qualitative data.

Continuous data was represented as mean and standard deviation.

Graphical representation of data: MS Excel and MS word was used to obtain various types of graphs.

Survival analysis done with Kaplan Meier method and log rank statistics were used to make comparisons between groups. Uni variable survival analyse were carried out using Cox proportional Hazards Model.

P value (Probability that the result is true) of <0.05 was considered as statistically significant after assuming all the rules of statistical tests.

Statistical software: MS Excel, SPSS version 22 (IBM SPSS Statistics, Somers NY, USA) was used to analyse data.

RESULTS

Table 6: Distribution of cases according to age group.

Age of the patients	Frequency	Percentage
<40yrs	7	10.0
41-50yrs	26	37.1
51-60yrs	21	30.0
>60yrs	16	22.9
Total	70	100.0

In our study, maximum cases were from age group 41-50 years constituting total 26/70 (37.1%) cases. This was followed by age group 51-60 years comprising of total 21/70 (30%) of the cases. The minimum number of cases were seen in age group less than 40 years which comprised of only 7 (10%) cases.

Chart 1: Pie chart showing distribution of subjects according to age group

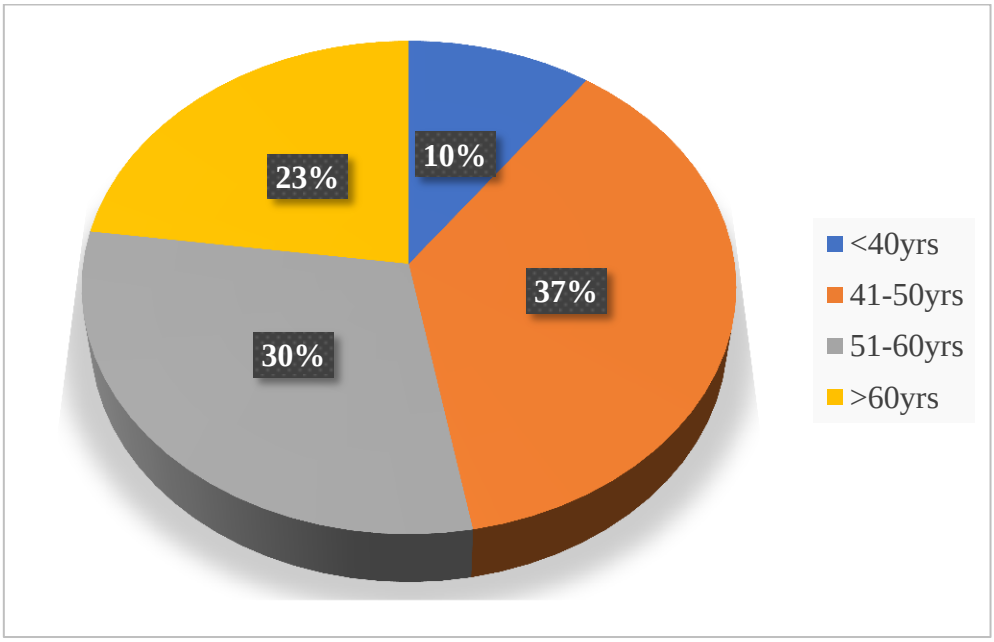


Table 7: Distribution of patients according to their sex.

Sex	Frequency	Percentage
Female	53	75.7
Male	17	24.3
Total	70	100.0

The predominant study population in our study were females which constituted a total of 53/70 (75.7%) cases.

Chart 2: Pie chart showing distribution of patients according to sex

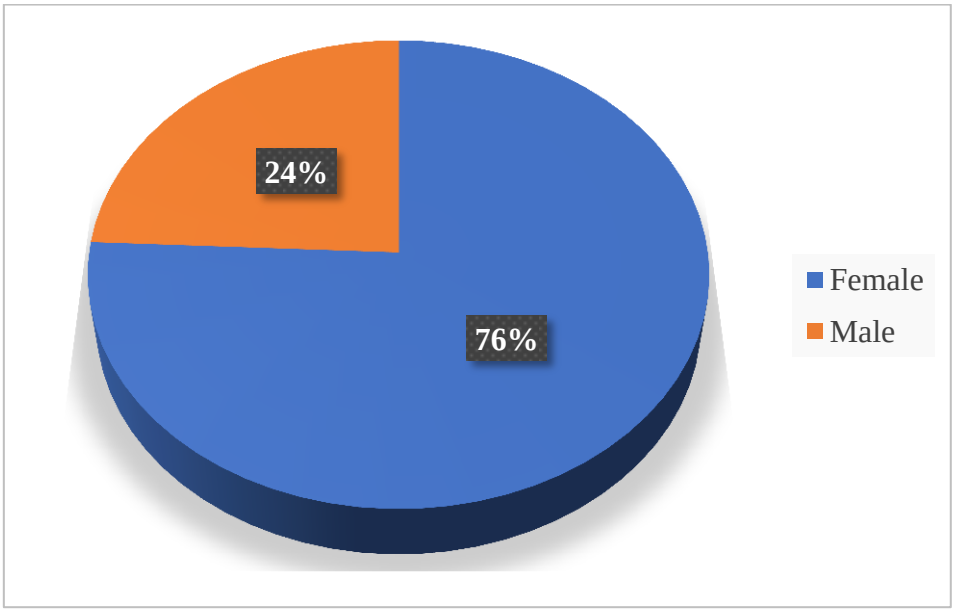


Table 8: Distribution of patients according to occupation.

Occupation	Frequency	Percentage
Business	10	14.3
Farmer	8	11.4
Homemaker	36	51.4
Labour	9	12.9
Service	7	10.0

In our study, the largest group of cases were of homemaker by occupation 36/70 (51.4%) followed by business 10/70 (14.3%) and labour 9/70 (12.9%).

Chart 3: Pie chart showing Distribution of patients according to occupation.

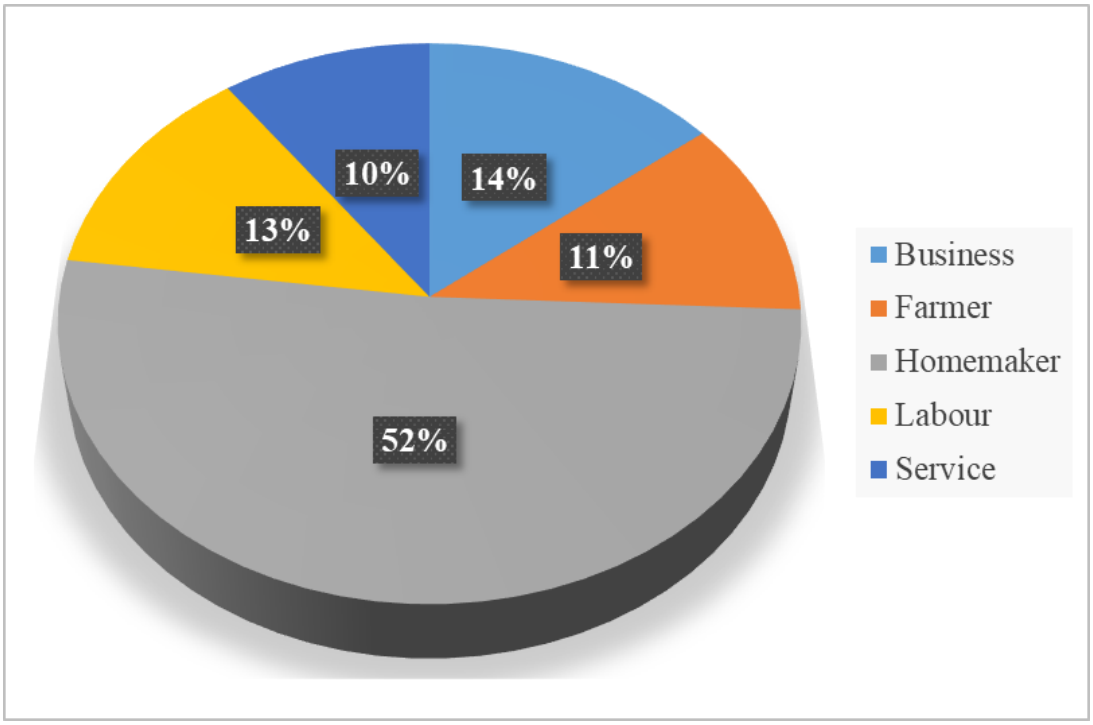


Table 9: Distribution of cases according to habits

Habits	Frequency	Percentage
No habits	29	41.4
Alcohol	8	11.4
Alcohol + smoking	3	4.3
oral tobacco	21	30.0
Smoking	9	12.9

The history of addictive habits of patients were taken and based on that majority of study population had no habits i.e., 29/70 (41.4%) followed by habit of chewing oral tobacco that was seen in 21/70 (30%) patients.

Chart 4: Bar diagram showing distribution of cases according to habits

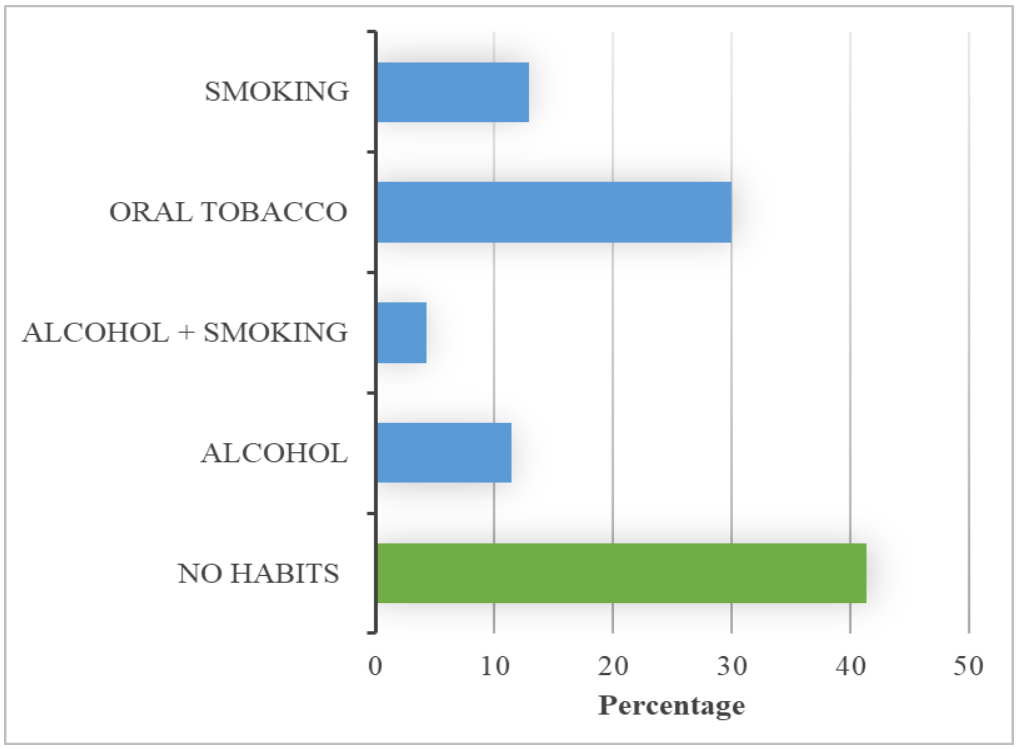


Table 10: Distribution of patients according to site of the lesion

Site	Frequency	Percentage
Alveolus	8	11.4
BM & GBS	3	4.3
Buccal mucosa	27	38.6
GBS	17	24.3
LARYNX	2	2.9
RMT	2	2.9
Tongue	11	15.7

In present study, maximum cases were from buccal mucosa 27/70 (38.6%) followed by gingiva buccal sulcus 17/70 (24.3%), tongue 11/70 (15.7%) and alveolus 8/70 (11.4%). The least number of cases were seen in larynx and retromolar triangle with 2/70 (2.9%) cases each.

Chart 5: Bar diagram showing Division of patients according to site of the lesion

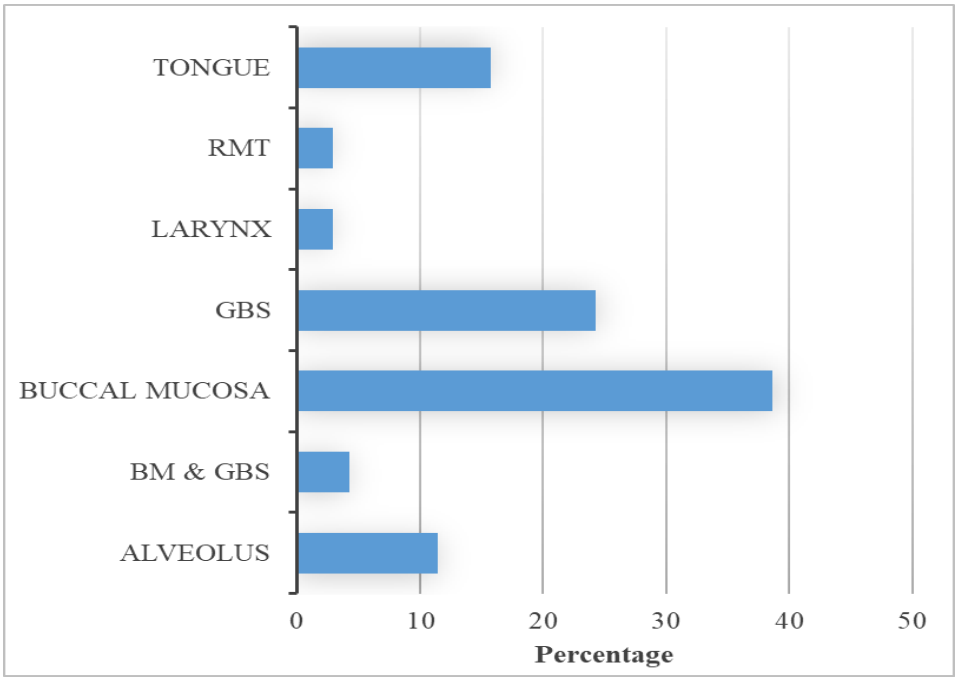


Table 11: Distribution of patients according to side of the lesion

Side	Frequency	Percentage
Left	40	57.1
Right	27	38.6

Left side was the predominant side with 40/70 (57.1%) cases. Right side constituted only 27/70 (38.6%) of cases.

Chart 6: Pie chart showing distribution of patients according to side of the lesion

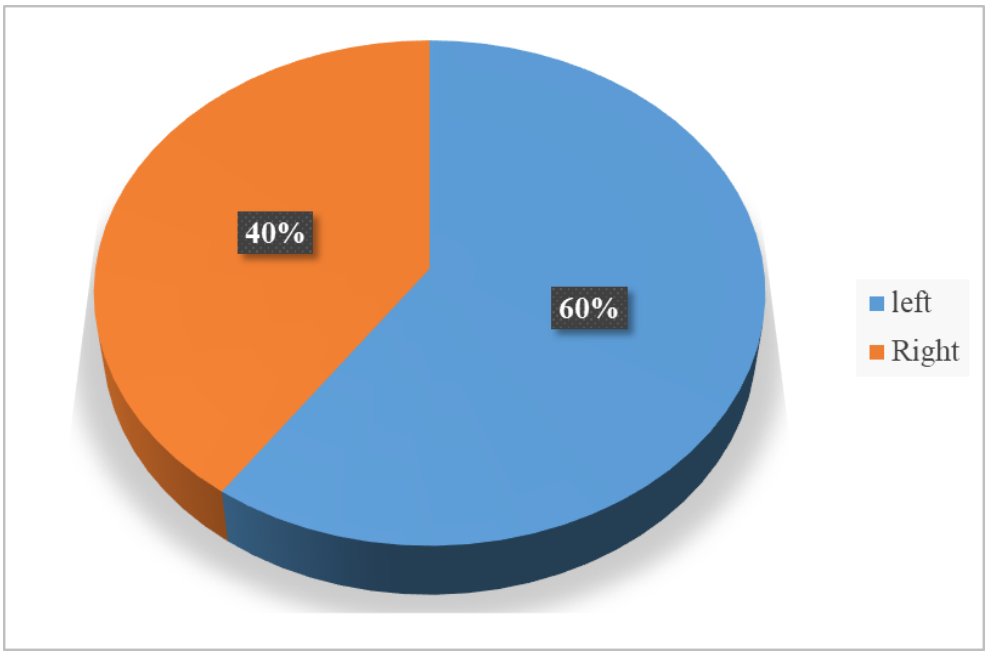


Table 12: Distribution of patients according to histological grade of the tumour

Histological grade	Frequency	Percentage
MDSCC	12	17.1
WDSCC	58	82.9
Total	70	100.0

Well differentiates squamous cell carcinoma constituted 58/70 (82.9%) cases and were the predominant histological grade. Only 12/70 (17.1%) of the cases were MDSCC.

Chart 7: Pie chart showing distribution of patients according to histological grade of the tumour

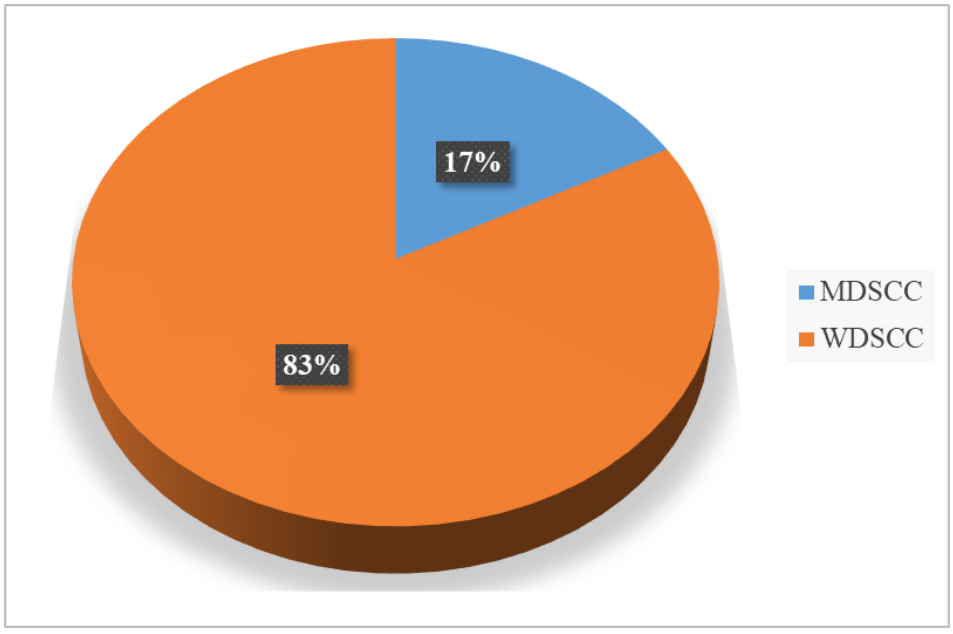


Table 13: Distribution of patients according to depth of invasion of the tumour

DOI (mm)	Frequency	Percentage
<20	42	60.0
>20	28	40.0
Total	70	100.0

Majority of the tumour 42/70 (60%) had depth of invasion less(DOI) than 20 mm and total 28/70 (40%) tumour had DOI more than 20 mm.

Chart 8: Pie chart showing distribution of subjects according to depth of invasion of the tumour

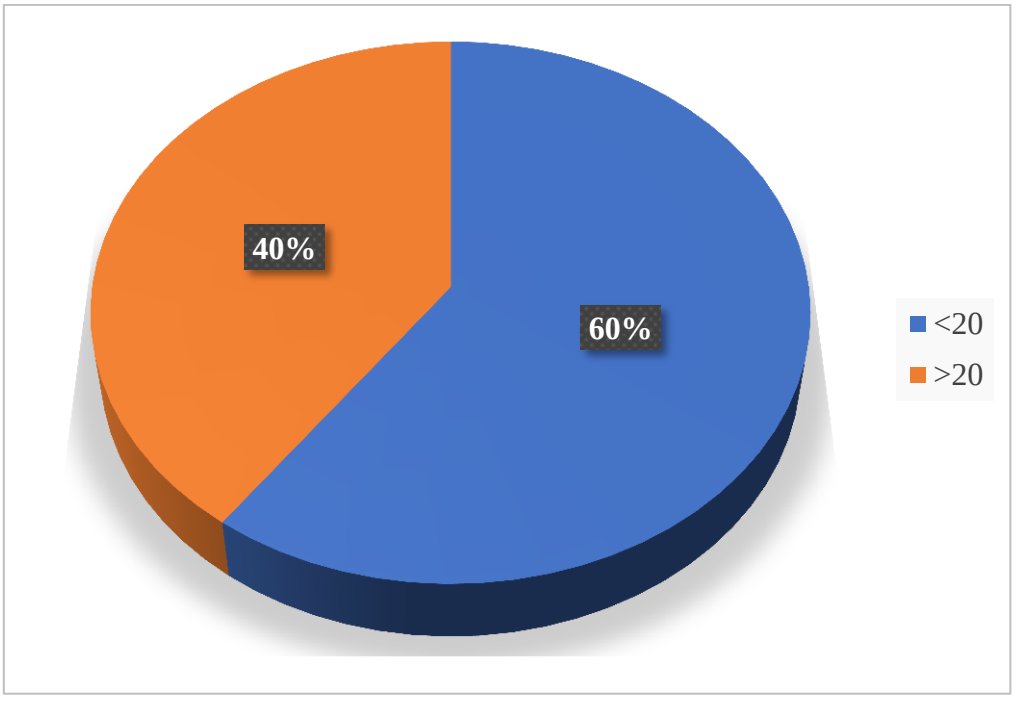


Table 14: Distribution of patients according to primary tumour stage (based on AJCC 8th edition)

Primary tumor stage	Frequency	Percentage
T1	4	5.7
T2	14	20.0
T3	23	32.9
T4	29	41.4

29/70 (41.4%) cases were of T4 grade followed by 23/70 (32.9%) cases of T3. The least cases were of T1 4/70 (5.7%).

Chart 9: Pie chart showing distribution of patients according to primary tumor stage (based on AJCC 8th edition)

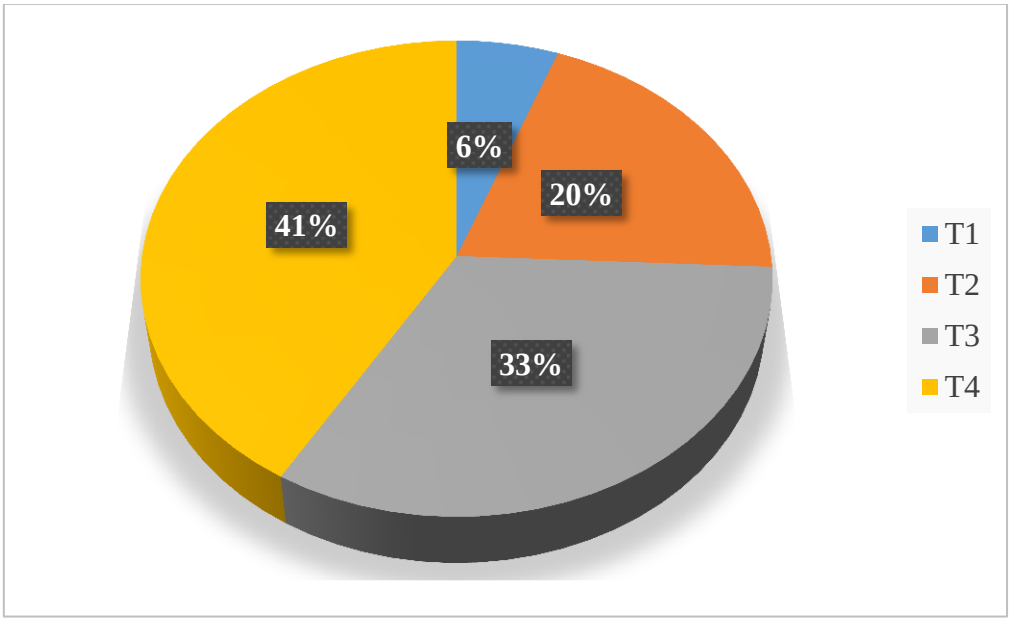


Table 15: Distribution of patients according to lymph node status.

Lymph node status	Frequency	Percentage
Negative	30	42.9
Positive	38	54.3

Based on the metastases to lymph node, majority of the cases 38/70 (54.3%) showed positive involvement of one or more nodes. 30/70 (42.9%) cases were negative for any nodal metastases.

Chart 10: Pie chart showing distribution of patients according to lymph node status.

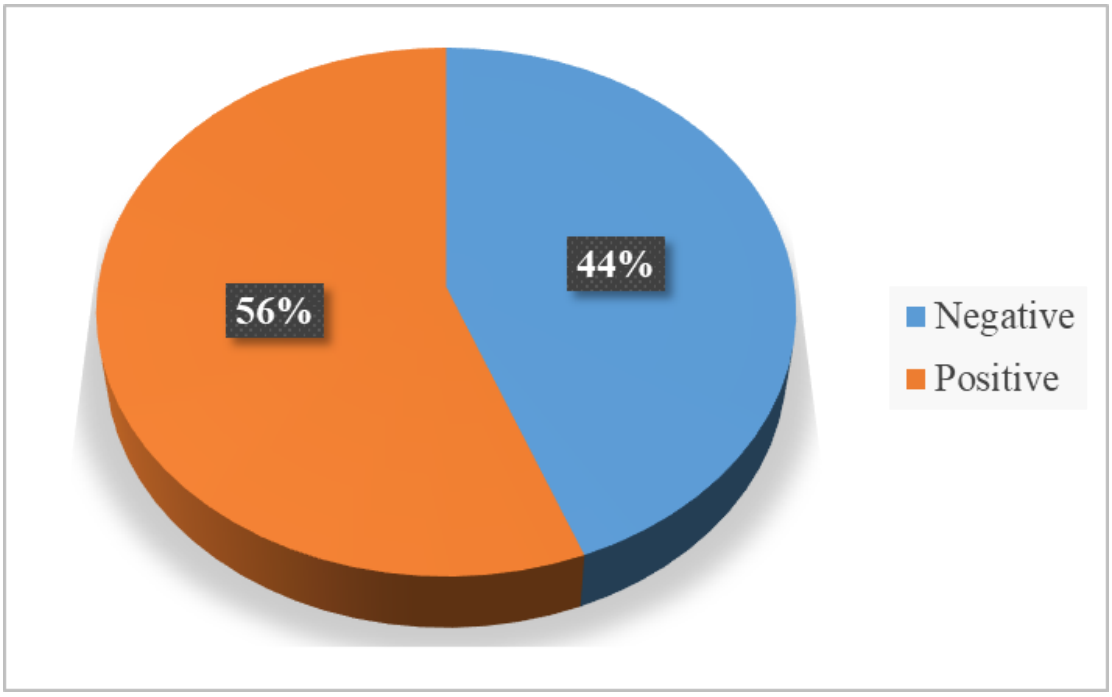


Table 16: Distribution of patients according to bone metastasis.

Status of bone metastases	Frequency	Percentage
Absent	60	86.1
Present	9	12.9

With regards to bone metastases, only 9/70 (12.9%) cases were positive. 60/70 (86.1%) cases were negative for any bone involvement.

Chart 11: Pie chart showing distribution of patients according to bone metastasis.

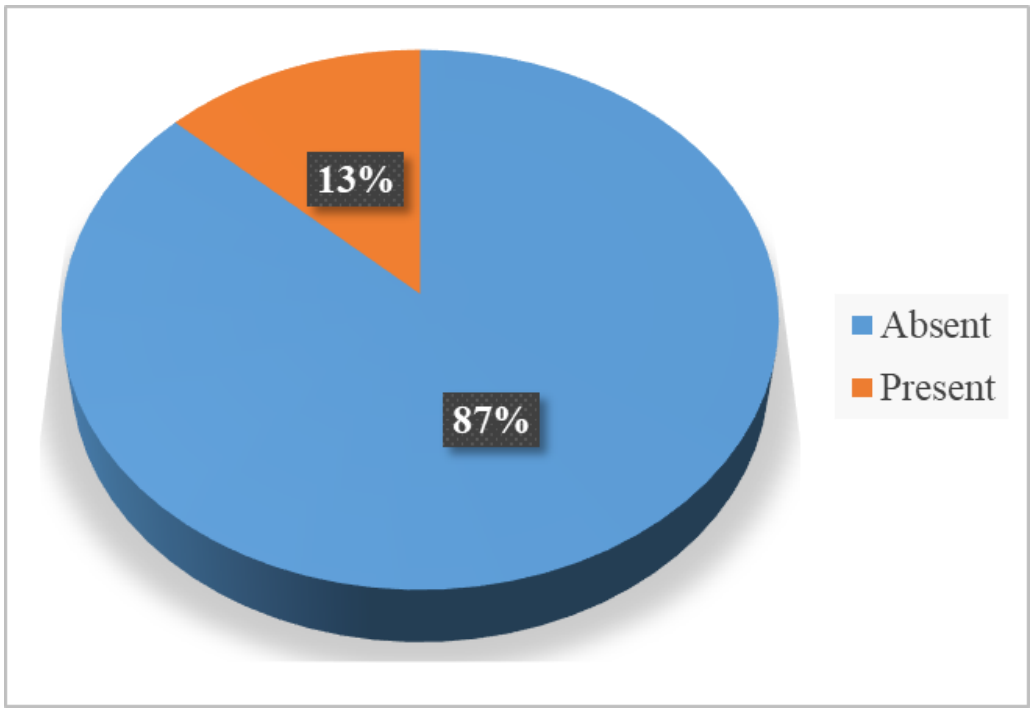


Table 17: Distribution of patients according to pathological TNM stage of the tumour. (Based on AJCC 8th edition)

Stage	Frequency	Percentage
I	1	1.4
II	5	7.1
III	21	30.0
IV	43	61.4

43/70 (61.4%) cases belonged to stage IV carcinoma, 21/70 (30%) to stage III. Stage II and stage I had only 5/70 (7.1%) and 1/70 (1.4%) cases respectively.

Chart 12: Pie chart showing distribution of patients according to pathological TNM stage of the tumour. (Based on AJCC 8th edition)

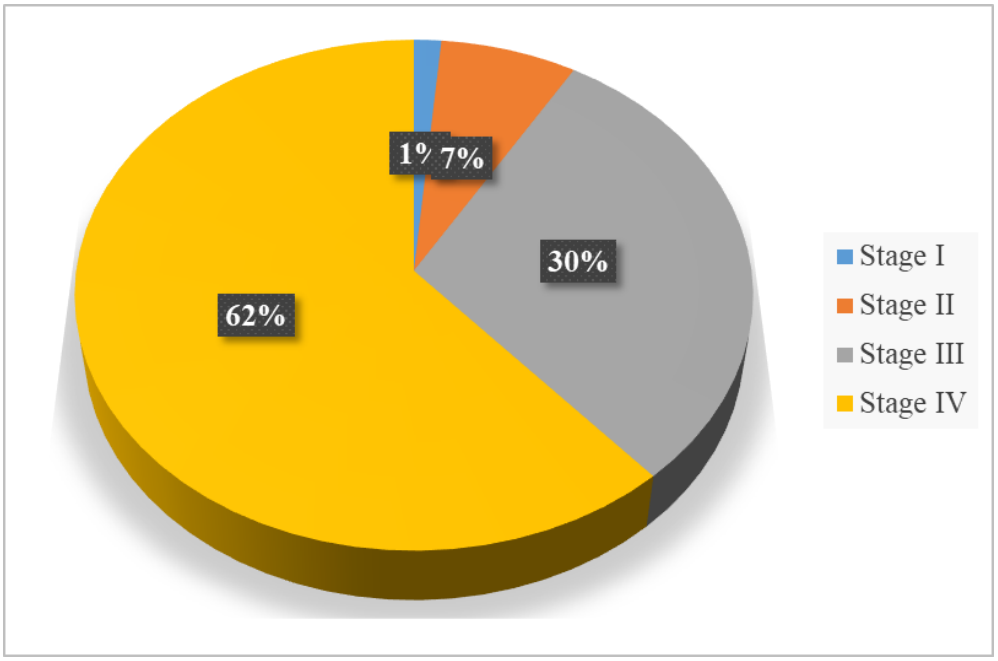


Table 18: Distribution of subjects according to Cyclin D1 expression.

Grading of cyclin D1 score	Frequency	Percentage
Strong	38	54.3
Moderate	16	22.9
Weak	16	22.9
Total	70	100.0

The total score for cyclin D1 was categorised as weak, moderate and strong. Majority 38/70 (54.3%) of the cases showed strong positivity for cyclin D1 that is showing expression in more than 50 % of cells with strong intensity. 16/70 (22.9%) of cases each showed moderate and weak score of cyclin D1 positivity.

Chart 13: Pie chart showing distribution of subjects according to Cyclin D1 expression.

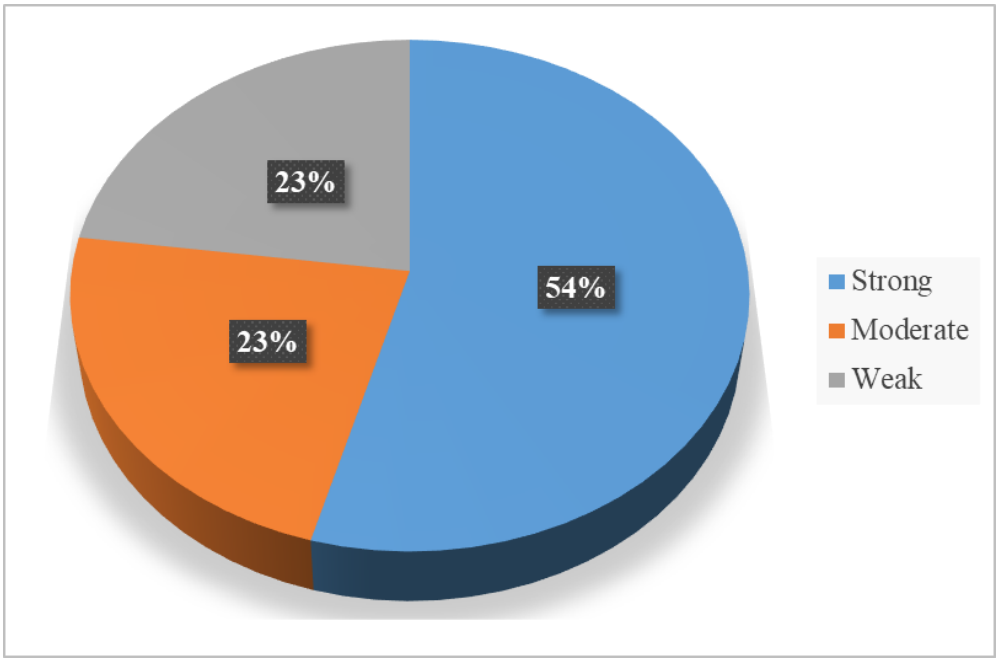


Table 19: Distribution of subjects according to histological grade of tumour and cyclin D1 expression

	Weak		moderate		strong	
Grade	**N**	**percentage**	**N**	**percentage**	**N**	**percentage**
WDSCC	13	22.4	13	22.4	32	55.17
MDSCC	3	25	3	25	6	50

32/58 (55.17%) cases of well differentiated squamous cell carcinoma showed strong positivity for cyclin D1 immuno-expression followed by 13/58 (22.4%) each WDSCC showing weak and moderate immuno-expression. 6/12 (50%) cases MDSCC showed strong immune-expression for cyclin D1. The p value for association of cyclin D1 with the grade of the tumour was 0.157 which was statistically insignificant and there was no association found between the grade of the tumour and the expression of the marker.

Chart 14: Bar diagram showing distribution of subjects according to histological grade of tumour and cyclin D1 expression

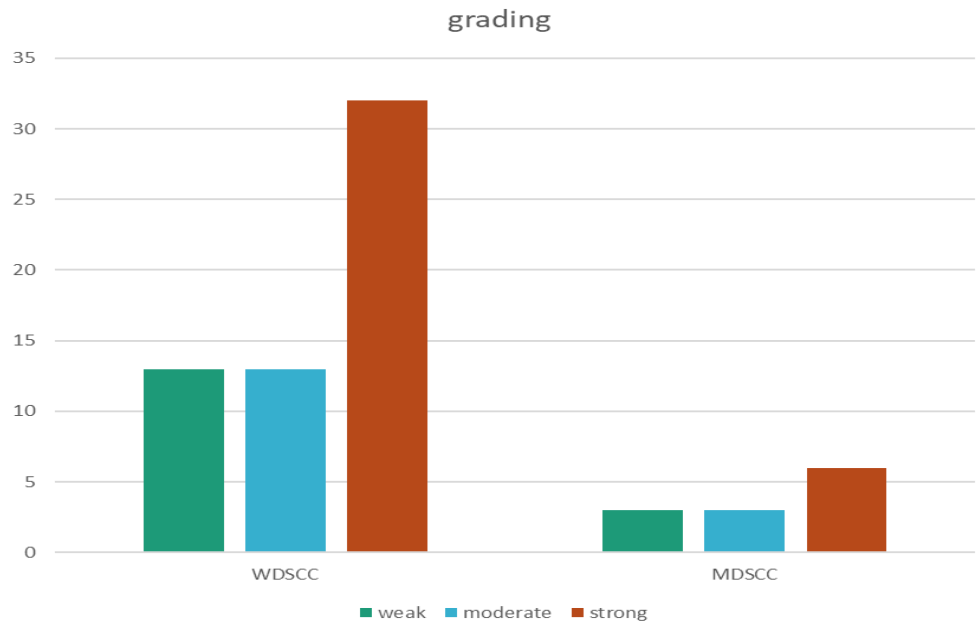


Table 20: Distribution of subjects according to depth of invasion and cyclin D1 expression

DOI (mm)	Weak		Moderate		Strong	
	N	%	N	%	N	%
<20	13	31.0%	12	28.6%	17	40.5%
>20	3	10.7%	4	14.3%	21	75.0%

Maximum cases 21/27 (75%) showing strong positivity for cyclin D1 with depth of invasion more than 20 mm. Among DOI less than 20mm group, only 17/42 (40.5%) tumours showed strong positivity for cyclin D1. The p value was 0.017 which was statistically significant.

Chart 15: Bar diagram showing distribution of subjects according to depth of invasion and cyclin D1 expression.

Table 21: Distribution of subjects according to primary tumour stage and cyclin D1 expression. (Based on AJCC 8th edition)

Primary tumour stage	Weak		Moderate		Strong	
	N	%	N	%	N	%
T1	2	50.0%	1	25.0%	1	25.0%
T2	6	42.9%	4	28.6%	4	28.6%
T3	4	17.4%	6	26.1%	13	56.5%
T4	4	13.8%	5	17.2%	20	69.0%

T4 tumours showed maximum number of strong immuno-expression for cyclin D1 with total 20/29 (69 %). Among T3 tumour stage, 13/23 showed strong expression for cyclin D1. 6/14 (42.9%) of T2 tumours showed weak immuno-expression followed by only 1 / 4 (25%) tumours of T1 category showed strong immuno-expression for cyclin D1. P value was 0.157, and there was no statistical significance difference found between primary tumour stage and expression of cyclin D1.

Chart 16: Bar diagram showing distribution of subjects according to primary tumour stage and expression of cyclin D1. (Based on AJCC 8th edition)

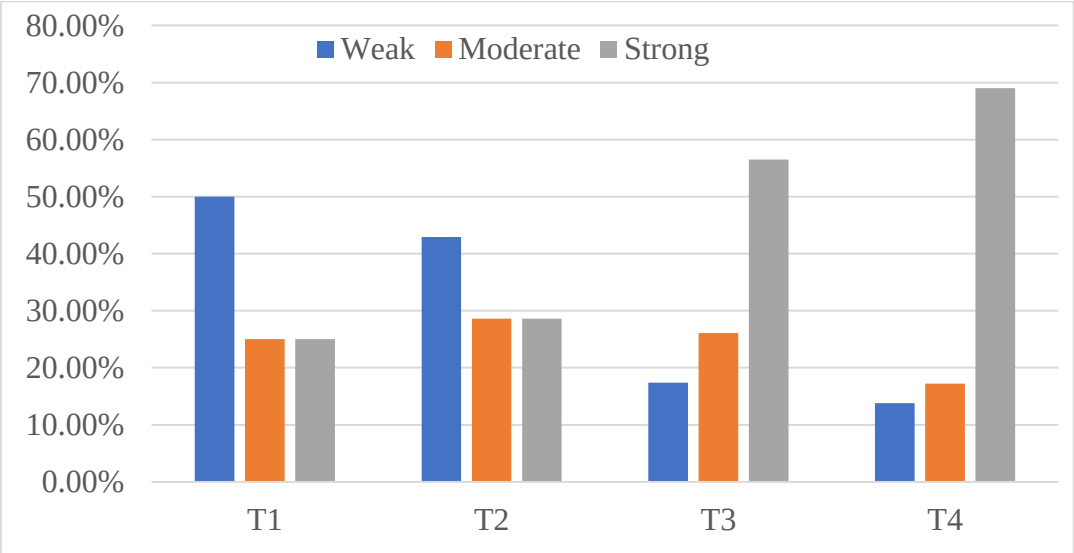


Table 22: Distribution of subjects according to stage of tumor and cyclin D1 expression. (Based on AJCC 8th edition)

TNM Stage	Weak		Moderate		Strong	
	N	%	N	%	N	%
stage I	1	100.0%	0	.0%	0	.0%
stage II	4	80.0%	1	20.0%	0	.0%
stage III	7	33.3%	7	33.3%	7	33.3%
stage IV	4	9.3%	8	18.6%	31	72.1%

Only 1 case was in stage 1 group which showed weak immune-expression for cyclin D1. 4/5 (80%) cases of stage 2 showed weak immune-expression followed by 7/21 (33.3%) cases each of stage 3 showing weak, moderate and strong immune-expression for cyclin D1. For stage 4 carcinoma, 31/43 (72.1%) cases were strongly positive for cyclin D1. P value was 0.001 and a statistical significance association was found between stage of tumour and cyclin D1 immuno-expression.

Chart 17: Bar diagram showing distribution of subjects according to grade of tumor and cyclin D1. (Based on AJCC 8th edition)

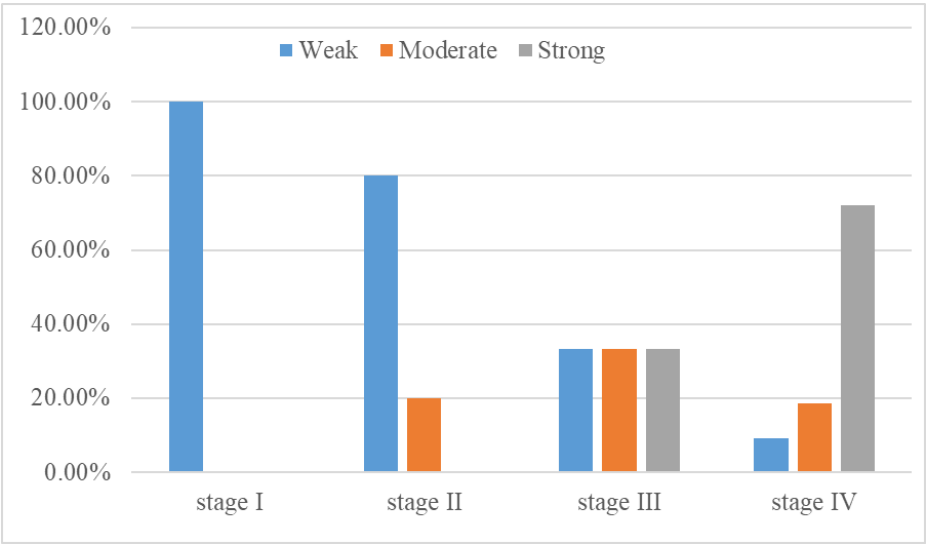


Table 23: Distribution of subjects according to Lymph node metastases status and cyclin D1 expression

Lymph node status	Weak		Moderate		Strong	
	N	%	N	%	N	%
Negative	10	33.3%	8	26.7%	12	40.0%
Positive	4	10.5%	8	21.1%	26	68.4%

12/30 (40%) cases of negative lymph node status showed strong immune-expression for cyclin D1 followed by 8/30(26.7%) and 10/30 (33.3%) cases showing moderate and weak expression respectively. 26/38 (68.6%) cases showed strong immune-expression for cyclin D1. P value was 0.032. There was a statistical significance found between positive lymph-node metastases and cyclin D1 expression.

Chart 18: Bar diagram showing distribution of subjects according to Lymph node status and cyclin D1

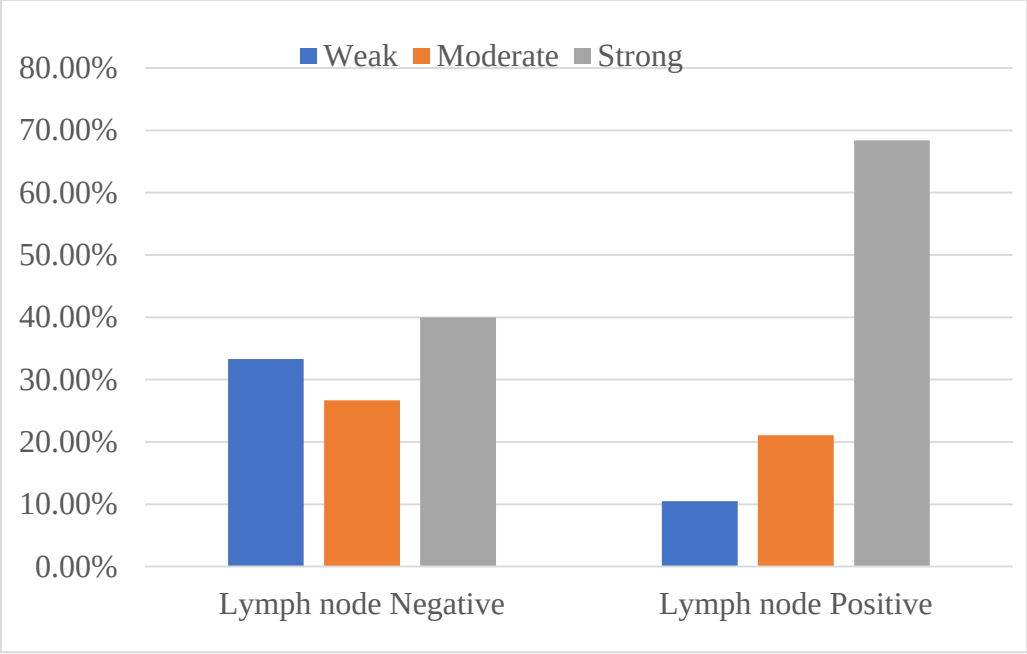


Table 24: Distribution of subjects according to Her 2 Neu expression.

Her2 neu expression	Frequency	Percent
Negative	65	92.9
Positive	5	7.1
Total	70	100.0

Only 5/70 cases were positive for her2 expression showing equivocal positivity. Majority of the tumour 65/70 (92.9%) were negative for HER2 neu expression.

Chart 19: Pie chart showing distribution of subjects according to Her 2 Neu.

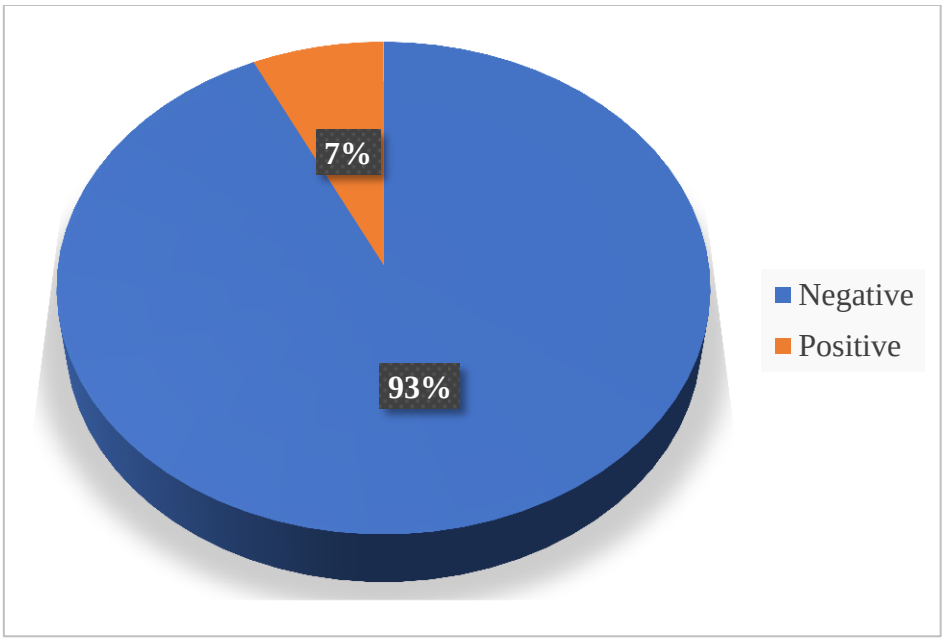


Table 25: Distribution of subjects according to DOI and Her 2 neu expression.

Depth of invasion (mm)	Negative		Positive	
	N	%	N	%
<20	42	100.0%	0	.0%
>20	23	82.1%	5	17.9%

All 5 tumors which were positive for HER2 neu expression were of more than 20 mm depth of invasion. 42/42 (100%) of the tumors less than 20 mm DOI were negative for HER2neu immune-expression. P value was 0.008, and was a significant association found DOI and Her 2 neu expression.

Chart 20: Bar diagram showing distribution of subjects according to depth of invasion and Her 2 neu expression.

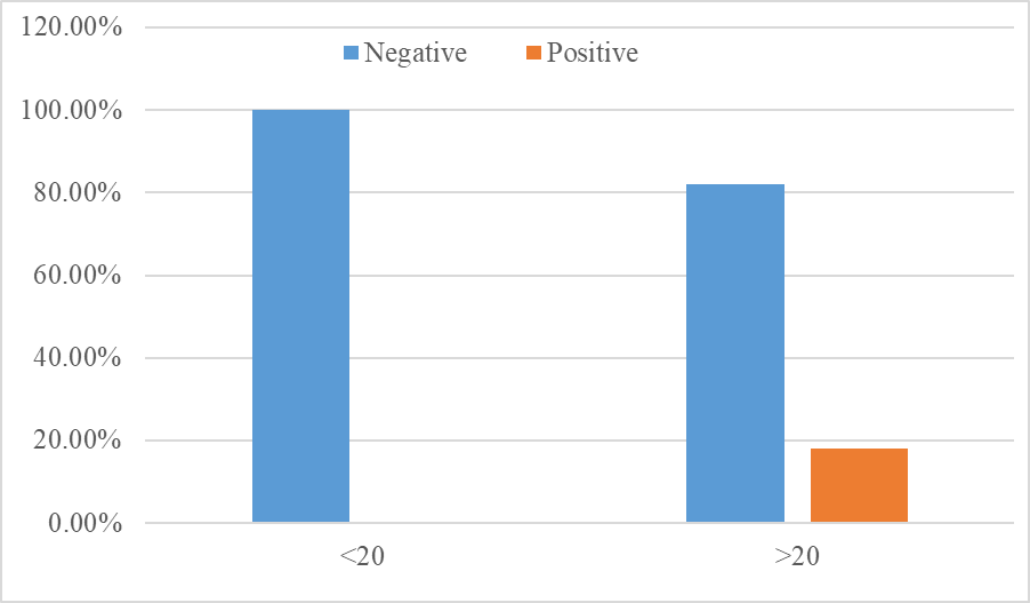


Table 26: Distribution of subjects according to histological grade and HER2neu expression

Grade	Positive		Negative	
	N	%	N	%
WDSCC	5	8.62	53	91.37
MDSCC	0	100	12	100

Among well differentiated squamous cell carcinoma group, 5/58 (8.62%) were positive for HER2 neu immuno-expression and the other 53/58 (91.37%) were negative for the HER2 neu. 12/12 (100%) cases of moderately differentiated squamous cell carcinoma were negative for HER2 neu. The p value was 0.932 which was statistically insignificant and no association was established between grade of the tumour and HER2 neu expression.

Chart 21: Bar diagram showing distribution of subjects according to histological grade and HER2neu expression.

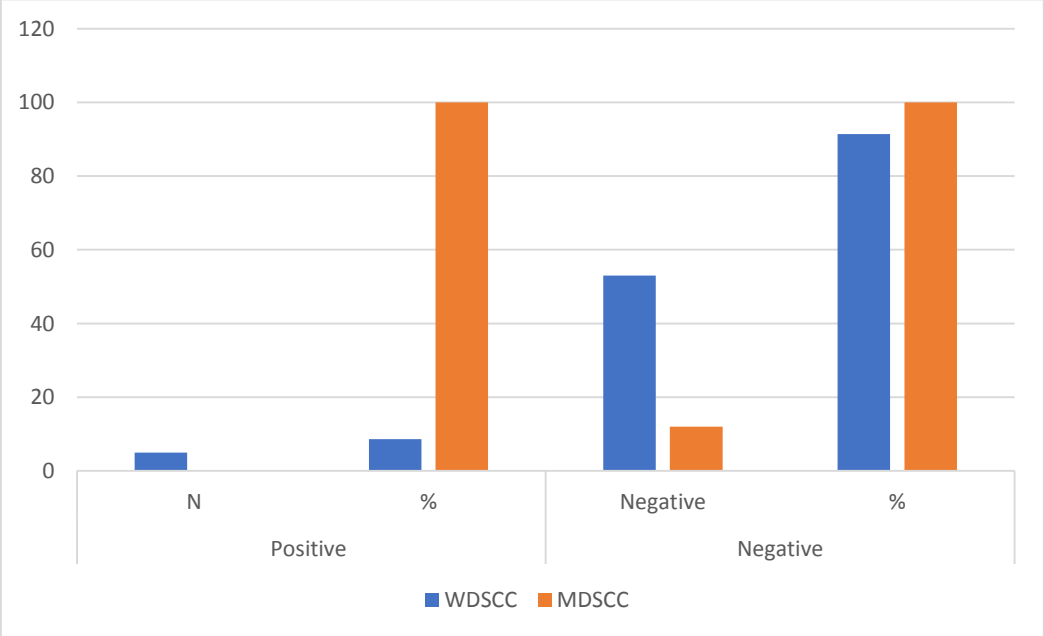


Table 27: Distribution of subjects according to Primary tumour stage and Her 2 neu expression. (Based on AJCC 8th edition)

Primary tumour stage	Negative		Positive	
	N	%	N	%
T1	4	100.0%	0	.0%
T2	14	100.0%	0	.0%
T3	22	95.7%	1	4.3%
T4	25	86.2%	4	13.8%

All T1 4/4 (100%) and T2 14/14 (100%) were negative for HER2 neu expression. Among T3 tumours only 1/23 (4.3%) was positive for HER2 neu expression. 4/29 (13.8%) of T4 tumours were positive for immuno-expression and 25/29 (86.2%) were negative for HER2 neu immune-expression. The p value was 0.309. There was no statistically significant association found between Primary tumour grade and Her 2 neu expression.

Chart 22: Bar diagram showing distribution of subjects according to grade of tumor and Her 2 neu expression. (Based on AJCC 8th edition)

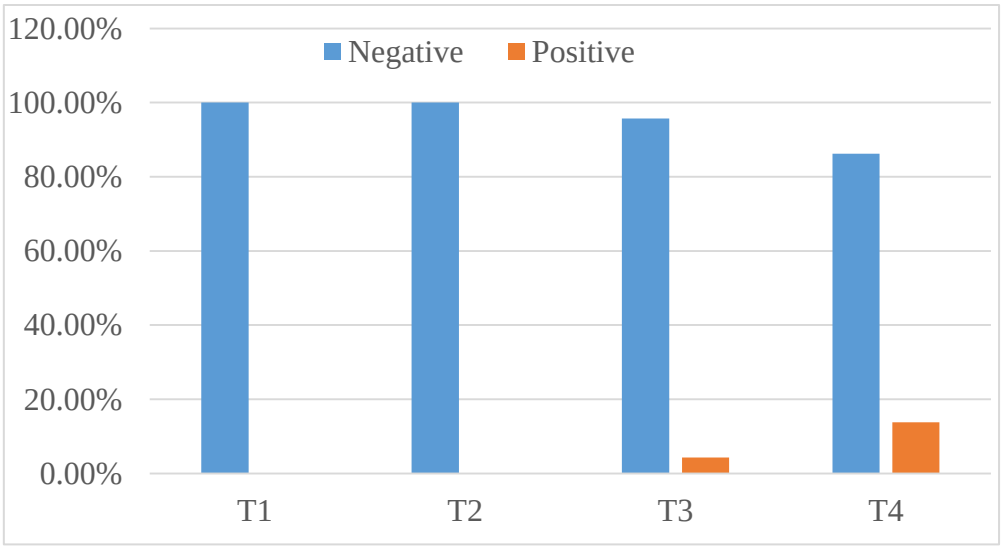


Table 28: Distribution of subjects according to TNM Stage of tumor and Her 2 neu expression. (Based on AJCC 8th edition)

TNM stage	Negative		Positive	
	N	%	N	%
stage I	1	100.0%	0	.0%
stage II	5	100.0%	0	.0%
stage III	20	95.2%	1	4.8%
stage IV	39	90.7%	4	9.3%

4/43 (9.3%) of stage IV tumour were positive for HER2 neu expression and 39/43 (9.07%) were negative for the same. When seen in stage III group, only 1/21 (4.8%) were positive and 20/21 (95.2%) were negative for HER2 neu expression. 5/5 (100%) and 1/1(100%) tumours from stage II and stage I respectively were negative for HER2 neu expression. P value was 0.819 and there was no statistically significant association found between Stage of tumor and Her 2 neu expression.

Chart 23- Bar diagram showing distribution of subjects according to Stage of tumor and Her 2 neu expression. (Based on AJCC 8th edition)

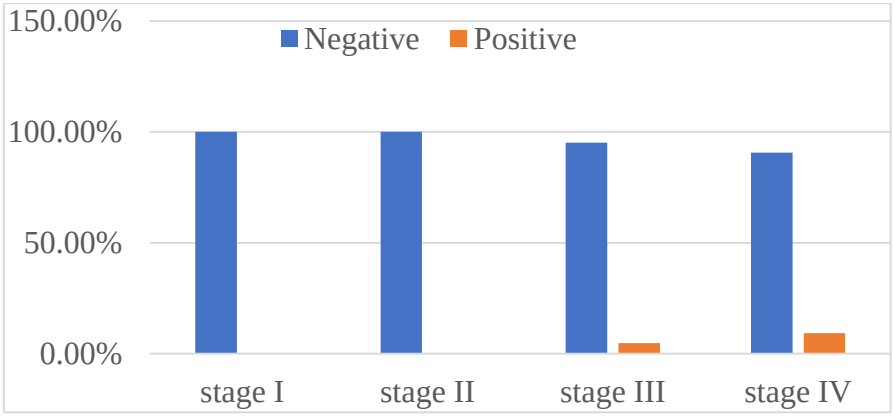


Table 29: Distribution of subjects according to Lymph node status and Her 2 neu

Lymph node metastases	Negative		Positive	
	N	%	N	%
Negative	28	93.3%	2	6.7%
Positive	35	92.1%	3	7.9%

2/30 (6.7%) of cases were from lymph node negative group were positive for HER2neu expression and 3/38 (7.9%) cases were positive from lymph node positive group. The p value 1.00, and no statistically significant difference was found between lymph node metastases and Her 2 neu expression.

Chart 24: Bar diagram showing distribution of subjects according to Lymph node status and Her 2 neu expression.

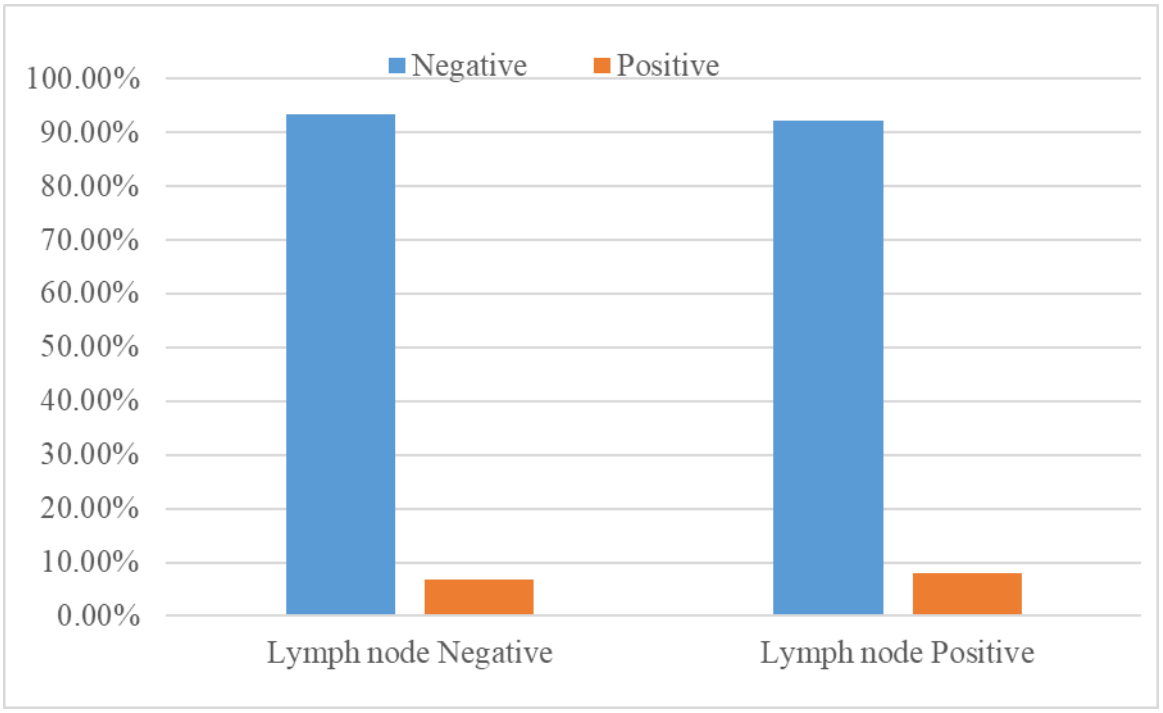


Table30: Overall Survival with histopathological parameters.

		Hazard ratio	95% CI	P Value
DEPTH OF INVASION (mm)	<20	0.874	0.327-2.336	0.789
	>20			
LYMPH NODE STATUS	Negative	6.853	1.56-30.100	**0.011**
	Positive			
PRIMARY TUMOUR	T1-T2	1.446	0.406-5.152	0.569
	T3-T4			
HISTOLOGICAL GRADE	WDSCC	0.431	0.097-1.914	0.268
	MDSCC			

Hazard ratio was 0.874 for increase in depth of tumour with overall survival of the patient. The p value for increase in depth of invasion and overall survival was 0.789 which was statistically insignificant.

With respect to lymph node status, the hazard ratio was 6.853 which indicated a poor overall survival with presence of positive lymph node metastases. The p value for the same was 0.011. which was statistically significant and hence presence of lymph node metastases in patients with HNSCC is associated with poor overall survival.

For primary tumour grade, the study population was divided into two groups, i.e., patients with stage I and II were put in one group and patients with stage III and IV in another group. The hazard ratio was 1.446 with a p value of 0.569 which was statistically insignificant.

Hazard ratio for histological grade was 0.431 with p value 0.268 which was statistically insignificant, stating that no association is found between histological grade and overall survival of the patients.

Table 31: Disease Free Survival for histopathological parameters.

		Hazard ratio	95% CI	P Value
DEPTH OF INVASION (mm)	<20	0.578	0.225-1.48	0.255
	>20			
LN STATUS	Negative	9.03	2.03-40.11	**0.004**
	Positive			
PRIMARY TUMOUR	T1-T2	0.952	0.320-2.82	0.929
	T3-T4			
HISTOLOGICAL GRADE	WDSCC	0.390	0.088-1.73	0.390
	MDSCC			
TNM STAGE	<IV	6.17	1.32-28.78	**0.020**
	≥IV			

The hazard ratio for increase in depth of invasion with disease free survival was 0.578 and p value for the same was 0.255 which was statistically insignificant.

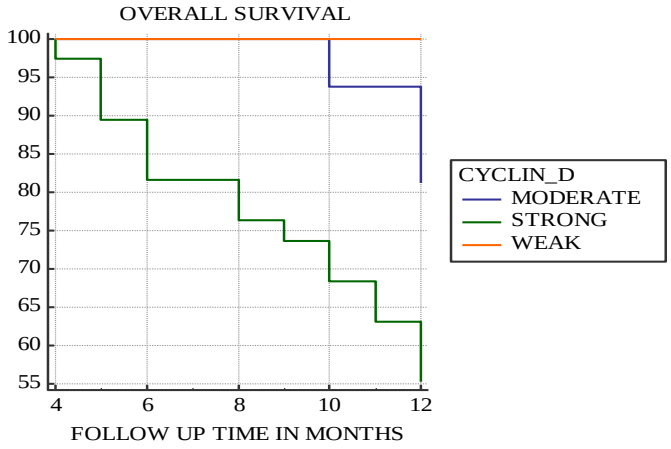
For presence of lymph node metastases, it was observed that with presence of lymph node metastases, the disease-free survival decreases. The hazard ratio was 9.03 and p value was 0.004 which established a significant association between presence of metastatic lymph node deposits and poor disease-free survival.

For primary tumour stage, the study population was divided into two groups, one group consisting of patients with T1 and T2 and another of patients with T3 and T4. The hazard ratio was 0.952 with a p value of 0.929 indicating that tumour stage is not related to disease free survival of the patients.

With respect to histological grade, the hazard ratio was 0.390 and p value was 0.390. There was no statistically significant relationship found between histological grade and disease-free survival of the patients.

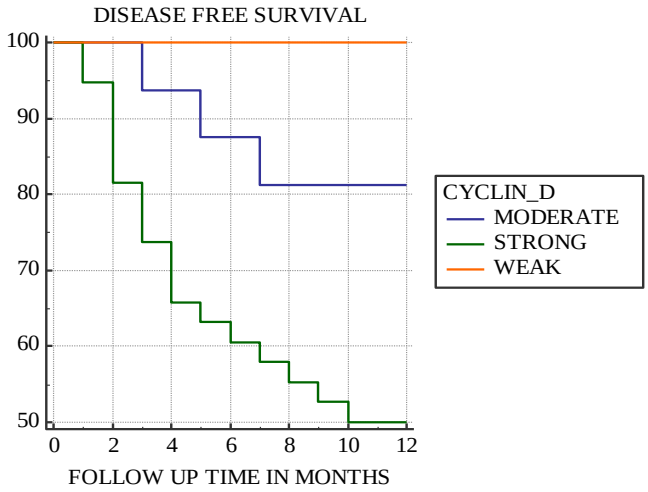
The study population was divided into two groups to find the association between disease free survival and stage of the disease. One group comprised of patients with stage I, II, III and another of patients with stage IV. The hazard ratio was 6.17 which indicated that the presence of more than twice the risk of poor disease-free survival in patients with stage IV carcinoma. The p value for the same was 0.020 which is statistically significant and hence a positive association was found between poor disease-free survival and increase in stage of the carcinoma.

Chart 25: Kaplan meier curve for overall survival of the patients with cyclin D1 expression



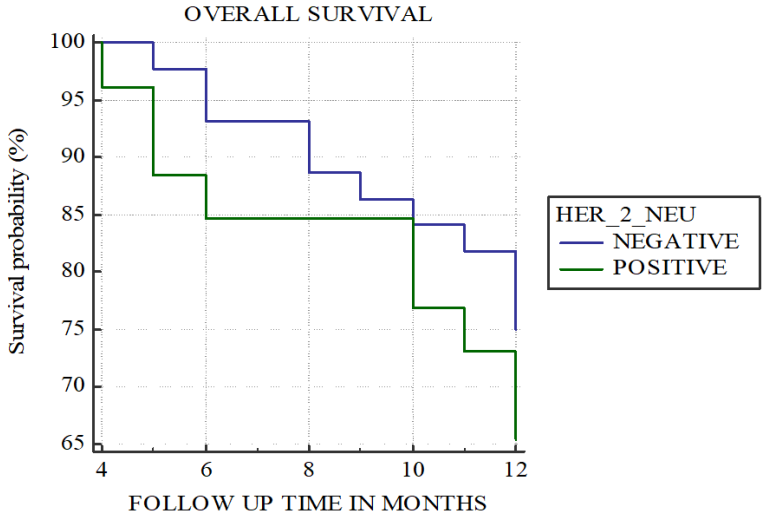
The overall survival proportion decreased with strong expression of cyclin D1 and patients with weak cyclin D1 immuno-expression had a better overall survival. The p value was 0.002. a statistically significant association was found between immuno-expression of cyclin D1 and poor overall survival.

Chart 26: Kaplan meier curve for disease free survival and cyclin D1 immuno-expression.



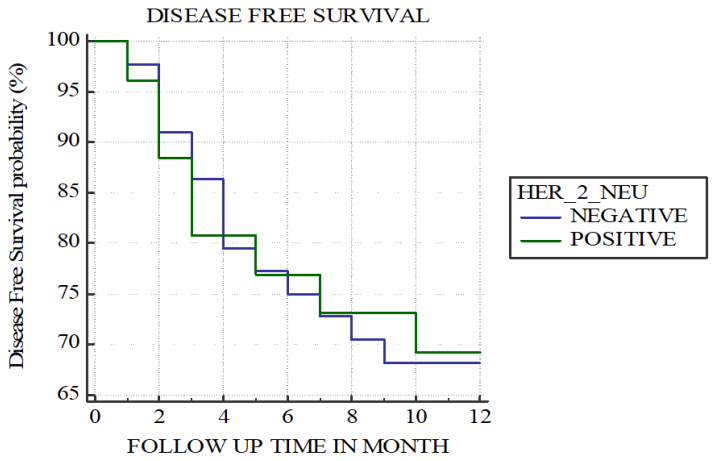
P value for expression of cyclin D1 and poor disease-free survival was 0.008 which was statistically significant.

Chart 27: Kaplan meier curve for overall survival of the patients with HER2 neu expression.



The HER2 neu positive tumours showed no relationship with overall survival of the patients and there was poor overall survival regardless of positive or negative immuno-expression. The p value was 0.363, which was statistically insignificant.

Graph 28: Kaplan meier curve for disease free survival and HER2 neu immuno-expression.



P value for association of HER2 neu with disease free survival was 0.955 and there was no statistically significant difference found with Her 2 Neu and disease-free survival.

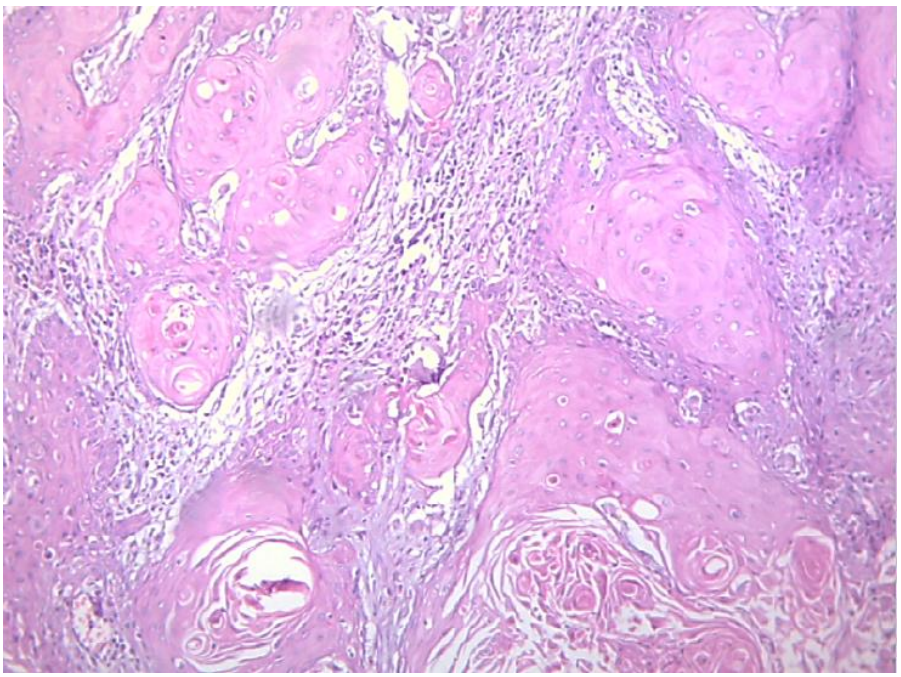


Figure 11: Well differentiated squamous cell carcinoma (H&E, x10)

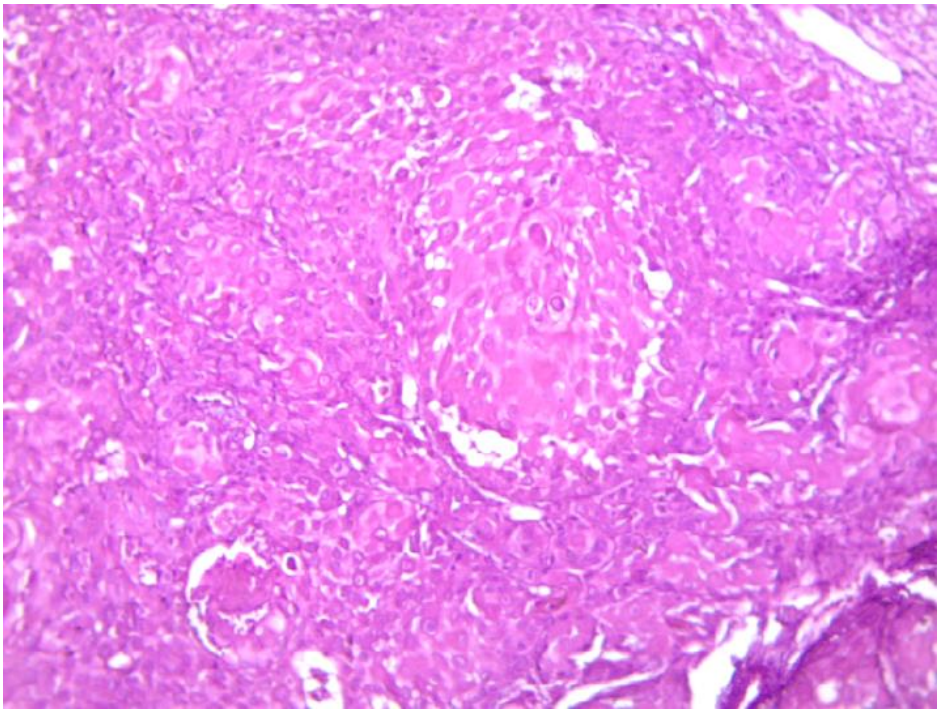


Figure 12: Moderately differentiated squamous cell carcinoma (H&E, x10)

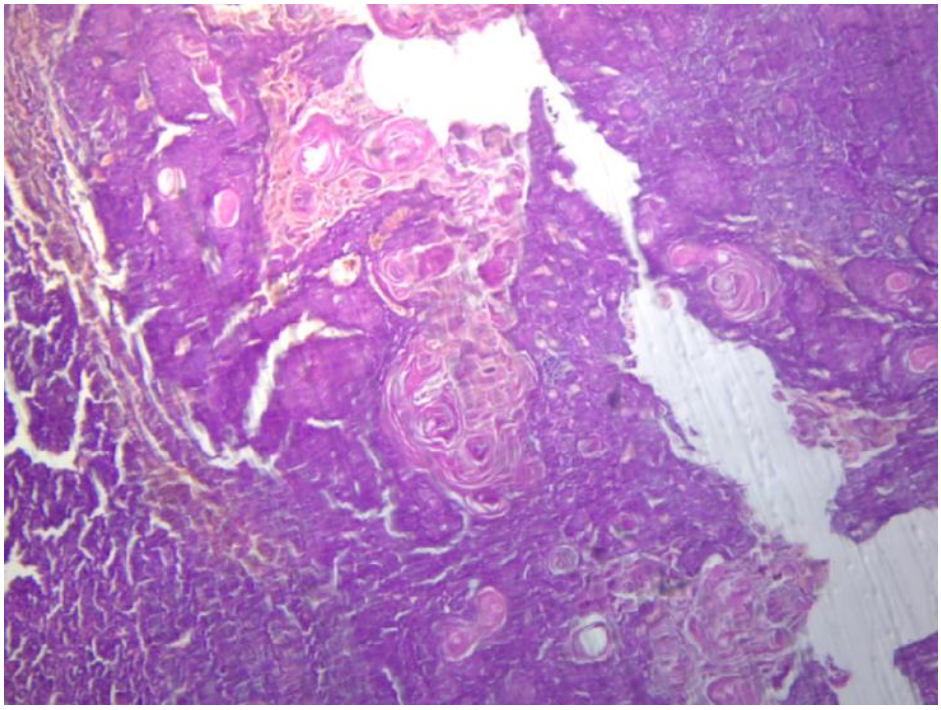


Figure 13: Metastatic lymph node of well differentiated squamous cell carcinoma (H&E x10)

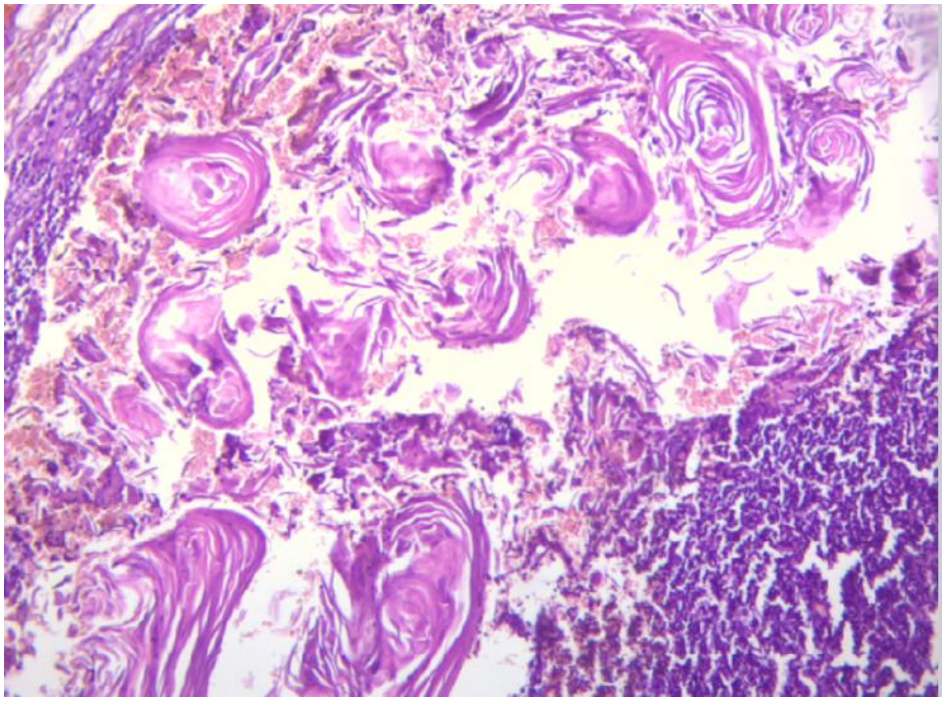


Figure 14: Metastatic lymph node of well differentiated squamous cell carcinoma (H&E x40)

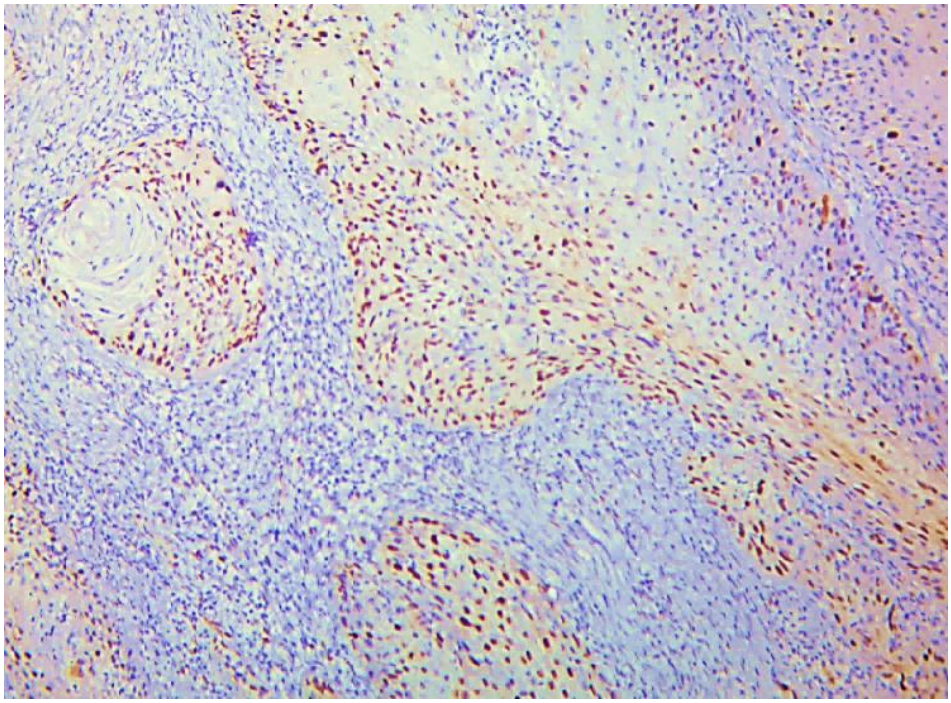


Figure 15: Strong expression of cyclin D1 IHC showing nuclear positivity. Expression-3, intensity: 3, total score 9 (IHC cyclin D1, x10)

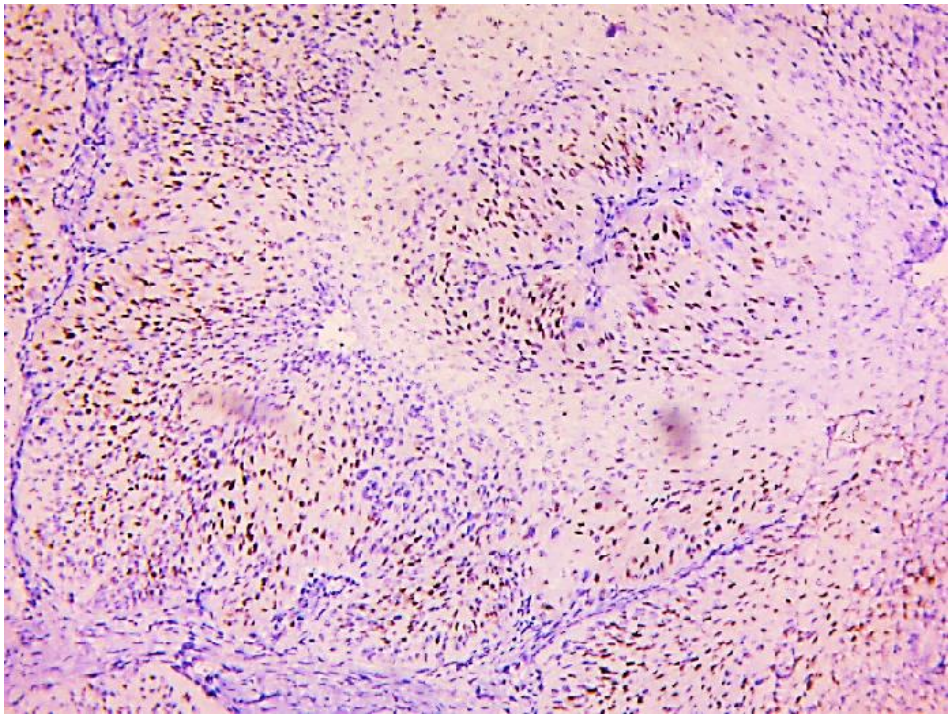


Figure 16: Strong expression of cyclin D1 IHC showing nuclear positivity. Expression-4, intensity: 3, total score 12 (IHC cyclin D1, x10)

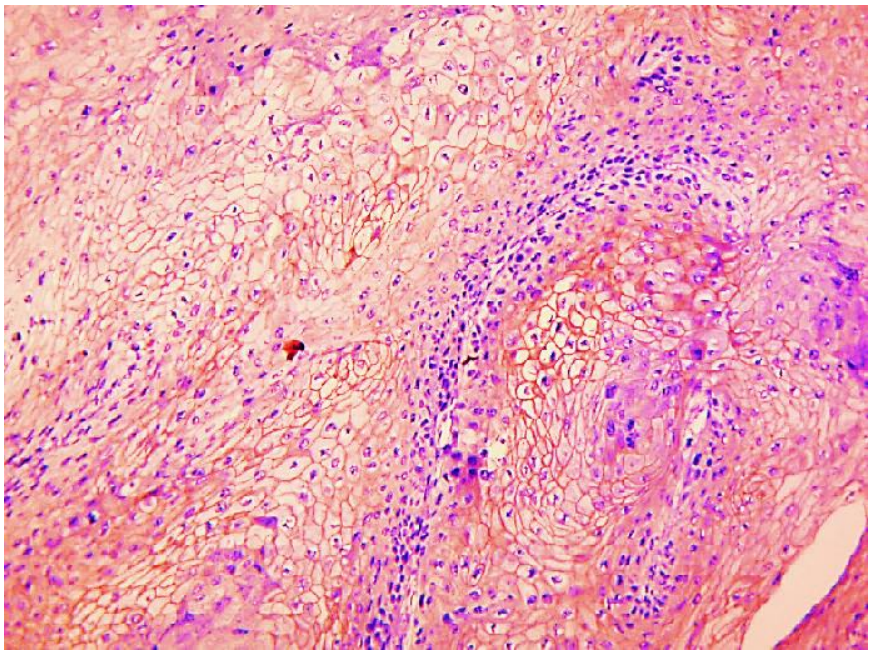


Figure 17: Positive expression of HER2neu IHC showing membranous staining, total score 2 (IHC HER2 neu, x10).

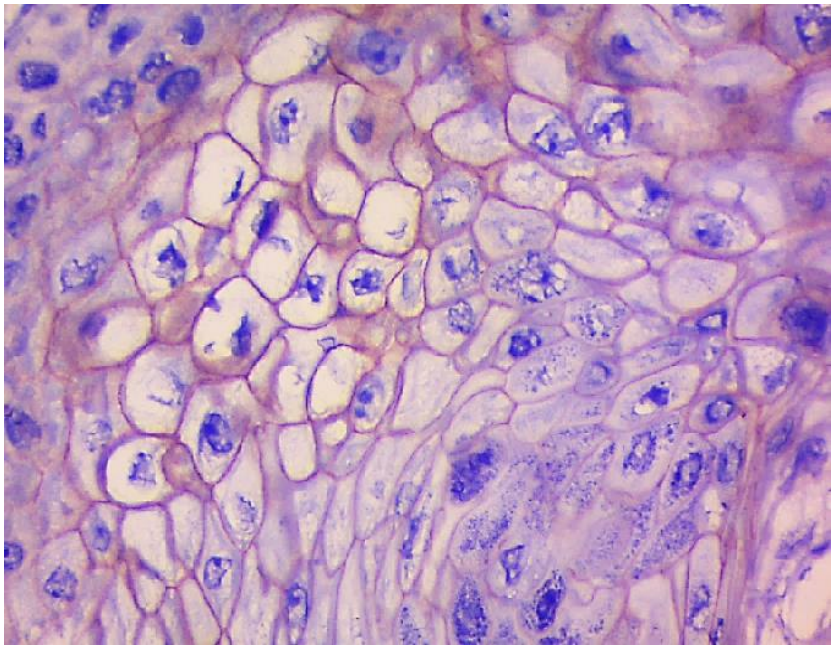


Figure 18: Positive expression of HER2neu IHC showing membranous staining, total score 2 (IHC HER2 neu, x40)

DISCUSSION

Table 32: Comparison of sex of subjects with different studies.

Study	Male	Female
Present study	17/70 (24.3%)	53/70 (75.7%)
Grilli g et al[54] (2021)	514/530 (96.9%)	16/530 (3.1%)
Chinnathambi P S et al[55] (2021)	108/150 (72%)	42/150 (28%)
Pandey m et al[56] (2018)	21/24 (87.5%)	3/24 (12.5)
Shergill K et al[57] (2018)	56/66 (84.8%)	10/66 (5.2%)

In present study, males comprised of 17/70 (24.3%) cases and females were 53/70 (75.7%). Based on gender, females were the predominant study group in present study which was discordant with all the other studies conducted on cyclin D1 and Her2 neu expression in head and neck squamous cell carcinoma. Studies done by Grilli g et al[54], Chinnathambi P S et al[55], Pandey m et al[56] and Shergill K et al[57] all showed male predominance in their study.

Table 33: Comparison of mean age distribution of head and neck squamous cell carcinoma with different studies.

Study	Mean age
Present study	53.21
Adnan Y et al[49] (2022)	51.42
Chinnathambi P.S. et al[55] (2021)	56
Zanaruddin S. N. et al[58] (2013)	59

Generally, carcinomas are more common in elderly age group or after 4th decade of life. The mean age for occurrence of head and neck squamous cell carcinoma in present study was 53.1 which was mostly consistent with all other studies. The maximum number of patients were seen in the age group of 45 to 55 years i.e., 4th and 5th decade of life. Study conducted by Adnan Y et al[49] had a mean age of 51.4 which was correlating with the present study. Similarly, studies conducted by Chinnathambi P. S. et al[55] and Zanaruddin S.N. et al[58] had mean age 56 and 59 respectively which was consistent with the present study.

The incidence of oropharyngeal carcinomas is increasing more in young individuals these days and relatively decreasing in older patients. The most common cause for shift in this trend is lifestyle of an individual which includes ingestion of tobacco in any form.[59] Moreover in rural society, the awareness about any form of disease is less which also attributes to high risk of acquiring human papilloma virus (HPV) infection among sexually active middle-aged individuals and this is further to be noted that infections by high-risk group of HPVS is the most frequent involved cause of squamous cell carcinoma.[60]

Table 34: Comparison of expression of Cyclin D1 with other studies.

Study	Weak expression	Moderate expression	Strong expression
Present study	16/70, 22.9%	16/70, 22.9%	38/70, (54.2%)
Siril et al[61] (2022)	23/84, 27.3%	39/84, 46.5%	22/84, 26.19%
Das S Net al[62] (2011)	15/45, 33.3%	10/45, 22.5%	20/45, 44.4%
Dhingra et al[52] (2017)	21/48, 43.75%	18/45, 37.5%	9/45, 18.8%
Menaka T R et al[63] (2022)	30%	30%	40%

Cyclin D1 immuno-expression was divided as weak, moderate and strong expression based on the product after multiplying the intensity and expression of the marker. In the present study, most of the cases showed strong immuno-positivity for cyclin D1 and when combined with moderate expression, the total constituted 54/70 (77.1%). In a study conducted by Siril et al[61] 46.5% tumour showed moderate positivity and 26.19% tumour showed strong positivity which was in accordance with the present study. Studies done by Das et al [62] and Menaka et al[63] also showed maximum number of cases with strong immuno-expression for cyclin D1 i.e., 44.4% and 40% respectively of the entire cases. The study by Dhingra et al[52] also showed maximum cases with moderate and strong over-expression 27/45. All these studies were in accordance with the present study which further also proves that cyclin D1 is expressed strongly in head and neck squamous cell carcinoma. Moreover, almost all the study, apart from Dhingra et al[52] showed maximum number of cases showing strong immuno-expression.

Table 35: Comparison of expression of cyclin D1 with histological grade in various studies.

	Intermediate and strong expression of cyclin D1			
Histological grade	**Present study**	**Dhingra et al[52] (2017)**	**Menaka T R et al[63] (2022)**	**Moharil R B et al[64] (2020)**
WDSCC	45/58, 77.5%	18/34, 52.9%	80%	9/14, 64.23%
MDSCC	9/12, 75%	8/13, 61.5%	50%	6/9, 66.66%
PDSCC	Nil	1/1, 100%	-	5/7, 71.4%

In present study, majority of the cases were of well differentiated squamous cell carcinoma i.e.; 58/70 and moderately differentiated squamous cell carcinoma constituted 9/70 cases. Out of these 58 well differentiated squamous cell carcinoma, 13 showed moderate score of positivity for cyclin D1 and 32 showed strong score of positivity for cyclin D1. In the present study, moderate and strong score were taken as positive. Similarly, total 9/12 (75%) cases of moderately differentiated squamous cell carcinoma showed strong score of positivity for cyclin D1. The p value for association with the tumour grade was 0.157 which was statistically insignificant. These findings were consistent with the findings of Dhingra et al[52], Menaka T R et al[63]. Dhingra et al[52] in his study on expression of cyclin D1 in head and neck squamous cell carcinoma did not find any statistical significance (p value 0.72) with the grade of the tumour and expression of cyclin D1. Further, in his study 18/34 (52.9%) cases of WDSCC, 8/13 (61.5%) cases of MDSCC and 1/1 (100%) case of PDSCC showed strong immunopositivity for cyclin D1. Similarly, in a study done by Menaka T R et al[63] 80% of WDSCC and 50 % of MDSCC showed strong and moderate score of positivity for cyclin D1. In both the studies, i.e.; Dhingra et al[52] and Menaka T R et al[63] maximum percentage of positive cases were seen in WDSCC which was in accordance with our study. Contrary to the

present study, Moharil et al's[64] study showed more percentage of cases positive with increase in grade of the tumour.this discrepancy can also be due to less number of cases of MDSCC and zero cases from PDSCC in the present study.

Table 36: Comparison of association of cyclin D1 expression with TNM stage of the tumour in various studies.

	Moderate and strong expression score of cyclin D1				
TNM stage	**Present study**	**Vahid Zand et al[65] (2020)**	**Lin et al[66] (2018)**	**Dhingra et al[52] (2017)**	**Saawarn et al (2012)[67]**
Stage 1	0/1	8/1, 66.6%	5/19, 26.3%	5/10, 50%	2/3,66.6%
Stage II	1/5, 20%	22/38, 57.8%		10/12, 83.3%	1/3,33.3%
Stage III	14/21, 66.6%	13/22, 59%	15/7, 68.2%	5/9, 55.55%	3/5, 60%
Stage IV	39/43, 90.7%	8/10, 80%	4/5, 80%	7/17, 41.1%	3/6, 50%
p value	**0.001**	**0.041**	**0.012**	**0.02**	0.866

In present study, majority of the tumour (43/70, 61.4%) were in stage IV, followed by stage III (21/70, 30%) which was consistent with the study conducted by Dhingra et al[52] and Saawarn et al[67]. The moderate and strong expression of cyclin D1 was seen in 39/43 (90.7%) cases of stage IV carcinoma and was in accordance with other studies. Majority of the cases 5/6 (83.33%) of stage I and II showed weak score of expression for cyclin D1. This present study can hypothesise that cyclin D1 immuno-expression increases with increase in stage of the disease. Studies conducted by Vahid Zand et al[65], Lin et al[66] and Dhingra et al[52] showed the similar result with p value of <0.05, showing a positive association for cyclin D1 expression with the increase in grade of the tumour. On the contrary, study by Saawarn et al[67] did not show any association between increase in stage and expression of cyclin D1. The p

value for the same was 0.866 and the maximum percentage of cases positive for expression of cyclin D1 belonged to stage I with total 66.6%.

Table 37: Comparison of association of cyclin D1 expression with metastatic lymph node of the tumour in various studies.

	Moderate and strong expression of cyclin D1	
Studies	**N %**	**P value**
Present study	34/38,89.47%	**0.032**
Chinnathambi et al[55] (2021)	13/19, 68.4%	**<0.05**
Huang et al[68] (2012)	35/71, 49.29%	**0.002**
Dhingra et al[52] (2017)	15/21,71.4%	**0.008**

As the previous result of the present study clearly indicates that cyclin D1 is overexpressed in advanced cases of head and neck squamous cell carcinoma. Total 38/70 cases presented with lymph node metastases, out of which 34 showed strong and moderate score for overexpression of cyclin D1. The result was statistically significant with p value of 0.032 proving an association with overexpression of cyclin D1 in tumours with positive lymph node metastases. Various other studies have also been done with the same parameter and the results obtained were statistically significant. In a study done by Chinnathambi et al[55] 13/19 (6804%) cases showed strong and moderate score for overexpression of cyclin D1 giving a positive association with overexpression and positive lymph node metastases. This finding was significant with present study. Similarly, studies conducted by Huang et al[68] and Dhingra et al[52] also showed positive association with present of lymph node metastases with p value of 0.002 and 0.008 respectively. All these findings were correlating with the findings of the

present study and it can be hypothesised that Cyclin D1 expression increases with presence of positive lymph node metastases.

Table 38: Comparison of expression of HER2 neu in various studies

Studies	Positive	negative
Present study	5/70, 7.1%	65/70, 92.9%
Adnan Y et al[49] (2022)	21/100,21%	79/100, 79%
Vats S et al[53] (2018)	14/70, 20%	56/70, 80%
Doley P et al[69] (2020)	17/50, 34%	33/50, 66%
Nayar R C et al[70] (2016)	2/20, 10%	18/20, 90%
Rout T et al[71] (2022)	14/35, 40%	21/35, 60%

In the present study, maximum of the tumours (65/70, 92.9%) were negative for expression of HER2neu and only 5/70 (7.1%) of the tumour were positive for HER 2 neu overexpression. This finding was consistent with all the other studies on HER 2 neu expression in head and neck squamous cell carcinoma. Studies done by Adnan Y et al[49] (79/100, 79%), Vats S et al[53] (56/70, 80%), Doley P et al[69] (33/50, 66%) Nayar R C et al[70] (18/20, 90%) and Rout T et al[71] (21/35, 60%) showed more cases with no immune expression of HER 2 neu in the patients. The finding of all these studies was consistent with the findings of the present study. So based on the results, it can be hypothesised that HER 2 neu is not expressed in head and neck squamous cell carcinoma. Moreover, there is no standardised scoring system for analysing HER2 neu in head and neck tumours. More studies are needed to further investigate the expression of HER 2neu in head and neck carcinoma and a standardised scoring system for the assessment.

Table 39: Comparison of Her2 neu with grade of the tumour in various studies

	Her 2 neu positive			
Grade	**Present study**	**Mohanapure et al[72] (2022)**	**Doley P et al[69] (2020)**	**Rout T et al[71] (2022)**
WDSCC	5/58, 8.62%	7/25,28%	10/24, 41.6%	5/17, 29.4%
MDSCC	0/12	25/45,55.5%	6/18, 33.3%	9/18, 50%
PDSCC			1/8, 12.5%	
p value	0.932	**<0.05**	>0.05	0.214

In the present study, the only cases positive for HER 2 neu overexpression were from well differentiated squamous cell carcinoma (5/58, 8.62%). All the cases from moderately differentiated squamous cell carcinoma were negative for the expression. The p value for association was 0.932, which was statistically insignificant. Other studies done by Doley P et al[69] and Rout T et al[71] showed no association between expression of HER2 neu and grade of the tumour. On the contrary, study done by Mohanapure et al[72] showed a positive association with the grade of the tumour with p value less than 0.05.

Comparison of expression of HER2neu with lymph node status in various studies.

In the present study, 38/70 (54.3%) cases showed positive lymph node metastases and 30/70 (42.9%) cases were negative for it. Her 2 expression was seen in 3/38 (7.9%) lymph node positive cases and 2/30 (6.7%) in lymph node negative cases. The expression showed no association with presence of lymph node metastases. The other studies in the literature including Vats et al[53], Mohanapure et al[72] and Doley P et al[69] showed similar result with no association of HER2neu expression with lymph node metastases.

Table 40: Comparison of HER2 neu expression with TNM stage in various studies.

	Her 2 neu positive		
Stage	**Present study**	**Vats S et al[53] (2018)**	**Mirza S et al[73] (2020)**
Stage I-II	0/6	5/28,17.85%	0/45
Stage III-IV	5/64, 7.8%	9/40,22.5%	1/95, 1.05%
P value	0.819	0.664	0.388

In present study, all 5/70 cases which were positive for HER2 neu expression belonged to stage III and stage IV carcinoma, 5/64 (7.8%) which is identical to the studies done by Vats S et al[53] and Mirza S et al[73] where 9/40 (22.5%) and 1/95 (1.05%) cases were of stage III and IV carcinoma respectively. However, there was no positive association establish between the expression of HER2neu and stage of the disease. This finding is consistent with findings in literature where most of the studies have failed to demonstrate a positive association between the two.

Association of histopathological parameters, cyclin D1 expression and HER2 neu expression with overall survival of the patients.

Follow up of patients was done for one year and association of overall survival was taken out with respect to histopathological parameters, cyclin D1 expression and HER2 neu expression. In the present study, depth of invasion, lymph node status, primary tumour grade and histological grade of the tumour were taken as the histological parameters. Univariate survival analysis was done using COX proportional hazard models. Out of all the histopathological parameters taken for consideration, only metastatic lymph node status showed a positive association with overall survival. Inference was drawn that with positive metastatic lymph node, the hazard for death increases six times. The p value for the same was

0.011 and was statistically significant. Other parameters like depth of invasion, primary tumour grade and histological grade showed no association with overall survival. This positive lymph node association with overall survival correlated with study conducted by Adnan Y et al[49] in which there was association of metastatic lymph node with decreased overall survival. The p value was 0.001. Cavalot et al[74] also found a positive association of poor overall survival and presence of metastatic lymph node. Based on the above results, an inference can with withdrawn that with presence of metastatic lymph node, overall survival of the patient decreases.

Comparison of overall survival with expression of cyclin D1 and HER2 neu was done using Kaplan Meier curve and log rank statistics were used to make comparison between the two groups.

It was seen that the patients with strong to moderate expression of cyclin D1 had decreased overall survival. A positive association was present with decrease in overall survival and strong and moderate expression of cyclin D1 with p value of 0.002. similar result was seen in a study by Lin X et al[66] where he stated that increase in expression score of cyclin D1 is associated with poor overall survival and hypothesised that cyclin D1 expression influences the survival and prognosis of patients. A similar study by Juliette B et al[75] also showed same result and a statistical significance was found with poor overall survival and cyclin D1 expression.

In the present study, no significant association was derived between expression of HER2neu and overall survival. Similar results were seen in studies conducted by Warren EAK et al[50] and Vats S et al[53] however, Cavalot et al[74] suggested that HER2neu expression can also be helpful in prognosis as an independent risk factor in head and neck squamous cell carcinoma.

Association of histopathological parameters, cyclin D1 expression and HER2 neu expression with disease free survival of the patients.

The association between disease free survival and histological parameters, cyclin D1 expression and HER2 neu expression was taken out after follow up of patients for 1 year.

Histopathological parameters like depth of invasion, lymph node status, histological grade, primary tumour grade and TNM stage of disease were considered for disease free survival. A positive association was seen with presence of lymph node metastases with p value of 0.004 and hazard ratio was 9.03 which stated that with presence of metastatic lymph nodes there is nine times more chance of recurrence of the disease. A positive association was also seen with increase in TNM stage of the disease with p value 0.020. It was also seen from the result that with increase in stage of the disease the rate of recurrence increases 6 times. Stage IV carcinomas has a higher chance of recurrence than stage I carcinomas. Similar results were seen in study by Adnan Y et al[49] where the p value was 0.018 and 0.03 for association of disease-free survival with metastatic lymph node and increase in TNM stage of the disease.

It was observed that patients who showed strong expression of cyclin D1 had poor disease-free survival and rate of recurrence was high. There was a statistical significance found for the association of strong cyclin D1 expression score and poor disease-free survival with a p value of 0.008. Similar result was seen in a meta-analysis by Pablo Ramos et al[43] where a statistical significance was found between cyclin D1 expression and poor disease-free survival.

For HER2 neu, there was no statistical significance found between disease-free survival and HER2 neu expression. Studies done by Adnan Y et al[49], Vats S et al[53] showed no correlation with disease-free survival HER2neu expression and correlated with the results of present study. On the contrary, Cavalot et al[74] in his study proved a significant association with

HER2 neu expression and poor disease-free survival. He further stated that HER2 neu expression and lymph node positive status were the only independent variable which were associated with disease-free survival.

SUMMARY

The present study was conducted in Department of Pathology of R L Jalappa hospital, with all resected specimen of histologically proven head and neck squamous cell carcinoma. The total sample size was 70.

Out of 70 cases, maximum cases were females (53/70, 75.7%). 26/70 (37.1%) cases were from the age group 41-50 years. Majority of the patients (36/70; 51.4%) were homemaker. 27/70 (38.6%) cases i.e., maximum number of cases were from buccal mucosa followed by gingiva buccal sulcus (17/70, 24.3%). Well differentiated squamous cell carcinoma constituted 58/70, (82.9%) cases and T4 stage were 29/70 (41.4%). Total 38/70 (54.3%) cases presented with lymph node metastases.

Cyclin D1 immuno-stain was done and the association with histopathological parameters were done. There was a positive association found between expression cyclin D1 with stage of the tumour, depth of invasion and lymph node metastases of the tumour with p value 0.001, 0.017 and 0.032 respectively.

With respect to HER2 neu, only 5/70 (7.1%) cases were positive and a positive association was found with depth of invasion and HER2 neu expression with p value 0.008.

Patients were followed up for one year. Overall survival was analysed with histopathological parameters, cyclin D1 expression and HER2 neu expression. The results were significant for presence of positive lymph node status with p value, 0.011hence indicating that presence of metastatic lymph node is associated with poor over-all survival.

Kaplan meier analysis was done for association of overall survival with cyclin D1 and HER2 neu expression. The p value for association with cyclin D1 was 0.001 which was statistically significant. There was no association found between overall survival and HER2 neu expression.

Disease free survival was taken out and association was found with increase in stage of the tumour and presence of lymph node metastases with p value 0.020 and 0.004 respectively. Kaplan meier analysis was also done for disease free survival and expression of the marker. A positive association was found with expression of cyclin D1 and poor disease-free survival with p value 0.008. no significant relationship was found with expression of HER2 neu and disease-free survival.

CONCLUSION

Based on the present study, conclusion can be drawn that cyclin D1 expression increases with increase in depth of invasion, TNM stage and lymph node metastases. More importantly, cyclin D1 expression is associated with poor overall survival and disease-free survival. Hence based on the above result, cyclin D1 can be used as a prognostic marker in head and neck squamous cell carcinoma.

With respect to HER2 neu, a positive association was seen only with increase in depth of invasion and there was no association with overall survival and disease-free survival with HER2 neu expression. More extensive research is needed to know the role of HER2 neu in head and neck squamous cell carcinoma. Moreover, a standardized system for scoring of Her2 neu on head and neck cancer is needed. There have conflicting results on expression of HER2 neu because of lack of a standardized scoring system. With further research, HER2 neu can be used as prognostic and therapeutic purpose in head and neck squamous cell carcinoma.

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INFORMED CONSENT FORM

STUDY TITLE: EVALUATION OF CYCLIN D1 AND HER2 NEU PROTEIN EXPRESSION IN HEAD AND NECK SQUAMOUS CELL CARCINOMA.

I, _____have read or have been read to me the patient information sheet and understand the purpose of the study, the procedure that will be used, the risk and benefits associated with my involvement in the study and the nature of information will be collected and disclosed during the study.

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

I, the undersigned, agree to participate in this study and authorize the collection and disclosure of my personal information for the dissertation.

Name and signature / thumb impression Date:

(subject) Place:

Name and signature / thumb impression Date:

Place:

(Witness/Parent/ Guardian/ Husband)

PATIENT INFORMATION SHEET:

STUDY TITLE: EVALUATION OF CYCLIN D1 AND HER2 NEU PROTEIN EXPRESSION IN HEAD AND NECK SQUAMOUS CELL CARCINOMA.

PLACE OF STUDY: Department of Pathology, Sri Devaraj Urs Medical College, Kolar.

The main aim of the study is to find the role of cyclin D1 and Her 2 neu as a predictive marker for head and neck squamous cell carcinoma. The specimens will be collected from the department of pathology, SDUMC,Kolar. This study will be approved by the institutional ethical committee. The information collected will be used only for dissertation and publication. There is no compulsion to agree to participate. You are requested to sign / provide thumb impression only if you voluntarily agree to participate in the study. All information collected from you will be kept confidential and will not be disclosed to any outsider. Your identity will not be revealed. You will not receive any monetary benefits to participate in this research. This informed consent document is intended to give you a general background of study. Please read the following information carefully and discuss with your family members. You can ask your queries related to study at any time during the study. If you are willing to participate in the study you will be asked to sign an informed consent form by which you are acknowledging that you wish to participate in the study and entire procedure will be explained to you by the study doctor. You are free to withdraw your consent to participate in the study any time without explanation and this will not change your future care.

For any clarification you are free to contact the investigator.

PRINCIPAL INVESTIGATOR: Dr. Snigdha

PATIENT PROFORMA

Age:	
Sex:	Hospital Number:

Anonymized Sample No:

Chief complaint:

History of presenting illness:

Past history:

Personal history:

Local examination:

Biopsy Number:

Microscopy:

Tumor side:

Tumor site:

Tumor size:

Depth of invasion:

Perineural invasion:

Lympho-vascular invasion:

Number of lymph nodes studied:

Number of lymph nodes involved:

Bone metastases:

Histopathological diagnosis:

Stage of disease:

TMN staging:

Cyclin D1:

Intensity of staining:

Proportion of staining:

IHC grading:

HER2neu:

Intensity of staining:

Proportion of staining:

IHC grading:

KEYS TO MASTER CHART

DOI – Depth of invasion

LN – Lymph node

WDSCC – Well differentiated squamous cell carcinoma

MDSCC – Moderately differentiated squamous cell carcinoma

T – T staging according to 8^{h} AJCC edition of head and neck carcinoma

N – N staging according to 8^{h} AJCC edition of head and neck carcinoma

M – M staging according to 8^{h} AJCC edition of head and neck carcinoma

UHID: Hospital number

																					cyclin D1				her2 neu				
sl.no	age	sex	UHID	biopsy no	occupation	habits	site	side	tumor size	DOI	perineural	lympho vascular	bone	LN status	LN positive	extranodal spread	histological grade	primary tumor (t)	stage	pTNM	intensity	proportion	score	grade	score	grade	follow up period (months)	recurrence (lymph node positive)	overall survival (12 months)
1	35	F	861016	1403/20	homemaker	oral tobacco	buccal mucosa	left	40x45x20mm	23mm	absent	absent	absent	23	1	absent	WDSCC	T4	stage IVa	T4aN1Mx	3	3	9	strong	0	neagtive	12	absent	survived
2	66	F	870242	1417/20	homemaker	oral tobacco	GBS	right	50x30x36mm	36 mm	absent	absent	absent	29	2	absent	WDSCC	T4	stage Iva	T4aN2aMx	3	4	12	strong	0	negative	12	at 8 month	survived
3	55	F	862849	1474/20	homemaker	oral tobacco	RMT	right	12x10x10mm	9 mm	absent	absent	absent	21	1	absent	WDSCC	T2	stage III	T2N1Mx	3	4	12	strong	1	weak	12	absent	survived
4	60	F	863578	1559/20	homemaker	smoking	BM & GBS	left	50x30x24 mm	23 mm	absent	absent	absent	21	0	absent	WDSCC	T4	stage IVa	T4aN0Mx	3	4	12	strong	0	negative	12	absent	survived
5	68	F	863293	1579/20	homemaker	no	BM & GBS	right	60x30x22 mm	21mm	absent	absent	absent	37	0	absent	WDSCC	T4	stage Iva	T4aN0Mx	3	4	12	strong	0	negative	12	at 6 th month	deceased (10th month)
6	73	F	864846	1580/20	homemaker	no	BM	right	45x40x23mm	21mm	absent	absent	absent	39	0	absent	WDSCC	T4	stage Iva	T4aN0Mx	2	4	8	moderate	1	weak	12	absent	deceased (12th month)
7	43	F	870693	1598/20	homemaker	oral tobacco	Tongue	Left	37x33x18mm	15 mm	absent	absent	absent	13	2	absent	WDSCC	T3	stage Iva	T3N2bMx	3	3	9	strong	0	negative	12	at 9th month	survived
8	60	F	869349	1692/20	homemaker	oral tobacco	lower alveolus	Left	35x27x20mm	16 mm	absent	absent	absent	14	0	absent	WDSCC	T3	stage III	T3N0Mx	3	4	12	strong	0	negative	12	absent	survived
9	53	F	871431	1762/20	business	no	Lower GBS	left	25x10x20mm	9mm	absent	absent	absent	12	0	absent	WDSCC	T2	stage II	T2N0Mx	2	2	4	weak	1	weak	12	absent	survived
10	45	F	874827	1899/20	business	no	Buccal mucosa	right	27x20x23mm	17mm	absent	absent	absent	18	1	absent	WDSCC	T3	stage III	T3N1Mx	3	3	9	strong	0	negative	12	absent	survived
11	45	F	877285	1871/20	homemaker	no	Buccal mucosa	left	25x25x10mm	8mm	absent	absent	absent	33	1	absent	WDSCC	T2	stage III	T2N1Mx	3	2	6	moderate	0	negative	12	absent	survived
12	55	F	871913	1863/20	homemaker	oral tobacco	Buccal mucosa	right	50x55x22mm	18mm	absent	absent	absent	35	3	absent	MDSCC	T3	stage IVA	T3N2bMx	3	4	12	strong	0	negative	12	at 3rd month	deceased (11th month)
13	48	M	877280	1906/20	business	smoking	Tongue		55x45x20mm	8mm	absent	absent	absent	10	0	absent	WDSCC	T3	stage III	T3N0Mx	1	1	1	weak	0	negative	12	absent	survived
14	52	M	875121	1947/20	labour	smoking	Lower alveolus	right	50x42x29mm	26mm	absent	absent	absent	31	0	absent	WDSCC	T4	stage Iva	T4aN0Mx	3	3	9	strong	0	negative	12	absent	survived
15	46	M	880307	2029/20	labour	alcohol	Tongue	right	25x20x10mm	9mm	absent	absent	absent	15	0	absent	MDSCC	T2	stage II	T2N0Mx	3	2	6	moderate	0	negative	12	absent	survived
16	50	F	880849	B/178/21	labour	oral tobacco	Buccal mucosa	Left	35x30x26mm	18mm	absent	absent	absent	35	1	absent	WDSCC	T2	stage III	T2N1Mx	1	2	2	weak	0	negative	12	absent	survived
17	44	M	886617	119/21	farmer	smoking	buccal mucosa	left	55x40x30mm	30 mm	absent	absent	present	36	8	present	WDSCC	T4	stage IVB	T4aN3bMx	3	3	9	strong	0	negative	12	at 2nd month	deceased (6th month)
18	45	M	885365	142/21	business	smoking	lateral tongue	left	60x25x25mm	15mm	absent	absent	absent	54	0	absent	MDSCC	T4	stage IVa	T4aN0Mx	2	3	6	moderate	0	negative	12	absent	survived
19	58	female	889849	B/641/21	labour	no	lateral border of tongue	left	40x32x18 mm	12 mm	absent	absent	absent	29	0	absent	MDSCC	T3	stage III	pT3N0Mx	3	3	9	strong	0	negative	12	absent	survived
20	45	male	901647	B/608/21	farmer	alcohol	buccal mucosa	right	45x40x23mm	19 mm	absent	absent	absent	30	0	absent	WDSCC	T4	stage IVa	pT4aN0Mx	2	2	4	weak	1	negative	12	absent	survived
21	49	female	903390	B/660/21	homemaker	smoking	BM+GBS	right	32x26x19 mm	15mm	absent	absent	absent	33	0	absent	WDSCC	T3	stage III	pT3N0Mx	2	3	6	moderate	1	negative	12	absent	survived
22	60	female	901536	B/776/21	homemaker	no	right buccal mucosa	right	32x30x20	18mm	absent	absent	absent	15	0	absent	WDSCC	T3	stage III	pT3N0Mx	3	2	6	moderate	1	negative	12	absent	survived
23	40	female	901774	B/781/21	homemaker	oral tobacco	lower GBS	left	25x20x10mm	4mm	absent	absent	absent	29	1	absent	WDSCC	T2	stage III	PT2N1Mx	2	4	8	moderate	1	negative	12	at 5th monh	survived
24	45	female	910059	B/786/21	labour	oral tobacco	retromolar trigone		35x14x20mm	7 mm	absent	absent	absent	19	2	absent	WDSCC	T2	stage IVa	pT2N2bMx	3	3	9	strong	0	negative	12	at 4th month	survived
25	65	female	905174	B/713/21	homemaker	smoking	upper alveolus	left	40x13x14 mm	12mm	absent	absent	present	14	4	present	WDSCC	T4	stage IVa	pT4aN2bMx	3	2	6	moderate	0	negative	12	at 7th month	deceased (12th month)
26	55	female	929261	B/1240/21	labour	no	lower GBS	right	40x30x25mm	22mm	absent	absent	absent	19	4	absent	WDSCC	T3	stage IVa	pT3N2bMx	3	4	12	strong	0	negative	12	absent	deceased (8th month)
27	65	female	917638	B/860/21	homemaker	no	left lower GBS	left	35x30x27 mm	27 mm	absent	absent	present	20	1	absent	WDSCC	T3	stage III	pT3N1Mx	2	4	6	moderate	2	positive	12	absent	survived
28	50	female	918421	B/826/21	homemaker	alcohol	left buccal mucosa	left	40x40x24mm	23mm	absent	absent	absent	28	1	absent	MDSCC	T4	stage IVa	pT4aN1Mx	3	3	9	strong	0	negative	12	at 3rd month	survived
29	50	female	919531	B/850/21	labour	no	lower alveolus	left	85x55x40 mm	40mm	absent	present	present	30	0	absent	WDSCC	T4	stage IVa	pT4aN0Mx	3	4	12	strong	2	positive	12	absent	survived
30	50	female	917875	B/898/21	farmer	no	left buccal mucosa	left	26x18x15 mm	16mm	absent	absent	absent	19	3	absent	WDSCC	T3	stage IVa	pT3N2bMx	3	3	9	strong	0	negative	12	at 2nd month	deceased (9th month)
31	65	female	922091	B/955/21	homemaker	no	right buccal mucosa	right	42x35x24	24mm	absent	absent	present	7	0	absent	WDSCC	T4	stage IVa	pT4aN0Mx	2	4	8	moderate	1	weak	12	absent	survived
32	46	female	918829	B/958/21	farmer	no	buccal mucosa	left	55x45x25 mm	25 mm	absent	absent	absent	34	9	absent	WDSCC	T4	stage IVa	pT4aN2bMx	1	1	1	weak	2	positive	12	absent	survived
33	38	male	927947	B/1111/21	labour	alcohol + smoking	lateral border of tongue	left	28x20x7mm	5mm	absent	absent	absent	30	2	absent	WDSCC	T2	stage IVa	pT2N2bMx	2	2	4	weak	1	weak	12	absent	survived
34	56	male	923786	B/1143/21	business	alcohol + smoking	larynx (pyriform fossa)	right	40x20x40mm		absent	absent	present				WDSCC	T3	stage III	pT3N0Mx	1	2	2	weak	0	negative	12	absent	survived
35	40	female	926491	B/1163/21	homemaker	oral tobacco	GBS	left	45x24x30mm	23mm	absent	absent	absent	33	6	present	WDSCC	T4	stage IVB	PT4aN3bMx	3	3	9	strong	1	weak	12	at 2nd month	deceased (5th month)
36	35	female	927719	B/1180/21	homemaker	no	buccal mucosa	left	35x19x33mm	22mm	absent	absent	absent	22	1	absent	WDSCC	T3	stage III	pT3N1Mx	3	4	12	strong	0	negative	12	absent	survived
37	49	female	925793	B/1195/21	homemaker	no	upper alveolus	left	30x20x32mm	22mm	absent	absent	present	10	3	absent	WDSCC	T3	stage IVa	pT3N2bMx	3	4	12	strong	1	weak	12	at 10 month	survived
38	48	male	927813	B/1196/21	service	alcohol	lateral border of tongue	left	28x15x23mm	19mm	absent	absent	absent	37	0	absent	WDSCC	T3	stage III	pT3N0Mx	1	3	3	weak	1	weak	12	absent	survived
39	60	female	928875	B/1133/21	labour	oral tobacco	GBS	left	15x13x5mm	3mm	absent	absent	absent	25	1	absent	WDSCC	T1	stage III	pT1N1Mx	1	2	2	weak	1	weak	12	absent	survived
40	55	female	925184	B/1021/21	farmer	oral tobacco	buccal mucosa	left	25x10x10 mm	10mm	absent	absent	absent	14	3	absent	WDSCC	T2	stage IVa	pT2N2bMx	3	4	12	strong	0	negative	12	at 2nd month	deceased (8th month)
41	53	female	926681	B/1086/21	farmer	no	buccal mucosa	right	30x10x3mm	3mm	absent	absent	absent	30	2	absent	WDSCC	T2	stage IVa	pT2N2bMx	3	3	9	strong	1	weak	12	at 3rd month	deceased (10th month)

sl.no	age	sex	UHID	biopsy no	occupation	habits	site	side	tumor size	DOI	perineural	lympho vascular	bone	LN status	LN positive	extranodal spread	histological grade	primary tumor (t)	stage	pTNM	intensity	proportion	score	grade	score	grade	follow up period (months)	recurrence (lymph node positive)	overall survival (12 months)
42	48	female	929512	B/1232/21	homemaker	no	post cricoid region larynx		40x30x27mm		absent	absent		65	3		MDSCC	T3	stage IVa	pT3N2cMx	2	4	8	moderate	0	negative	12	absent	survived
43	55	male	927721	B/1266/21	service	no	upper GBS	left	25x15x12 mm	11mm	absent	absent	absent	19	2	present	WDSCC	T3	stage IVb	PT3N3bMx	3	4	12	strong	0	negative	12	at 4th month	deceased (12th month)
44	54	female	934884	B/1248/21	homemaker	no	buccal mucosa	left	20x15x8mm	11mm	absent	absent	absent	15	0	absent	WDSCC	T3	stage III	pT3N0Mx	2	4	8	moderate	0	negative	12	absent	survived
45	43	female	932192	B/1283/21	homemaker	oral tobacco	buccal mucosa	right	20x15x19	5mm	absent	absent	absent	14	3	present	WDSCC	T1	stage IVb	pT1N3bMx	3	2	6	moderate	0	negative	12	absent	survived
46	72	female	933008	B/1363/21	homemaker	alcohol	lower GBS	left	25x20x10mm	4mm	absent	absent	absent	29	1	absent	WDSCC	T2	stage III	PT2N1Mx	3	2	6	moderate	0	negative	12	absent	survived
47	61	female	923854	B/1399/21	business	alcohol	upper alveolus	left	35x22x25 mm	9mm	absent	absent	absent	not retrieved			WDSCC	T2	stage II	pT2NxMx	2	2	4	weak	0	negative	12	absent	survived
48	50	male	931871	B/1419/21	farmer	smoking	lateral border of tongue	left	33x28x13 mm	9 mm	absent	absent	absent	13	0	absent	WDSCC	T2	stage II	pT2N0Mx	2	2	4	weak	0	negative	12	absent	survived
49	72	female	932303	B/1431/21	homemaker	oral tobacco	buccal mucosa	right	48x23x15	11mm	absent	absent	absent	12	0	absent	WDSCC	T4	stage IVa	pT4aN0Mx	2	3	6	moderate	1	weak	12	absent	survived
50	34	female	335771	B/1457/21	farmer	no	gingivobuccal sulcus	left	32x4x18 mm	18mm	absent	absent	present	31	0	absent	WDSCC	T3	stage III	pT3N0Mx	3	4	12	strong	0	negative	12	absent	survived
51	69	female	937022	B/1555/21	homemaker	no	buccal mucosa	right	35x25x12mm	13mm	absent	absent	absent	19	0	absent	MDSCC	T3	Stage III	pT3N0Mx	2	2	4	weak	0	negative	12	absent	survived
52	45	F	891721	B/1502/21	homemaker	no	GBS	right	48x23x15mm	7mm	absent	absent	absent	21	0	absent	WDSCC	T3	stage III	T3N0Mx	3	4	12	strong	1	weak	12	at 5th monh	survived
53	45	F	890479	B/274/21	homemaker	oral tobacco	GBS	right	35x12x20mm	5mm	absent	absent	absent	15	0	absent	MDSCC	T2	stage II	T2N0Mx	1	2	2	weak	0	negative	12	absent	survived
54	55	F	929261	B/1503/21	homemaker	oral tobacco	Lower GBS	right	40x30x25mm	22mm	absent	absent	absent	19	4	Absent	WDSCC	T4	stage IVa	T4aN2bMx	3	4	12	strong	2	positive	12	at 2nd month	deceased (11th month)
55	56	F	931769	B/1245/21	homemaker	no	BM	right	45x40x27mm	23mm	absent	absent	absent	28	1	Absent	WDSCC	T4	stage IVa	T4aN1Mx	3	3	9	strong	1	weak	12	absent	deceased (6th month)
56	47	F	941207	B/1607/21	business	no	Lower GBS	Left	45x50x35mm	26mm	Absent	absent	absent	29	0	Absent	WDSCC	T4	stage IVa	T4aN0Mx	3	3	9	strong	1	weak	12	absent	survived
57	55	F	938650	B/1658/21	business	no	Lower alveolus	Right	35x40x40mm	34mm	absent	absent	absent	15	0	Absent	WDSCC	T4	stage IVa	T4aN0Mx	3	4	12	strong	0	negative	12	absent	survived
58	66	F	946913	B/1677/21	homemaker	oral tobacco	Lateral border of tongue	Left	85x45x40mm	36mm	absent	absent	absent	15	1	Absent	MDSCC	T4	stage IVa	T4aN1Mx	3	4	12	strong	1	weak	12	absent	deceased (5th month)
59	60	F	941733	B/1690/21	homemaker	oral tobacco	Lower GBS	Left	41x20x35mm	30mm	absent	absent	absent	41	4	present	WDSCC	T4	stage IVb	T4aN3bMx	3	4	12	strong	1	weak	12	at 7th month	deceased (12th month)
60	65	male	945476	B/1815/21	service	alcohol+smoking	buccal mucosa	right	45x20x30 mm	30mm	absent	absent	absent	10	0	absent	WDSCC	T4	stage IVa	pT4aN0Mx	1	2	2	weak	2	positive	12	absent	survived
61	47	male	43977	B/2293/21	service	oral tobacco	upper GBS	left	50x38x35 mm	15mm	absent	absent	present	19	0	absent	MDSCC	T4	stage IVa	PT4aN0Mx	3	4	12	strong	1	weak	12	absent	survived
62	29	female	20285	B/1953/21	business	none	buccal mucosa	left	35X25X10 mm	7mm	absent	absent	absent	27	1	present	WDSCC	T3	stage IV b	pT3N3bMx	3	2	6	moderate	1	weak	12	at 3rd month	deceased (10th month)
63	44	male	41022	B/2319/21	business	none	buccal mucosa	right	45x25x25mm	23mm	absent	present	absent	24	5	absent	MDSCC	T4	stage IVa	pT4aN2bMx	3	3	9	strong	1	weak	12	absent	survived
64	54	male	37156	1924/21	service	none	buccal mucosa	left	54x25x24mm	21mm	absent	absent	absent	19	3	absent	WDSCC	T4	stage IVb	PT4aN3bMx	3	4	12	strong	0	negative	12	at1st month	deceased (6th month)
65	74	F	37560	B/1986/21	homemaker	oral tobacco	Lower GBS	Right	15x10x2mm	2mm	absent	absent	absent	15	0	Absent	WDSCC	T1	stage I	T1N0Mx	1	1	1	weak	0	negative	12	absent	survived
66	65	F	40134	B/1988/21	homemaker	oral tobacco	Buccal mucosa	Right	11x8x3mm	2mm	absent	absent	absent	29	7	present	WDSCC	T1	stage IVb	T1N3bMx	3	3	9	strong	0	negative	9	at 4th month	deceased (5th month)
67	72	F	48691	B/2247/21	homemaker	smoking	Upper alveolus	Left	60x50x35mm	15mm	present	absent	absent	37	0	present	WDSCC	T4	stage IVa	T4aNoMx	3	3	9	strong	1	weak	9	absent	survived
68	65	F	35280	B/1917/21	homemaker	none	Buccal mucosa	Left	15x10x15mm	11mm	absent	absent	absent	20	2	present	WDSCC	T3	stage IVb	T3N3bMx	3	4	12	strong	1	weak	9	at 1st month	deceased (4th month)
69	45	M	23366	B/1939/21	service	alcohol	Lateral border of tongue	Left	60x35x28mm	11mm	absent	absent	absent	17	0	Absent	MDSCC	T4	stage III	pT4aN0Mx	1	3	3	weak	0	negative	9	absent	survived
70	58	M	932885	B/1725/21	service	alcohol	Lateral border of tongue	Left	50x35x30mm	26mm	absent	absent	absent	36	1	absent	WDSCC	T4	stage IVa	T4aN1Mx	3	4	12	strong	0	negative	9	absent	deceased (12th month)