"STUDY OF KI67 EXPRESSION IN ORAL AND OROPHARYNGEAL SQUAMOUS CELL CARCINOMA"

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DR. Vijayalaxmi Dhorigol MD.
Professor and HOD
Department of Pathology,
J. N. Medical College,
Belagavi, Karnataka

Professor & Head
Department of Pathology
J.N. Medical College, BELAGAVI.

Date: 28/6/24
Place: Belagavi.

DR.(Mrs) N. S. Mahantashetti MD (Paed)
Principal
J. N. Medical College,
Belagavi, Karnataka.

PRINCIPAL
J.N. Medical College,
BELAGAVI- 590 010

Date: 28/06/2024
Place: Belagavi.

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Nehru Nagar, Belagavi- 590 010, Karnataka, INDIA

0831 - 2471350 0831 - 2470759 www.jnmc.edu principal@jnmc.edu

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Principal,
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To,
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Postgraduate Student,
2021-22 Batch,
Department of Pathology
J. N. Medical College, Belagavi.

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JAWAHARLAL NEHRU MEDICAL COLLEGE,
NEHRU NAGAR, BELAGAVI-590010 (KARNATAKA-INDIA)

Website: http//www.jnmc.edu
E-Mail : dome@jnmc.edu

Phone: (+ 91-(0)831 Office : 2472550
Principal: 2471701
Fax No. +91 (0)831 – 2470759

Ref No.MDC/JNMCIEC/ 137

Date: 27/09/2022

To

BN0121004

PG Student in Pathology,
J. N. Medical College,
BELAGAVI.

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(Dr. Smita Sonoli)
Member Secretary
JNMC Institutional Ethics Committee
J.N.Medical College, Belagavi.

(Dr. Harsha Hegde)
Chairman,
JNMC Institutional Ethics Committee
J.N.Medical College, Belagavi

LIST OF ABBREVIATIONS USED

- AdSCC – Adenoid squamous cell carcinoma
- ASC – Adenosquamous carcinoma
- BSCC – Basaloid squamous cell carcinoma
- DAB – Diaminobenzidine
- DPX – Dibutylphthalate Polystyrene Xylene
- EGFR – Epidermal growth factor receptor
- H & E – hematoxylin and eosin
- HN SCC – Head and neck squamous cell carcinoma
- HPV – Human Papilloma virus
- HSGAG – Heparin sulfate glycosaminoglycan
- HSV – Human Simplex Virus
- IHC – Immunohistochemistry
- Ki67 LI – Ki67 Labelling Index
- MDSCC – Moderately differentiated squamous cell carcinoma
- OC-SCC – Oral cavity squamous cell carcinoma
- OP-SCC – Oropharyngeal squamous cell carcinoma
- OIN – Oral Intraepithelial neoplasia
- OSCC – Oral Squamous cell carcinoma
- PDSCC – Poorly differentiated squamous cell carcinoma
- pKi67 – Phosphorylated ki67
- PSCC – Papillary squamous cell carcinoma
- SCSC – Spindel cell squamous cell carcinoma
- TNM – Tumor, Node and Metastasis

- VC – Verrucous carcinoma
- VEGF – Vascular endothelial growth factor
- WDSCC – Well-differentiated squamous cell carcinoma
- WHO – World Health Organization

ABSTRACT

"STUDY OF KI67 EXPRESSION IN ORAL AND OROPHARYNGEAL SQUAMOUS CELL CARCINOMA"

Introduction: Oral and oropharyngeal squamous cell carcinomas are a common cancers in India, particularly in males, and are a significant cause of death. The major risk factors include tobacco and alcohol. Ki67 is a nuclear protein seen in proliferating cells but is absent in resting cells. Ki67 can be used as a prognostic marker for predicting the patient's survival. Hence, the current study aims to examine the expression of Ki-67 in oral and oropharyngeal squamous cell carcinomas.

Objectives-

1. To estimate the prevalence of Ki67 expression in oral and oropharyngeal squamous cell carcinomas.
2. To correlate the expression of Ki67 in oral and oropharyngeal squamous cell carcinoma with histological grading.

Material and methods: A one-year prospective study of 44 cases of oral and oropharyngeal squamous cell carcinomas was taken and histological grading was done as per modified Broder's grading system. All the oral and oropharyngeal squamous cell carcinoma cases were studied for Ki67 expression and were scored as 1+ (10-30%), 2+ (30-50%), and 3+(>50%). Cases showing Ki67 positivity and its association with histological grading were studied. A p-value of <0.05 was considered statistically significant.

Results: A total of 44 cases were studied, out of which 33 were oral squamous cell carcinoma and 11 were oropharyngeal squamous cell carcinoma. The mean age in our study was 51.50±10yrs with the youngest being 30 years and the oldest 72 years of age. Males comprised 79.55% of total cases. Out of 44 cases, 23 were moderately differentiated, followed by 12 cases of well-differentiated and 9 cases of poorly differentiated carcinoma. Ki67 expression was highest in poorly differentiated carcinoma and lowest in well-differentiated carcinoma.

Conclusion: In our study, both oral and oropharyngeal squamous cell carcinomas showed male predominance. There was a statistically significant association between Ki67 expression and histological grading (pvalue<0.0001). No statistically significant correlation between Ki67 expression and age, sex, or type of cancer was seen. Thus, it can be used as a prognostic and proliferative marker in oral and oropharyngeal squamous cell carcinoma.

Keywords – Squamous cell carcinoma, Ki67, oropharyngeal, oral

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INTRODUCTION

Oral cancer ranks as the second most prevalent form of cancer in developing nations such as India.[1] In the year 2022, India reported 143,759 new cases and 79,979 fatalities due to oral and lip cancer, while the global statistics for that year revealed 389,485 new cases and 188,230 deaths[.1,2] In India, oropharyngeal carcinoma holds the 16th position among common carcinomas, with 106,316 new cases and 52,268 deaths globally in 2022, including 23,174 new cases and 14,202 deaths[.1,2]

The escalating burden of oral cancer cases and associated mortality underscores the need for an in-depth examination of factors that can enhance survival rates and the prognosis for oral squamous cell carcinoma. Risk factors linked to oral carcinoma encompass tobacco and alcohol consumption, with HPV infection also playing a role.[3]Notably, the high consumption of tobacco and alcohol, especially tobacco quid placement in the gingivobuccal sulcus, is associated with an increased likelihood of carcinoma development[.4,5, 6,7] Squamous cell carcinoma of the lip (42%), tongue (22%), intraoral carcinoma (17%), gingiva (6%), and palate (5.5%) are the most commonly affected areas in decreasing order. The most frequent main location of oropharyngeal squamous cell carcinoma is the palatine tonsils, followed by the pharynx, soft palate, base of the tongue, and uvula.[3]

Ki-67, a gene on human chromosome 10 (10q25), exhibits maximum expression during mitosis, while absent during the G1 and early S phases. [8,9,10,11] During mitosis, most of the protein is found on the surface of chromosomes, whereas in interphase, it is exclusively present within the cell nucleus.[4] Ki-67 is not present in resting cells. Still, it is detected in proliferating cells, making it a potential tool for rapidly estimating the extent of proliferation in neoplasms and a promising prognostic marker[.4,8]

In developing countries like India, where there is a rising prevalence of oral and oropharyngeal carcinomas leading to increased mortality, this study seeks to explore the potential of Ki-67 as a prognostic marker to improve the prognosis of patients with these conditions.

In cases of oral cancer where treatment decisions based on factors like lymph node metastasis, histopathological type, and grade have been deemed unsatisfactory, there is a growing recognition of the importance of biomolecular markers as valuable diagnostic and prognostic indicators. Scholzer and Gerdes, identified Ki67 antigen in mid 1980s.The atomic weight of 2 proteins encoded by ki67 are 395 and 345 kDa. It has a relatively short half-life of approximately 1 to 1.5 hours. [12]

Ki67 is demonstrated in all the phases in the cell cycle except Go. Furthermore, there is a sharp decline in its levels during later stages of mitosis, specifically in anaphase and telophase.[12] In the context of human oral squamous cell carcinoma (OSCC), histologic grading strongly correlates with cell proliferation in invasive tumors, as measured by Ki-67. This correlation provides valuable information regarding the degree of aggressiveness and the prognosis of OSCC, highlighting the significance of Ki-67 as a biomarker in this context.[12]

Prognostic assessment of pKi-67 has been investigated in multiple tests; its potential as a reliable marker has been observed in the prostate, cervix, lung, delicate tissue, and focused sensory system.[13,14,15,16]

OBJECTIVES

1. To estimate the prevalence of Ki-67 expression in oral and oropharyngeal squamous cell carcinomas.
2. To correlate the expression of Ki-67 in oral and oropharyngeal squamous cell carcinoma with histological grading.

REVIEW OF LITERATURE

EMBRYOGENESIS-

The development of the head and neck involves branchial or pharyngeal arches that appear during the fourth and fifth weeks of embryonic development. These arches give rise to various structures in the head and neck region. The ectodermal extension of these arches is referred to as the pharyngeal cleft, while the endodermal extension is known as the pharyngeal pouch.

The internal pharyngeal gut develops along the lateral walls of these arches, particularly within the pharyngeal pouches. This intricate process contributes to the formation of the complex anatomy of the head and neck.[17,18] (Figure 1)

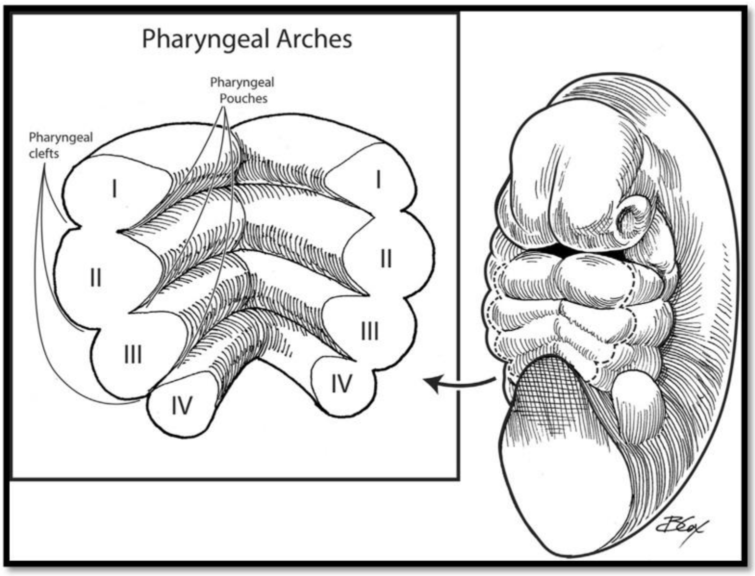


Figure 1- Pouch and cleft formations in the pharynx[17]

The oral cavity is developed from the first branchial arch during embryonic development. By the end of the fourth week of development, several processes become visible, including two maxillary processes, two mandibular processes, and the frontonasal process. The fusion of the midline structures between the face and the palate is completed between the 6th and 12th weeks of gestation. By the 6th week, there is already a fusion of the upper lip. During weeks 6 to 10 of gestation, the anterior part of the hard palate begins to form. In the sixth week of development, the palatine shelves—which are essentially extensions of tissue—decline on both sides of the tongue. These shelves protrude from the maxillary prominences and are essential in the development of the intricate anatomy of the oral cavity as well as the construction of the hard palate.[17, 18]

Failed midline fusion during embryogenesis results in a variety of disorders such as mucous cleft like clefting of the palate, and lips.[19]

The development of the palate and the palatine tonsils continues during embryonic growth. By the seventh week of development, the palatine shelves, which are located above the tongue, become horizontal and fuse, forming the secondary palate. The palatine tonsils arise from the epithelium of the second pharyngeal pouch. Lymphatic tissue starts to invade the primitive palatine tonsil during the third and fifth months of development.[17,18]

The tongue's formation involves three lingual swellings by the end of the fourth week. These include one medial lingual swelling, known as the tuberculum impar, and two lateral lingual swellings. These three swellings originate from the second branchial arch.[17,18]

Due to the outgrowth of the tuberculum impar, the anterior two-thirds of the tongue becomes enlarged. A terminal sulcus, in the shape of a shallow V-shaped groove, separates the enlarged anterior part from the posterior 1/3rd of the tongue. It is derived from the second, third, and fourth branchial arches. A second median swelling called the hypobranchial eminence, is formed by mesoderm originating from the second, third, and fourth arches. The epiglottis develops as a third median swelling that originates from the posterior region of the fourth branchial arch. This complex process results in the formation of the tongue and its associated structures.[17,18] (Figure 2)

After around five to six weeks, the single layer of epithelial cells that lines the oral cavity divides into two layers. Shortly after, dense ectomesenchyme begins to secrete the irregular extracellular fibers.[20] By week 10, the epithelium has several layers.[21] At this stage, the surface features of the oral mucosa also start to emerge, including the incisive papilla, palatal rugae, and papillae of the front two-thirds of the tongue. In the connective tissues, capillary buds and collagen also begin to form. The masticatory mucosae differ noticeably from the lining mucosae in that the latter has more cells and fibers.[20]

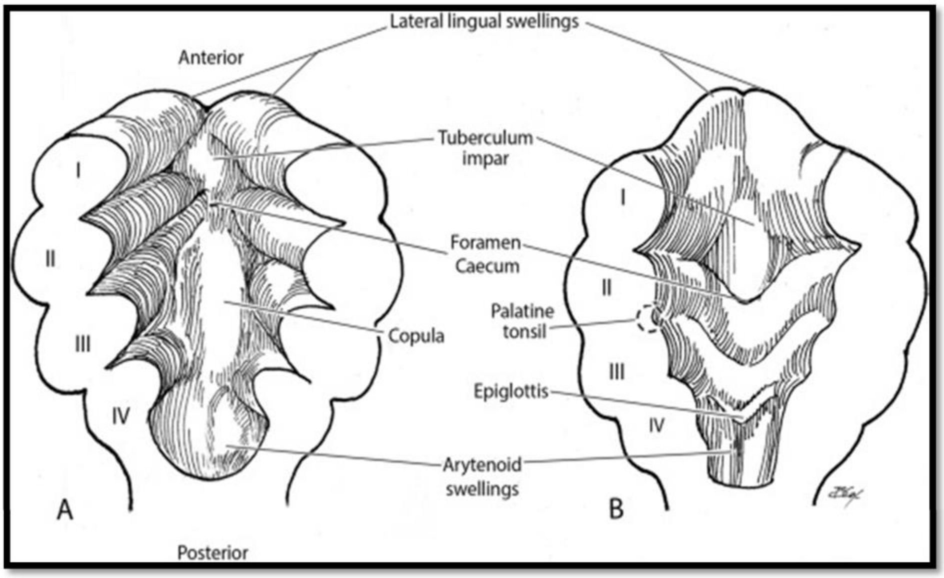


Figure 2- Tongue, palatine tonsils, and epiglottis development[17]

ANATOMY-

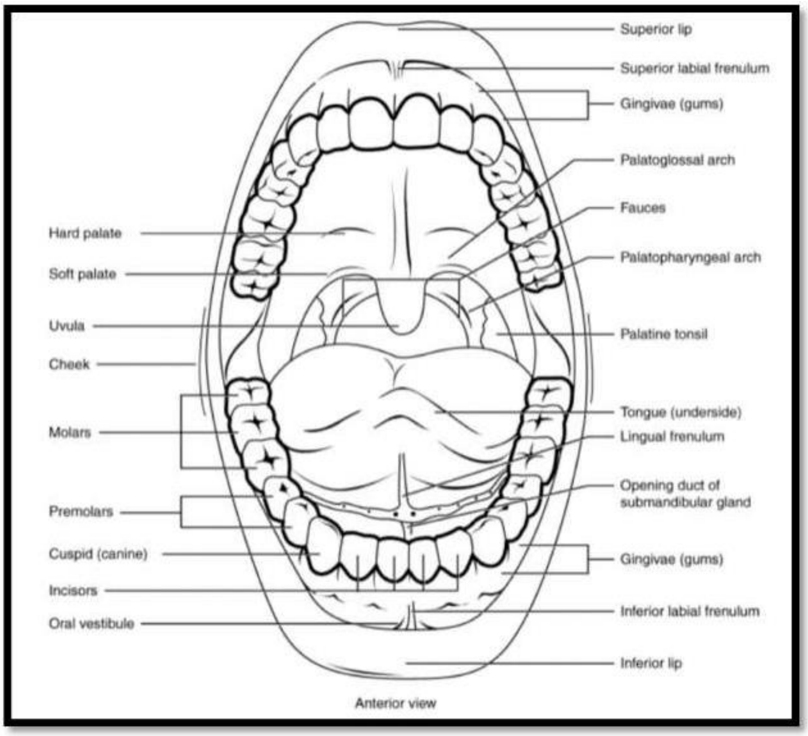


Figure 3- Anterior view of Oral cavity[22]

The oral cavity is divided into the Vestibule and the Oral cavity Proper

VESTIBULE-

This is a narrow gap located between the cheeks and the gums, connecting to the outer surface through the oral fissure. The oral vestibule is delimited externally by the lips and the mucosa of the cheeks, while internally, it is bordered by the alveolar processes and the teeth. The vestibule links to the interior of the mouth through the space behind the third molar tooth and extends to the outside via the oral fissure. Opposite the upper second molar tooth, you can find the opening of the parotid gland duct into the vestibule, which releases saliva.[23,24,25]

ORAL CAVITY PROPER-

ROOF

The hard palate and the soft palate are the two components that make up the actual roof of the mouth.

The front of the mouth is where the hard palate is located. This bony structure acts as a partition between the oral and nasal cavities. Oral mucosa, which is made up of stratified squamous epithelium, covers the lower surface, while respiratory mucosa, which is lined on the upper surface, is distinguished by ciliated pseudostratified columnar epithelium.

On the other hand, the hard palate continues into the soft palate in the posterior region. It is mostly made up of muscles, as opposed to hard palate. It serves like a valve that can be raised to divide the nasopharynx and oropharynx. It is depressed to close off the oropharyngeal isthmus.[24]

LATERAL WALL – CHEEKS

The buccinator muscle, which is internally covered by the oral mucous membrane, makes up the cheeks. The buccal branches of the facial nerve innervate the buccinator muscle, which contracts to help hold food between the teeth during chewing (Cranial Nerve VII).[24]

FLOOR

The oral cavity floor is composed of the following elements:

- The bilateral mylohyoid muscles comprise the muscular diaphragm. These muscles help move the larynx forward during swallowing and provide structural support to the lower part of the mouth.
- The geniohyoid muscles are crucial in propelling the larynx forward when swallowing.
- The tongue, which stays attached to the floor via the tongue's frenulum, an oral mucosal fold.
- Salivary ducts and glands.[24]

1 . LIPS-

The labial commissures join the longer upper lip and the shorter lower lip where they meet at the corners of the mouth. The nasolabial fold divides the lips from the cheeks. Every lip has two separate sections: an exterior darker dry vermilion and an interior pale wet vermilion. The red line divides these two areas apart. A white line denoting the lip's purely cutaneous section is also evident on the exterior.[24]

2. FLOOR OF MOUTH

With a posterior base, the floor of the mouth resembles a quadrangular pyramid. It has been divided up into three different zones:

1. The front floor of the mouth, which is located before the lingual frenulum.
2. Between the mandibular gingiva and the lateral edges of the tongue are two sublingual folds.
3. In addition, the submandibular gland's excretory salivary duct and several tiny ducts that come from the sublingual gland are located on the floor of the mouth.[24]

3. TONGUE

The tongue is comprised primarily of muscle and has a mucous membrane covering it almost completely. It is innervated by the 5th cranial nerve.

There are three primary sections of the tongue:

The tip.

The body

The base

The dorsal (superior) and ventral (inferior) surfaces make up the tongue's two surfaces. The median sulcus divides it into left and right halves, forming the terminal sulcus, a V-shaped furrow that separates the tongue's base from its body.

The foramen caecum, a vestige of the proximal thyroglossal duct, is located at the tip of the terminal sulcus. Furthermore, the lingual tonsils are located at the base of the tongue.

The superior surface of the oral part of the tongue is covered by numerous papillae, each with its distinctive characteristics:

- Filiform papillae are small cone-shaped projections of the mucosa that end in one or more points. They do not contain taste buds.[24]
- Fungiform papillae are rounder and larger than filiform papillae. They tend to cluster along the edges of the tongue and do contain taste buds.[24]
- Vallate papillae are the largest of all. They are arranged immediately in front of terminal sulcus in V shaped manner. They are cylindrical blunt-ended invagination. They also contain taste buds.[24,25]

Foliate papillae are found near the terminal sulcus on sides of the tongue., They also have taste buds.

In summary, all types of papillae on the tongue contain taste buds except for the filiform papillae.[24]

The undersurface of the oral part of the tongue does not have papillae but instead features several linear mucosal folds. Among these folds, there is a single median fold known as the frenulum of the tongue, which connects to the mucosa covering the floor of the oral cavity. The frenulum overlies a midline sagittal septum, which internally separates the right and left sides of the tongue.

On either side of the frenulum, there is a lingual vein. Adjacent to each vein, there is a rough and fimbriated fold[.24]

The muscles that make up the inside of the tongue are called the intrinsic muscles. They regulate the tongue's functions, such as speaking, chewing, and any other action that involves tongue movement. The muscles that are external to the tongue and only attached to specific areas of it are known as its extrinsic muscles. These facilitate the tongue's ability to perform more complex motions, such as protrusion and retraction.[.24] The superior longitudinal, inferior longitudinal, transverse, and vertical muscles are the four intrinsic muscles of the tongue. The genioglossus, hyoglossus, styloglossus, and palatoglossus muscles are the four extrinsic tongue muscles[.24]

BLOOD SUPPLY

Arteries

Tongue along with other structures like gingiva, sublingual gland, and oral cavity mucosal surface are supplied by lingual artery .[24]

VEINS

The drainage of the tongue is facilitated by two main veins:

- Dorsal lingual vein
- Deep lingual vein.

Deep lingual vein runs on outer surface of hyoglossus muscle. It exits the floor of the oral cavity through an aperture known as the oropharyngeal triangle. This triangle is formed by the margins of the mylohyoid, superior constrictor, and middle constrictor muscles. The deep lingual vein eventually drains into neck via internal jugular vein.[24]Lingual artery along with dorsal lingual vein travels admist 2 muscles namely genioglossus and hypoglossus.. It plays a role in the tongue's venous drainage.[24]

INNERVATION

MOTOR SUPPLY

The hypoglossal nerve (XII) supplies the majority of innervation to the tongue muscles. One muscle, the palatoglossus, is an exception, as it receives innervation from the tenth cranial nerve.)

SENSORY SUPPLY

The general sensation and taste from the posterior portion is transmitted by ninth cranial nerve. A major branch of the mandibular nerve (V3), the lingual nerve provides general sensory innervation from the anterior two-thirds. The chorda tympani branch of the facial nerve carries taste and other special senses.[24]

LYMPHATIC DRAINAGE

The lymphatic drainage of the tongue is organized as follows:

- The posterior one-third directly drains into the jugulodigastric node of the deep cervical chain.
- The anterior two-thirds is drained by deep cervical nodes directly and indirectly into submental and submandibular lymph nodes.
- In lymphatic channels eventually drain into nodes of deep cervical chain situated beside internal jugular vein. [24]

PALATE:

The palate is the U- or V-shaped arched roof of the oral cavity. The front portion of the hard palate and the oral mucosa are firmly joined. The hard palate divides the nasal and oral chambers..[26] Together with the uvula and the moveable part of the roof of the mouth, the soft palate forms the posterior side of the palate and helps differentiate the oropharynx from the nasopharynx. This region of the palate controls swallowing and prevents food from re-entering the nostrils.

There are two ways to expand the soft palate. The front segment connecting the soft palate to the tongue is known as the palatoglossal arch, and the posterior segment connecting the palate to the pharynx is known as the palatopharyngeal arch. These arches are often referred to as the anterior and posterior palatal pillars. The palatine tonsils are usually located in between these two arches.[26]

Hard Palate

The hard palate forms the bottom of the nasal cavities and is covered above by the respiratory mucosa.

Below, it forms a large percentage of the oral cavity's roof, which is made up of a closely linked layer of oral mucosa.

Soft Plate

The soft palate acts as a valve, helping to close the oropharyngeal isthmus when it is depressed. It divides the oropharynx through the nasopharynx when it is raised. This is made up of 5 muscles. The mucosal lining of the nasal, oral, and throat cavities is continuous with the mucosa covering it. These muscles descend into the palate through the base of the skull and include the tensor and levator veli palatini. Palatoglossus and palatopharngeus other 2 muscles via tongue and pharynx ascend into palate. The musculus uvulae, is the last muscle. It is associated with the uvula.

All the muscles are supplied by tenth cranial nerve except tensor veli palatini . This is innervated by mandibular nerve.[24]

OROPHARYNGEAL ISTHMUS

The oropharyngeal isthmus serves as the gateway between the oral cavity and the oropharynx. It is defined by its borders:

- Palatoglossal arches bounds it laterally
- Soft palate makes the superior border
- On inferior surface it is delinated by the sulcus terminalis.[24]

TEETH AND GINGIVAE

The gingivae, or gums, are specialized areas of oral mucosa that surround the teeth and extend over adjacent portions of the alveolar bone.[26,27] The Maxillary artery directly or indirectly via its branches gives supply to teeth. The gingivae receive their blood supply from different sources depending on whether they are facing towards buccal side or palatal side.

More specifically, branches from the tongue's lingual artery supply the lingual side of the lower teeth's buccal gingiva, while the inferior alveolar artery supplies the lingual side.

The posterior superior and anterior alveolar arteries send branches to buccal gingiva of the upper teeth. The incisor and canine teeth receive blood from branches of the nasopalatine artery, however, premolar and molar teeth receive blood from larger palatine artery, which supplies blood to the palatal gingiva.

Venous drainage has similar passage as arteries. It eventually drain into facial vein. Lymphatics drain into the submandibular, submental, and deep cervical lymph nodes.

Regarding innervation, all the nerves that provide sensory input are branches fifth cranial nerve.[24]

HISTOLOGY

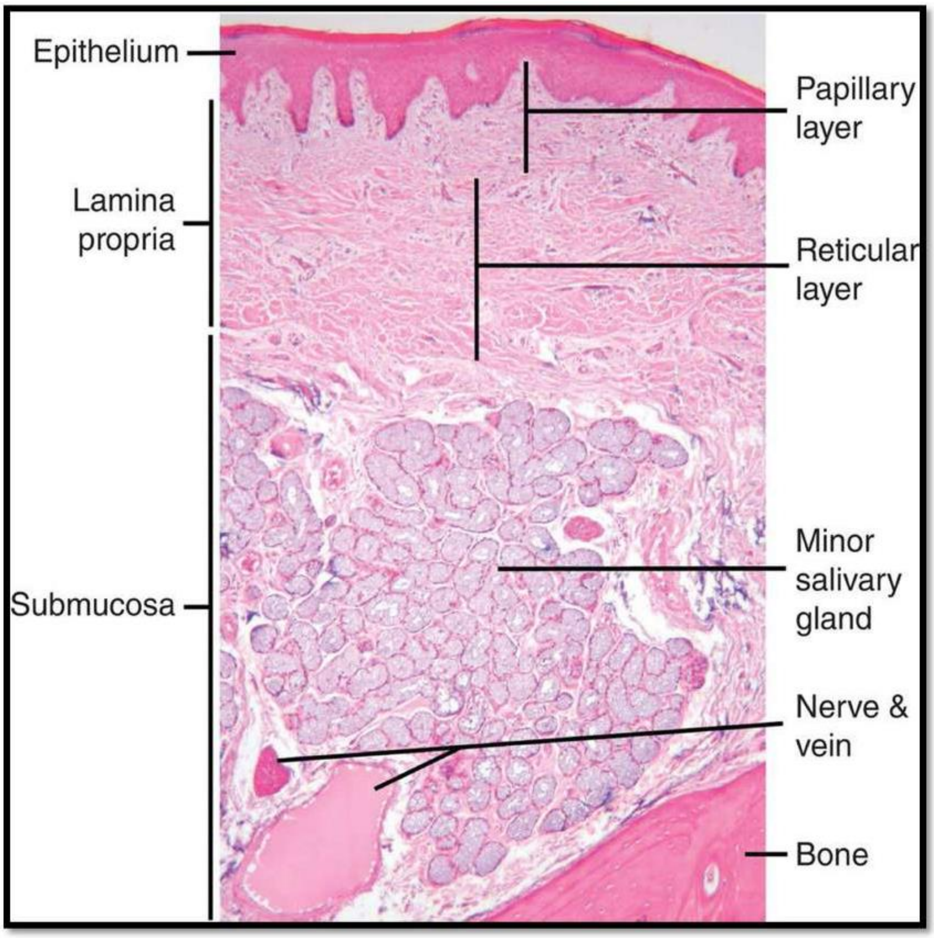


Fig 4 - Histology of Oral Mucosa

(Image retrieved on 15.04.2022, from https://pocketdentistry.com/12-oral-mucosa)

Oral mucosa is made of 3 layers histologically: (Figure 4)

- Oral epithelium is formed by stratified squamous epithelium
- The lamina propria which is present underneath the epithelium
- Submucosa which is deepest connective tissue which is arranged irregularly and is dense. It is absent in certain regions of oral cavity. In this part, lamina propria directly adheres to muscle or bone below it.[28]

There are 3 types of oral mucosa

- Lining
- Masticatory
- Specialized

This has varying thickness. This depends on its location, functional and mechanical needs in oral cavity. It is semipermeable and avascular.

Interdigitations define the interface between lamina propria and epithelium. The epithelium is firmly bound by a non-cellular basement membrane, which is located between these two tissues. Epithelium and underlying connective tissue are separated by a basement membrane It provides support to the epithelium. Electron microscopy reveals a more detailed view, showing the basal lamina, which consists of

- lamina lucida
- lamina densa.

The oral mucosa has 3 types. Each one has distinctive histological, clinical, and functional properties

- Lining (movable) mucosa, It is covered by stratified squamous epithelium which is non-keratinized. It is seen at various sites like the floor of the mouth, soft palate, cheeks, lips, alveolar mucosa, and vestibular fornix. [28]
- Masticatory mucosa, It is tightly adherent to bone below it. It is found in the gingiva and hard palate. It is keratinized or para-keratinized, enabling it to better withstand the stress during mastication.[28,29]

- Specialized mucosa, It is covered by keratinized or non-keratinized stratified squamous epithelium. It is observed in the dorsum part of tongue. It contains taste buds which help in taste sensation. Due to its involvement in mastication, it is sometimes classified as masticatory mucosa.

Masticatory, mucosa is keratinised oral mucosa. It is made up of 4 layers from deepest to most superficial:

1. Stratum basale
2. Stratum spinosum
3. Stratum granulosum
4. Stratum corneum.

In epithelium which is non-keratinized, like that found in lining mucosa, there are two additional layers above the stratum basale:

- The stratum filamentosum
- Stratum distendum.

It does not have granular layer and has a thinner spinous layer. Desmosomes hold the epithelium's cells together. They gradually flatten from the stratum basale to the stratum corneum. They lose their nuclei and take on a scaly or squamous appearance. The primary component of these squamous epithelial cells is cytokeratin.

The stratum basale is situated above basement membrane. It consists of cuboidal or columnar cells It is anchored to basement membrane by hemidesmosomes. These cells are mitotically active. Just above the stratum basale, the stratum spinosum is formed by several layers of larger, prickle-shaped cells. The stratum granulosum follows, with cells containing small keratohyalin granules. These granules stain intensely with hematoxylin. It is composed of very flat cells which do not have a nucleus. They stain pink with eosin stain .[28]

Lamina propria is situated below the epithelium. It is made up of fibroblasts, mast cells, blood vessels, inflammatory cells, and macrophages. All are engrossed within the amorphous substance made up of glycoproteins and proteoglycans.

Lamina propria is made up of 2 layers

- The superficial papillary layer
- the deeper reticular layer.

A larger surface area for the transportation of nutrients is provided by the undulating papillae ridges, which are generated by thin collagen fibers that are orientated unevenly and join with the epithelium to form the papillary layer. Numerous capillary loops can be observed in the papillary layer. The basal fibers are eventually arranged perpendicularly and are connecting to the periosteum. Thick collagen fibers are seen in reticular layer. These are oriented parallelly in context with surface.

These fibrous attachments, known as mucoperiosteum, give the oral mucosa the ability to withstand shear and compression because of their strong bond with the bone. The fibroblast acts as a main cell type in the lamina propria. It takes part in wound healing. Certain diseases, such as drug-induced gingival overgrowth, are caused by medicines that activate and multiply the fibroblasts in gingival tissues. This causes an increase in the amount of the amorphous substance's glycosaminoglycan being secreted. During wound healing, macrophages primarily engage in phagocytic activities and also promote fibroblast growth. The cytoplasmic granules that hold histamine and heparin are what make them unique. It is well known that histamine starts the inflammatory process's vascular alterations. Collagen and elastin are the two primary fiber types found in the lamina propria's connective tissue. The main constituents of this tissue are collagen fibers, specifically types I and III. They are essential for preserving the oral mucosa's strength and structural integrity. The layer of fibro collagenous and elastic tissue is situated below the lamina propria. It consists of blood vessels and nerves. This is referred to as the submucosa. It is common feature of buccal cavity except for the attached gingiva and the hard palate. These are covered by masticatory mucosa. Here because of the absence of submucosa, lamina propria have direct attachment with underlying bone. This forms a mucoperiosteum.

Submucosa may also have ectopic sebaceous glands, known as Fordyce granules at certain locations of oral cavity. They can be present as normal variation also. But few studies have observed that they are seen increased in number in individual with high lipid profiles. So clinical findings should not be ignored. They are typically observed on labial and buccal mucosa. Factors such as age and smoking have been associated with a reduction in their density.[28]

LIPS

The thin epidermis, or skin on the outside of the lip, is made up of stratified squamous keratinized epithelium. This category of epithelium comprises desquamate-shedding surface cells. The dermis, which is found beneath the epidermis, has simple tubular sweat glands as well as sebaceous glands connected to hair follicles. The smooth muscles known as the arrector pili, which adhere to the hair follicles, are also located in the dermis. An artery and a venule are two examples of blood vessels that are visible around the edge of the lip. The orbicularis oris, a layer of striated muscles located in the core of the lip, is essential to lip function.[30](Figure 5)

The mucocutaneous junction is the transition zone that is located between the oral epithelium and oral skin epidermis. Lips internally are covered by stratified squamous epithelium. The thickness of which is more than the epidermis. Reflecting its adaptation to the specific conditions and functions of the oral cavity.[30]

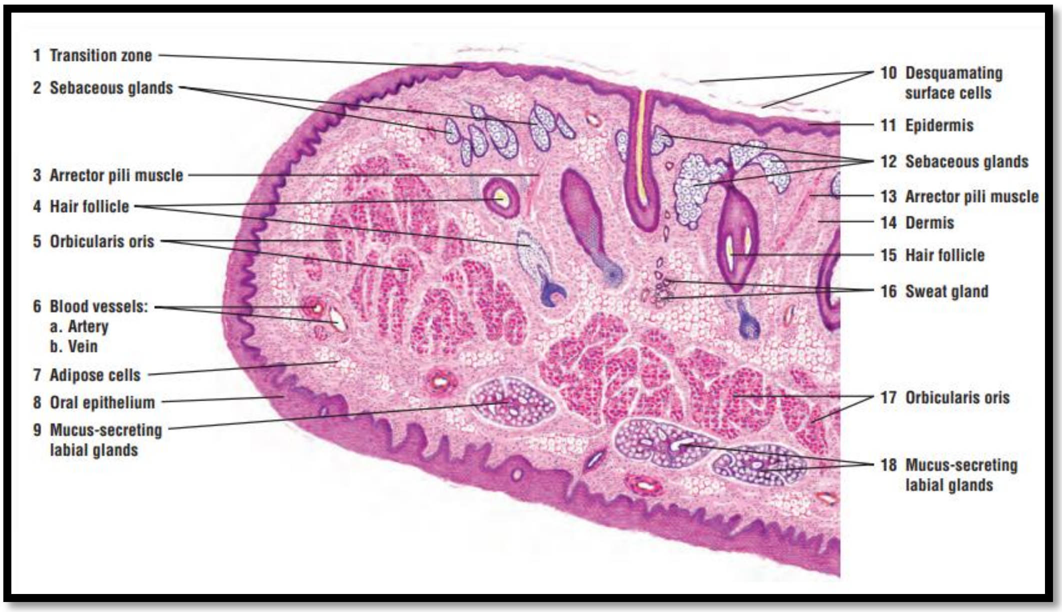


Figure 5- Lip (longitudinal section)- H&E, Low magnification. [30]

Anterior Region of the Tongue: Apex

The protective mucosa that lines the oral cavity is made up of the lamina propria, a layer of connective tissue, underneath the outer layer of epithelium. The tongue's texture is rough on the dorsal surface due to the many mucosal projections called papillae. Filiform papillae are slender and cone-shaped. These are maximum in number. They are seen on dorsal surface. Keratinization is seen on its tips. Other Fungiform papillae are less in number. These are having rounded surface and are broad. They have prominent lamina propria as a core .[25]

The tongue is mainly made up of crisscrossing bundles of skeletal muscle. These are typically observed in various planes. The muscle bundles are surrounded by connective tissue. These contain blood vessels as well as nerve fibers.

Anterior lingual gland is situated in its lower half. It has both mucinous as well as serous acini. The interlobular ducts pass into the larger excretory duct of the lingual gland via anterior lingual gland. This opens on ventral surface of tongue into the oral cavity.[25](Figure 6)

The stratified squamous epithelium of the tongue covers the circumvallate papillae and is known as the lingual epithelium. Numerous secondary papillae emerge from the lamina propria and project into the stratified squamous epithelium that covers the papilla. Each circumvallate papilla has a deep trench or furrow at its base, and the lateral surface of the circumvallate papilla, as well as the outer wall of the furrow, contains oval taste buds. Numerous tubuloacinar serous glands are located in the midline of the tongue, as well as in the lamina propria.[25]

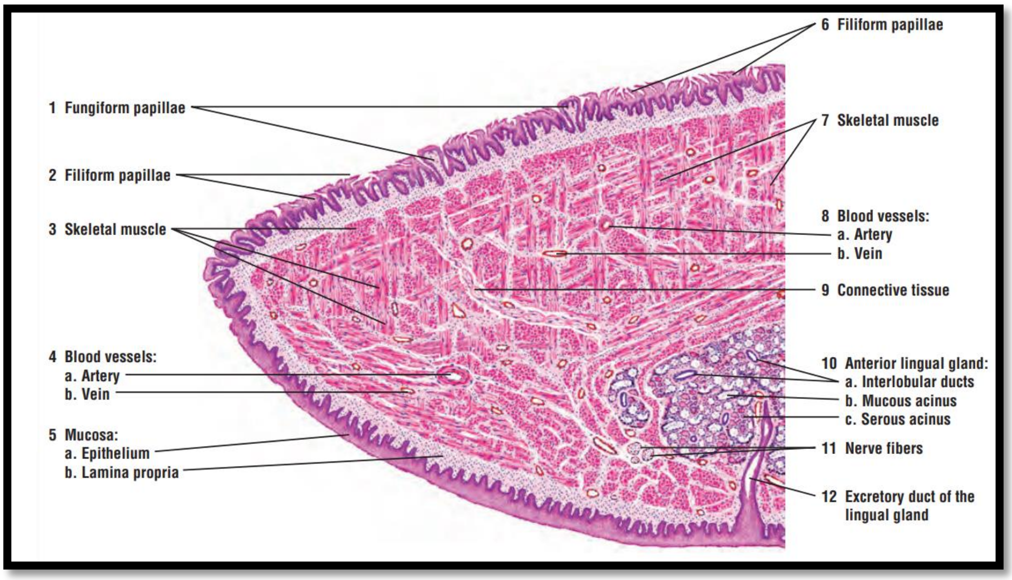


Figure 6 - Anterior region of the Tongue (longitudinal section)- H&E, Low magnification [30]

Figure 7 - Cross section of a posterior tongue: serous (von Ebner`s) glands, surrounding furrow and H&E, Low magnification.[30]

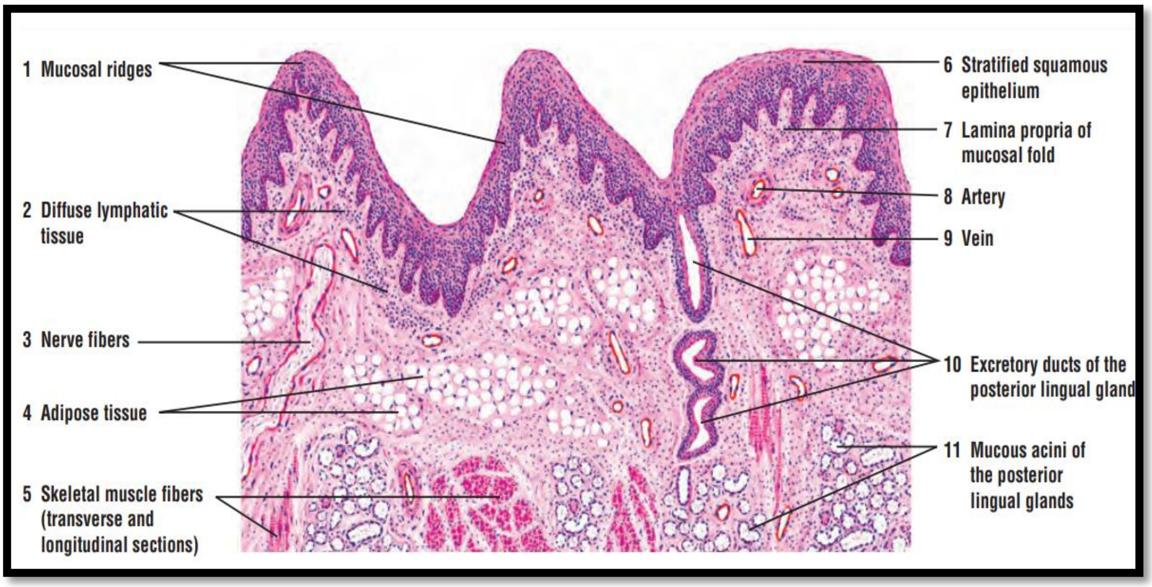


Figure 8 - Posterior tongue: Behind circumvallate papillae and near lingual tonsils (longitudinal section), H&E, low magnification.[30]

Posterior Tongue: Behind Circumvallate Papilla and Near Lingual Tonsil

The anterior two-thirds of the tongue is distinct from the posterior one-third by the presence of a depression called the sulcus terminalis. The posterior region of the tongue is located behind the circumvallate papillae and in the vicinity of the lingual tonsils. On the dorsal surface of the posterior tongue, there are typically large mucosal ridges, elevations, or folds that resemble the large fungiform papillae found on the anterior tongue. The mucosal ridges and folds are covered by a stratified squamous epithelium without keratinization. They may have lymphatic nodules of lingual tonsils. There is an absence of filiform and fungiform papillae.[29]

The lamina propria of the mucosa in the posterior tongue is wider than the anterior 2/3rd of tongue. Beneath epithelium contains -

- Lymphoid tissue in aggregates
- Adipose tissue
- Nerve fibers
- Blood vessels.

Mucinous acini of lingual glands are situated deeper in lamina propria. These are seen interlaced with skeletal muscle fibers. Between the base of mucosal ridges and fold on the dorsal surface there is an opening of excretory ducts of posterior lingual glands. In the anterior region, the posterior lingual gland comes in contact with the serous gland of circumvallate papillae. It extends via root of tongue on the posterior region of the tongue.[30](Figure 7,8)

LINGUAL TONSILS

These are seen on posterior region on dorsal surface of the tongue. They are lined by squamous epithelium of nonkeratinized stratified type. This lining is also seen in tonsillar crypts. These crypts folds at surface and can extend to lamina propria of the tongue.[30](Figure 9)

In the lamina propria which is situated below the epithelium have numerous lymphatics nodules, few of which can have germinal center.[30]

Figure 9 - Lingual tonsil (transverse section), H & E, low magnification[30]

CLASSIFICATION OF ORAL AND OROPHARYNGEAL SQUAMOUS CELL CARCINOMA

According to Globocan's 2022 estimate, there were approximately 20 million new instances of oral cancer and 9.7 million deaths. Every year, over 52,000 deaths and 77,000 new cases are reported in India.[2]

OSCC is the 16th most frequent cancer globally, accounting for around 389,485 cases annually, and the oropharynx ranks 24th in the list and accounts for 106,316 cases.[2] OSCC, with an age-standardized incidence rate of 4.3 per 100,000 in females and 13.9 per 100,000 in males, is the fourth most prevalent cancer in India in terms of incidence in females, following cancers of the breast, cervix, and ovary.[2]

The oral cavity comprises the floor of the mouth, the labial and buccal mucosa, the retromolar trigone, the anterior two-thirds of the tongue (before the circumvallate papillae), and the hard palate. The oropharynx consists of the posterior pharyngeal wall, palatine tonsils, palatoglossal folds, soft palate, base (or posterior third) of the tongue, and valleculae.[31]

WHO CLASSIFICATION [32] – In annexure

ETIOPATHOGENESIS - OSCC & OPSCC

There have been numerous descriptions of risk factors or potential causal agents for OC.

There is a strong correlation between OC and chemical factors like alcohol and tobacco use, biological factors like syphilis, human papillomavirus (HPV), Oro-dental factors, dietary inadequacies, chronic candidiasis, and viruses.[33]

Chemical Factors

Tobacco

Both oropharyngeal squamous cell carcinoma (OP-SCC) and oral cavity squamous cell carcinoma (OC-SCC) are significantly increased by tobacco use. Although cigarettes are the most widely used type of tobacco in the globe, other combustible tobacco products are popular in different parts of the world. Water pipes (hookahs or nargile), kreteks (clove cigarettes), and bidis (tobacco hand-rolled in tendu leaf) are a few common substitutes for cigarettes. Long-term use of smokeless tobacco is frequently linked to the development of oral cancer, with tobacco-specific nitrosamines being a common cause.[31,34,35] Almost 9500 chemical compounds are found in tobacco and tobacco smoke, many of which have been recognized by regulatory bodies as being harmful to human health.[35] Constantly eating paan and swallowing gutkha causes submucosal tissue to progressive fibrosis. Generally speaking, a variety of risk factors contribute to OSMF, including smokeless tobacco and its constituents, which include areca nuts, slaked lime, and betel quid, which are utilized in paan and gutkha.[36]

It is counted as a group 1 carcinogen .[37]Chewing betel quid (paan) is a prevalent habit among migrants and in many regions of Asia.[38]Betel quid can be made with tobacco, sweets, and/or spices. It is made up of a blend of betel leaf areca nut and slaked lime. Traditionally, tobacco has been blamed for the carcinogenicity of betel quid, although areca nut itself is carcinogenic.[39]

According to available studies, tobacco can alter the epigenetic makeup of oral epithelial cells, suppress a number of the host's systemic immunological responses, and promote OSCC in tissues via oxidative stress caused by its toxic metabolites.[40] N′-nitrosonornicotine (N) and 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) are two nicotine metabolites that have the potential to cause cancer By binding to the nicotinic acetylcholine receptor, NNK and NNN encourage cell division and foster the conditions necessary for tumor formation.[39,41,42]

Alcohol

Studies show a substantial correlation between alcohol intake and OC-SCC and pharyngeal SCC, especially in nonsmokers, making alcohol another independent risk factor.[35] Alcoholic drink ingredients like ethanol are broken down by microflora and epithelial cells producing the recognized carcinogen acetaldehyde.[43]

Alcohol may cause hyposalivation, which increases exposure to locally acting carcinogens, amplifies the toxicity of other carcinogenic agents, increases the permeability of the mucosa by dissolving the lipid component of the epithelium, and indirectly damages cells through acetaldehyde.[43]

Biological Factors

Viruses

Research on the function of oncogenic viruses in human cancer is only being initiated. Viruses can take control of the host's cellular machinery, alter DNA and chromosomal architecture, and cause changes in cell proliferation. Herpes simplex virus (HSV) and HPV have been identified as the primary causes of OC in recent years. [35]

HPV is an established etiological factor in one of the subtypes of HN-SCC. There are many molecular, clinicopathological, and epidemiological studies that acts as evidence for it. Most of these arise in oropharyngeal region mainly lingual and palatine tonsils. They have reduced expression of wild-type p53 and display increased p16 expression. [44,45] There are losses of 9p, 3p, and 17p chromosomes most often in early stage in HPV negative cases. They contain tumor suppressor gene TP53 and CDKN2A which leads to genomic instability and also cell cycle dysregulation.[36,44]

Syphilis

There is limited information regarding the causative relationship between syphilis and oral cancer (OC). Among patients with tongue cancer, there have been reports of 19 and 6% serological positive for syphilis, respectively.[46,47]

Candida

Clinical research has shown that nodular leukoplakia with a Candida infection is more likely to develop dysplasia and malignant transformation.[35,48]

Dental Hygiene and Related Factors

The development of oral cancer has been linked to poor oral hygiene, chronic irritation from sharp teeth, and ill-fitting dentures.[49,50]

Nutritional Factors

It appears that vitamin C and vitamin E, which are responsible for removing free radicals from the cell membrane. By preventing the synthesis of nitrosamine and the chromosomal damage caused by the combination of DNA and some carcinogens, vitamin C also lowers the chance of getting cancer through a variety of methods.[51-54] Vegetables have high concentrations of anti-cancer micronutrients (lycopene, beta- and alpha-carotene, and vitamins A, C, and E) and, in certain situations, the combination of many molecules amplifies their effects.[55]

MOLECULAR PATHOGENESIS OF ORAL AND OROPHARYNGEAL SQUAMOUS CELL CARCINOMA

Tumor suppressor genes, which encourage tumor formation when inactivated, and oncogenes, which stimulate tumor development when activated, are the two main categories of tumorigenic alterations in genetics.[56]

Larger sections of the genetic material, including chromosomes, can be harmed in addition to individual genes by prolonged exposure to carcinogens including alcohol, tobacco, oncogenic viruses, and inflammation. When these genetic changes accumulate, premalignant lesions may appear, which may then progress to aggressive cancer.[57]

Two major tumor suppressor proteins,

The tumor suppressor gene which are mostly inactivated are p16 and p53.[58-60]

P16 takes part in cell growth and cell cycle regulation. It is encoded by gene CDKN2A which is located at chromosome 9p21.In particular, it prevents the cell cycle from moving from the G1 to the S phase by blocking Cyclin D1, another protein. Hence, p16 activity disruption causes a decrease in cell senescence, which in turn causes dysplasia.[61,62]

When hypophosphorylated, the tumor suppressor protein pRb, which is encoded by RB1, prevents the cell cycle from moving from the G1 to the S phase. [63,64] An increased likelihood of malignant carcinoma transformation has been associated with early-stage deregulation of pRb in oral epithelial dysplasia.[65]

The protein p53 controls the cell cycle. When cells are under stress, such as when DNA is damaged, p53 is translocated to the nucleus, where it is involved in several biological events, such as apoptosis or cell growth arrest [66,67]. Most H&N tumors (84%) have mutations in either exon 4 or intron 6 of the TP53. This includes OCSCC. [68,69]

NOTCH pathway

NOTCH1 inactivating mutation is seen in 11 to 19 % of HNSCC. This indicates it may function as a tumor suppressor gene in HNSCC.[66,67,70]

Cell Proliferation

EGFR

When activated, the receptor tyrosine kinase known as the epidermal growth factor receptor (EGFR) upregulates multiple downstream signaling pathways. They play a critical role in tumor growth and proliferation. They are also associated with apoptosis, survival, angiogenesis, invasion, and metastasis. There are various pathways involved in it.[71-75]

Patients with OCSCC have a lower chance of survival and more aggressive tumors when there is a high expression of EGFR.[6,77,76]

AKT signaling activation has a role in OCSCC according to few studies.[56]

CyclinD1

Cyclin D1 protein, encoded by the CCND1 gene, is inhibited by p16 in its activity. In 25–43% of OCSCCs, CCND1 is amplified. Cyclin D1, its product, is overexpressed in oral carcinogenesis during early stage. This is linked to tumorigenic proliferation.[78-81]

STAT3

In OSCC cells, STAT3 is constitutively overexpressed. It regulates the transcription of its downstream target genes, which in turn promotes anti-apoptosis, migration and invasion, angiogenesis, radiotherapy resistance, immune escape, and stem cell self-renewal and differentiation.[82]

Angiogenesis

During the angiogenesis process, vascular endothelial growth factor (VEGF) protein is activated.[83] VEGFA is one VEGF protein family member that has shown promise as an oral tongue SCC prognosticator.82%. Its overexpression is associated with poor survival.[84]

Cell Adhesion and migration

In particular, mesenchymal proteins like N-cadherin and cytoskeletal proteins like vimentin are upregulated during the epithelial-mesenchymal transition, while molecules that control cell adhesion like E cadherin are reduced. Studies using immunohistochemistry have revealed that oral cavity tumors exhibit more vimentin and less E-cadherin. [85,86]

HISTOLOGICAL CLASSIFICATION ORAL CAVITY SCC

The most recent version of the Classification of Head and Neck Tumors mainly distinguishes between well-, moderately-, and poorly-differentiated variations of traditional OSCCs and provides a straightforward grading system based on the Broders criteria.[87]

Keratinizing-type SCC can have a very variable gross appearance, varying from exophytic, fungating tumors to endophytic, ulcerated tumors with elevated margins. Because most tumors cause stromal fibrosis, their cut surfaces are hard and tan-white. [88]

Under a microscope, these tumors are made up of sheets and nests of squamous-differentiated cells. The cells contain round to oval nuclei, frequently with large nucleoli, and definite cell boundaries. They are generally polygonal in shape. The cytoplasm is eosinophilic.

For squamous cell carcinoma of the lip, Broders originally developed histopathological grading based on variations in tumor differentiation .[89] Subsequently, Jakobsson et al., Anneroth et al., and Bryne et al. proposed even more intricate grading schemes.[90-92]

Squamous cell carcinoma has long been classified using a criterion based on the percentage of the tumor that resembles healthy squamous epithelium.

Well-differentiated

Both the cytological and histological features are quite similar to the squamous lining of the oral mucosa. Even though they are well-differentiated, these tumors often contain a lot of pink or clear cytoplasm, intercellular bridges that resemble desmosomes, and sometimes even keratin "pearls."[88]

Moderately differentiated.

They have invading sheets and tumor cell nests of varying sizes with eosinophilic cytoplasm, somewhat pleomorphic nuclei, and occasionally keratin formation or keratin pearls. Compared to well-differentiated forms, the cells often have less cytoplasm and more pleomorphism, and the nests are smaller. [88]

Poorly differentiated

It has strands, small nests, and single cells invading a thick, desmoplastic stroma. Compared to moderately differentiated carcinoma, the cells usually lack large nests and have higher nuclear-to-cytoplasmic ratios as well as greater nuclear pleomorphism.

Nonkeratinizing SCC is primarily seen in the base of the tongue and palatine tonsils, and it is almost always associated with HPV and p16-positive (HPV16 can be shown in over 90% of cases).[88]

VARIANTS OF SQUAMOUS CELL CARCINOMA

Approximately 10-15% of all squamous cell carcinomas (SCC) are variations of conventional oral squamous cell carcinoma (OSCC), which can appear in multiple ways. Verrucous carcinoma (VC), basaloid SCC (BSCC), adenosquamous carcinoma (ASC), spindle cell/sarcomatoid cancer (SCSC), adenoid/acantholytic/pseudo glandular SCC (AdSCC), and papillary SCC (PSCC) are some of these variations.[93] These variations are distinct in their histomorphological look. VC is a well-differentiated SCC with a 5-year survival rate of almost 75%. It does not spread and has a great prognosis.[93-95]

A rare kind of squamous cell cancer having characteristics of an adenoid pattern is called adenoid squamous cell carcinoma. The skin in sun-exposed areas is more typically damaged, especially on the head and neck of older males.[96]

Up to 25% of SCSC cases will eventually migrate to regional lymph nodes; distant metastasis occurs less frequently (5–15%). The 5-year survival rate ranges from 65 to 95 percent.[92,97,98]Aggressive behavior, a worse prognosis, and a tendency toward distant and locoregional metastases—particularly to the lungs—are characteristics of ASC. The larynx is affected 70% of the time, followed by the oral cavity (30%). The data indicates an around 55% 2-year survival rate.[92,99,100] It is believed that BSCC is a high-grade malignancy with a higher risk of distant metastasis. Treatment with intensive multimodality is necessary. 40% of people survive for two years.[92,101] The larynx is more commonly affected by PSCC. When compared to site and stage-matched conventional OSCC, it has a better prognosis.[92]

SQUAMOUS CELL CARCINOMA – PATTERNS OF SPREAD

In oral squamous cell carcinoma, the sites involved are lips (45%), tongue (16%), floor of mouth (12%), buccal mucosa (10%), lower gingiva 12% and upper gingiva and hard palate (5%).[3]

Lip SCC is most common type of oral cancer constituting 42% of cases. Most often the lower lip along its vermillion border of is involved in 90%. SCC of lip involves ipsilateral submandibular and submental lymph nodes, and in case of a midline lesion, it has the capacity for bilateral metastasis.

Preauricular and infraparotid lymph are involved in upper lip SCC.[3]

Twenty-two percent accounts for tongue SCC. Ipsilateral subdigastric, submandibular, and midjugular lymph nodes are involved in tumors affecting anterior $2/3^{rd}$ of tongue. Tumors of midline location and at base of tongue involves bilateral nodes. Level IV lymph node involvement has poor 5-year survival rate.[3]

The floor of the mouth is the third most common site. It makes up approximately 17% of all intraoral carcinomas. The submandibular triangle and subdigastric lymph nodes are the most commonly involved regional lymph nodes.[3]

Carcinoma of the gingiva account for 6% of intraoral carcinomas. It is mainly seen in mandibular bicuspid and molar areas. Metastasis commonly involves the submandibular lymph nodes.[3]

Buccal mucosa SCC is seen in approximately 2% of intraoral carcinomas. It can involve the adjacent soft tissues and even the underlying bony structures. The size of tumor is directly related to extent of involvement. Involvement of submandibular lymph nodes usually happens in late stages. [3]

Palate cancer is the most frequent malignant tumor of the palate, accounting for approximately 5.5% of intraoral Squamous Cell Carcinoma (SCC) cases. Both the soft and hard palates' lymphatic drainage pathways normally pass via the retromolar triangle and connect to the submandibular and retropharyngeal areas, as well as the internal jugular chain's lymph nodes. Approximately one-third of patients initially have cervical node metastases, and this prevalence is comparable for both.

Tonsillar carcinomas are prone to lymph node metastases in general. They can affect the nodes in the posterior cervical triangle, the middle and lower jugular lymph nodes, and the ipsilateral sub-digastric lymph nodes, which are situated beneath the digastric muscle. The aggressive nature of tonsillar carcinomas is highlighted by this pattern of lymph node involvement, which emphasizes the necessity of careful assessment and therapy to reduce the chance of metastasis[3]

Clinicopathologic Presentation

Squamous Cell Carcinoma of the Oral Cavity

Oral cavity squamous cell carcinoma is often preceded by mucosal changes, with the most common ones being leukoplakia and erythroplakia.[102]

Leukoplakia: A white patch or plaque on the oral mucosa that cannot be clinically or pathologically identified as any other particular disease is the standard description of leukoplakia.

It excludes conditions like pseudomembranous candidiasis, lichen planus, tobacco pouch keratosis, nicotine stomatitis, and oral hairy leukoplakia. One variant of leukoplakia called proliferative verrucous leukoplakia (PVL), is particularly concerning. PVL often presents with multifocal lesions that exhibit slow but relentless growth. Over time, these lesions may transform into verrucous carcinoma or conventional squamous cell carcinoma (SCC).[102]

Erythroplakia: It appears clinically as a red, velvety patch with well-demarcated borders., Nearly all true erythroplakias manifest premalignant lesions or invasive SCC. As invasion occurs, the mucosal surface typically becomes irregular, granular, and ulcerated. Exophytic or endophytic mass which has a raised, rolled border is seen when growth is continuously increasing..[103-105]

Oropharyngeal squamous cell carcinoma (OP-SCCs)

Oropharyngeal SCC most commonly develops in the tonsillar region and the base of the tongue. These tumors often present as ulcerated masses, fullness, or irregular erythematous mucosal changes. OP-SCCs are frequently diagnosed at a more advanced stage compared to OC-SCC because of their propensity to grow undetected and their high likelihood of metastasis. Common symptoms of OP-SCC include the presence of a neck mass (indicating metastatic disease), sore throat, and dysphagia.[106]

CLINICAL FEATURES FOR ORAL AND OROPHARYNGEAL SQUAMOUS CELL CARCINOMA (OSCC)-

LIPS-

Lesions on the lower lip that are ulcerative or exophytic are most frequently seen in patients with squamous cell carcinoma (SCC) of the lower lip. Pain and bleeding can occasionally result from these lesions. Slow-growing SCC of the lower lip is well-known. The chin's skin may go numb as the tumor spreads and starts to affect the mental nerve. Sensation of the lower lip as well as chin is provided by the mental nerve, a sensory nerve whose involvement can cause sensory abnormalities. For improved treatment outcomes, it's critical to identify and diagnose SCC of the lower lip as soon as possible. Sensation of the chin as well as is provided by mental nerve, a sensory nerve whose involvement can cause sensory abnormalities. For better treatment results, it's critical to identify and diagnose SCC of the lower lip as soon as possible.

ALVEOLAR RIDGE AND RETROMOLAR TRIGONE

Similar growth patterns can be seen in squamous cell carcinomas of the oral cavity and the alveolar ridge, a section of the gum where the upper teeth are fixed. Exophytic lesions, or infiltrative disease, which is frequently linked to the localized loss of bone, are the two possible presentations.

Usually, discomfort is the main sign of this illness, especially when chewing. Other signs and symptoms could be loose teeth and sporadic bleeding. Unfortunately, the earliest signs of periodontitis or gingivitis can be mistaken for inflammatory disorders, delaying diagnosis and necessary medical intervention. In these kinds of situations, early diagnosis and detection are critical to better treatment outcomes.[106]

FLOOR OF MOUTH

The most common symptom of squamous cell carcinomas of the floor of the mouth is infiltration of lesions that are quite painful. These lesions have the potential to spread in three different directions: deep into the floor of the mouth muscles, anteriorly, invasively into bone, and posteriorly, penetrating the tongue. Sometimes the first sign that points to this condition's diagnosis is an enlarged lymph node in the neck. The management and treatment of such carcinomas depend on an early diagnosis and immediate medical attention.[106]

TONGUE

Both infiltrative and exophytic growth patterns can be seen in cancers in this area. While certain lesions may not hurt, pain is the main and most frequent sign of malignant malignancies. Additionally, patients may have trouble deglutition and speaking.Compared to other malignancies of the oral cavity, these tumors frequently develop more quickly. Before symptoms appear, there may occasionally be a history of protracted leukoplakia, especially in younger female patients. For these illnesses to be treated and managed on time, early diagnosis is essential.[106]

HARD PALATE

Cancers of the hard palate can exhibit various growth patterns, which include deeply infiltrating and destructive lesions, as well as more diffuse superficial lesions associated with microscopic invasion. When it comes to metastatic squamous cell carcinoma of the hard palate, lymph node metastases are less frequently encountered compared to cancers arising in other sites within the oral cavity. The pattern and extent of metastasis can vary depending on the specific location and characteristics of the tumor.[106]

BUCCAL MUCOSA

Compared to other oral cavity malignancies, buccal mucosa tumors frequently have exophytic development behaviors. They are rarely identified as T1 lesions because they are usually quiet in the early stages. Usually, the first complaint is pain, which is followed by symptoms like bleeding and trouble chewing. Patients may experience trismus—a diminished capacity to open their mouth fully—as the disease worsens and spreads into the pterygoid musculature. Patients may find it challenging to chew correctly or open their lips widely as a result of this illness.tongue

BASE OF TONGUE

Because of their aggressive nature and relatively silent location, the base of the tongue cancers are frequently detected at an advanced stage. Usually, patients have pain and trouble swallowing. People who have lost weight in the past or who have a neck mass are frequently present. Cranial nerve involvement can result in referred ear discomfort, or otalgia. A muffled voice may occur in patients as laryngeal involvement advances.

TONSIL, TONSILLAR PILLAR, AND SOFT PALATE

Cancers of the tonsil, tonsillar pillar, and soft palate can present and spread in various ways. Tonsillar pillar cancers are usually more superficial and may progress over a broader region, including the lateral soft palate, retromolar trigone, buccal mucosa, tonsillar fossa, and the glossopalatini sulcus.

In contrast, tonsillar fossa cancers are more likely to be diagnosed at an advanced stage. Approximately 75% of patients present with stage III or stage IV disease. The disease in this area tends to be bulky and can extend to the base of the tongue and lateral pharyngeal wall. Common symptoms include pain, dysphagia, weight loss, and the presence of a neck mass. If the disease progresses to involve the pterygoid muscles posteriorly, patients may experience trismus, a reduced ability to open their mouth fully.

Primary disease of the soft palate may progress more slowly and may remain in the early stages. Tumors in this region can also stay superficial, often appearing as diffuse erythroplakia extending into the hard palate or inferiorly along the tonsillar pillar.[106]

Immunohistochemistry Markers of SCC of the oral cavity

Ki-67

There are two protein isoforms of the nuclear antigen Ki67, which have molecular weights of 345 and 395 kDa. This antigen, which is linked to proliferation, is only found in dividing cells in the G1, S, G2, and M phases of the cell cycle; quiescent cells in the G0 phase do not have it. Low in the G1 and S phases, Ki67 levels peak early in mitosis and then sharply decline in later stages of mitosis. Chromosome 10q25 contains gene for Ki67. This has continuous 29,965 bp sequence with 15 exons and 14 introns. Ki67 repetitions, or 16 homologous regions total of 366 bp, are found in exon 13.[107-110]

With a half-life of only one to one and a half hours, the amount of phosphorylated Ki67 (pKi67) present during the cell cycle is controlled by stability between synthesis and degradation. Systems for grading Ki67 rely on the proportion of tumor cells that have been stained with an antibody. Labeling index (LI) is a measure of the percentage of immunoreactive tumor cell nuclei that indicates the degree of proliferation. An indication of the possibility of tumor recurrence that is more accurate can be obtained by combining the Ki67 proliferation marker with well-established histopathological characteristics. While Ki-67 has shown promise as a diagnostic marker, data on its diagnostic use is limited. This difference is due to varying laboratory techniques and statistical methods. Pathogenesis in cancer is complicated. Hence early diagnosis is challenging since symptoms are generally manifested in advanced stages. Additional research into prognostic and diagnostic markers, including Ki-67, may contribute to early cancer detection. Tumor proliferation rate is directly associated with ki67 expression. It is also related to tumor initiation, progression, metastasis, and prognosis in various types of tumors.[107]

In the study by Sharma N et al., 50 histologically diagnosed oral squamous cell carcinoma cases were taken. of which 31 showed moderately differentiated carcinoma, followed by 16 showing well-differentiated carcinoma, and only 3 had poorly differentiated carcinoma. Ki-67 showed high expression in 36 cases, and 16 had low Ki-67 expression. In histological grading, out of 34 high grades (moderately diff and poorly diff), 28 cases had high Ki-67 expression and only 5 showed low expression. In the case of 16 low-grade (well-differentiated) cases, 7 had high expression and 9 showed low expression. There was a statistically significant association between Ki-67 and histological grades.[111]

The research conducted by Agrawal T. et al. studied 50 histologically confirmed cases of oral SCC to compare the histological grading of SCC with Ki-67 expression. The poorly differentiated carcinoma had more ki67 labelling index, followed by moderate diff ca and the lowest n-well diff ca. Ki-67 LI showed a statistically significant association with histological grades with a p-value <0.047. The comparison between Ki-67, lymphovascular, and perineural invasion was not statistically significant.[4]

The analysis by Omer S et al. took 50 cases of SCC. Out of 50 cases, 20 showed moderately differentiated carcinoma, 27 cases showed well-differentiated carcinoma and poorly differentiated carcinoma was seen in 3 cases. Ki-67 expression was seen in 76% of the cases. In this study, they did not find any correlation between Ki-67 expression and the age, sex, location, or pathological grade of the tumor. [12]

Kumar H et al. studied 40 biopsies of oral mucosa. Of 40 cases, 2 were normal oral mucosa cases, (10) well differentiated, (21) moderately differentiated, and (7) poorly differentiated. The Ki-67 labeling index was 29.52 ± 21.25 for normal mucosa, which increased to 68.57 ± 18.2 in moderate diff and 68.57 ± 17.6 in poorly differentiated carcinoma. There was a statistically significant correlation with a p-value <0.05 between Ki-67 expression and histological grading.[112]

The study by Ahmed M. et al. considered 35 cases of SCC of the oral cavity. The Ki-67 and CD147 expressions were analyzed. Ki-67 was scored 1 to 3, 1+ means low proliferation (10–30%), 2+ (30–50%), and 3+ >50%. Seventeen cases had high Ki-67 expression, compared to low and moderate in 18 cases. There was a statistically significant association between Ki-67 and tumor grade.[113]

Balani S et al. examined 112 cases, which comprised 12 benign cases, (19) premalignant cases (six oral intraepithelial grade 1, four OIN II, and nine OIN III), 41 cases of well-differentiated squamous cell carcinomas (WDSCC), (31) moderately differentiated SCC (MDSCC), (8) poorly differentiated SCC (PDSCC) and three verrucous carcinomas. In the malignant cases, Ki-67 immunoexpression was significantly higher in poorly differentiated squamous cell carcinoma cases in contrast to well-differentiated squamous cell carcinoma.[114] Other markers used in predicting course, progression, defining stages of dysplasia, and prognosis of squamous cell carcinoma include CD105, CD147,p16,p53, PNCA respectively.[113,114,116]

OTHER MARKERS [117]

Germ Cell markers -Carcinoembryonic antigen (CEACAM1) is expressed on the cell membrane and cytoplasm of tumor epithelial cells.[118]

Epithelial markers - E cadherin is expressed on the cell membrane of tumor cells. CK20 is expressed in the cytoplasm of Merkle cells (basal cells).[119,120]

Melanocyte markers – MIF (Macrophage Migration inhibitory factor) is expressed on tumor epithelial cells' cell membrane and cytoplasm.[121]

Mesenchymal markers- Fibroblast specific protein (FSP) is expressed on the nucleus and cytoplasm of tumor epithelial cells and used in squamous cell carcinoma of the tongue.[122] Vimentin is expressed in the cytoplasm of tumor epithelial cells.[119]

Muscle marker- Actinin 4 is expressed on the cell membrane and α SMA in the cytoplasm of tumor cells and myofibroblast in the stroma.[123,124]

Endothelial markers - Endoglin (CD105) is expressed on the cytoplasm of tumor cells and is used in oropharyngeal squamous cell carcinoma.[125]

Extracellular matrix markers –These include HS GAG (heparan sulfate glycosaminoglycan) and fibronectin. Both are expressed in the cytoplasm. HSGAG is expressed in carcinoma cells and fibronectin is expressed in stromal cells.[126,127]

Lymphoid markers- FAS ligand is expressed on the cell membrane of lymphoid cells in Oral SCC.[128]

Genetic Marker – p53 is expressed in the nucleus of tumor cells.[129]

Cytoplasmic inclusion marker – Galectin 3 is expressed in the nucleus and cytoplasm of tumor cells used in SCC of the tongue.[130]

TREATMENT

Oral SCC are treated surgically with 1-2 cm of clear margin in early and late stages along with lymph node dissection if necessary. In advanced cases, they may require a combination of surgery, radiation, and chemotherapy.[34]

For oropharyngeal SCC has a more complex treatment than oral SCC. The main treatment includes surgery and radiotherapy. A combination of both is used in the case of advanced tumors. Various resection methods used include transoral surgeries like video laryngoscopy, ultrasound endoscopic, laryngopharyngeal surgery, and robotic surgery.[131,132]

TNM STAGING OF ORAL SQUAMOUS CELL CARCINOMA[49] – In annexure

MATERIALS AND METHODS

The study was done on biopsy and specimens of clinically suspected cases of oral and oropharyngeal squamous cell carcinoma received in the Histopathology section of the Department of Pathology, at KLE Prabhakar Kore Hospital.

Study period: 1st January 2023 to 31st December 2024 (one year prospective study)

Study Design: Descriptive cross-sectional study

Sample size: 44 cases of oral and oropharyngeal SCC were studied in 1yr.

Within past 3 years 56,40,35 samples were collected respectively from KLE'S DR. PRABHAKAR KORE HOSPITAL, BELAGAVI. This amounts on average of approximately 40 to 44 samples year.

Using a formula for sample size

$n = N Z^2 P(1 – P)/ d^2(N-1) + Z^2 P(1- P)$

P – prevalence (p = 285)

d – precision error (15% of p = 0.15 x 28.5 = 4.27)

N – population size (approx. 44)

Z – Statistic for level of confidence

Here Z = 1.96 for 95% confidence

Selection criteria:

Inclusion Criteria: All the patients were diagnosed histopathologically with oral and oropharyngeal squamous cell carcinomas.

Exclusion Criteria:

1. All poorly preserved and inadequate specimens of patients.
2. Cases that were diagnosed with premalignant lesions or dysplasia.
3. Oral and Oropharyngeal carcinoma patients which are not squamous cell cancers.

Ethical clearance: The ethical clearance was acquired from Institutional Ethics

Committee, JNMC, Belagavi before the commencement of study.

Method of data collection

Procedure: All the biopsies and whole specimens of oral and oropharyngeal carcinoma received at the histopathology laboratory were collected, numbered, and kept for fixation in 10 % formalin overnight. The specimens were taken the next day for grossing and representative sections of tissue were given in different capsules. These capsules were taken for processing in the tissue processor. The tissues in the capsules underwent the process of dehydration in upgraded alcohol solutions, clearing in xylene, and impregnation with paraffin wax in the tissue processor.

The tissues were then taken from the capsule and embedded in molten wax for block preparation. Sections measuring 3-4 microns each were cut using microtome and taken onto the slides. 44 slides were stained using hematoxylin and eosin stain (H &E) for the histological grading and histopathological evaluation. The slides for IHC were pre-coated using Poly-L-Lysine and stained for IHC using a specific mouse monoclonal antibody to Ki-67. For positive control, tonsils were taken and for negative control, IHC staining was done without the use of primary antibody. After dipping slides in xylene, they were mounted with a coverslip using Dibutylphthalate Polystyrene Xylene (DPX).

Both H & E and Ki67 IHC staining procedures were performed and mounted.

PROCEDURE FOR IHC STAINING FOR Ki-67 ANTIBODY

1. Cut the sections at approximately 3-4 µm thickness and collect them on poly L Lysine-coated slides.
2. Then Bake the sections at 37 degrees Celsius overnight. Before the test bake it at 60 degrees Celsius for 1 hr.
3. Then Deparrafinize the slides-
    a. Xylene I – 10 min
    b. Xylene II – 10 min
    c. Absolute alcohol I -10min
    d. Absolute alcohol II – 10 min
    e. Rinse in water – 5min
    f. Rinse in distilled water – 1 min
4. Antigen retrieval by heat, using microwave and TRIS EDTA Buffer.
5. The required amount of the buffer is prepared and cook the slide in a pressure cooker for 3 whistles
6. Allow it to cool to room temperature for 15 min
7. Wash in water buffer two times with a gap of 30 sec each
8. Wash with water buffer 3 times with a gap of 30 sec
9. Treatment with 3% hydrogen peroxide for 10 minutes to block endogenous peroxidase.

10. Incubation with primary antibody (Ki-67) for 60 minutes in the closed chamber at room temperature.
11. Wash in TBS buffer two times for 3 times with a gap of 30 sec each.
12. Apply polymer Horseradish peroxidase for 25-30 min in a closed Chamber at room temperature.
13. Wash with wash buffer 3 times with a gap of 30 seconds
14. Treatment with DAB (secondary antibody) for 10 minutes to give a brown color to antigens
15. Wash in water for 2 minutes
16. Wash with distilled water for 1 minute
17. Counterstain with Harris hematoxylin for 3 minutes
18. Bluing in warm water for 1 minute.
19. Clearing with xylene for two minutes. Dry the slides and mount them with DPX

Ki-67 stained slides were scanned under 400x magnification for visual counting using a microscope.

All the slides were examined and reported using MODIFIED BRODER'S GRADING SYSTEM and graded as per degree of differentiation by a pathologist on H & E staining as:-

Well differentiated (Grade1)

Moderately differentiated (Grade 2)

Poorly differentiated (Grade3)

The Ki67 IHC slides were assessed under the Olympus BX41 microscope. Selected pictures were taken using the JENOPTIK SUBRA digital camera using the GRYPHAX software.

CRITERIA USED TO INTERPRET Ki67 OVEREXPRESSION INCLUDES

Pattern of staining

Intranuclear staining for Ki-67 was observed in each tumor cell, and Ki-67 antigen expression was considered positive as per the percentage of tumor cells stained for that specific slide. Regardless of the staining intensity, all the Ki-67stained nuclei were counted as positive.

Percentage of tumor cell staining[5]

1. +++ — High proliferation — >50% positive cells

2. ++ — Moderate proliferation — 30 to 50% positive cells

3. + — Low proliferation — 10 to 30% positive cells.

Statistical analysis

The data entry was done in the Microsoft EXCEL spreadsheet and the final analysis was done with the use of Statistical Package for Social Sciences (SPSS) version 23 .Cases showing ki67 positivity and its association with histopathological grading were studied. A p-value of less than 0.05 was considered statistically significant.

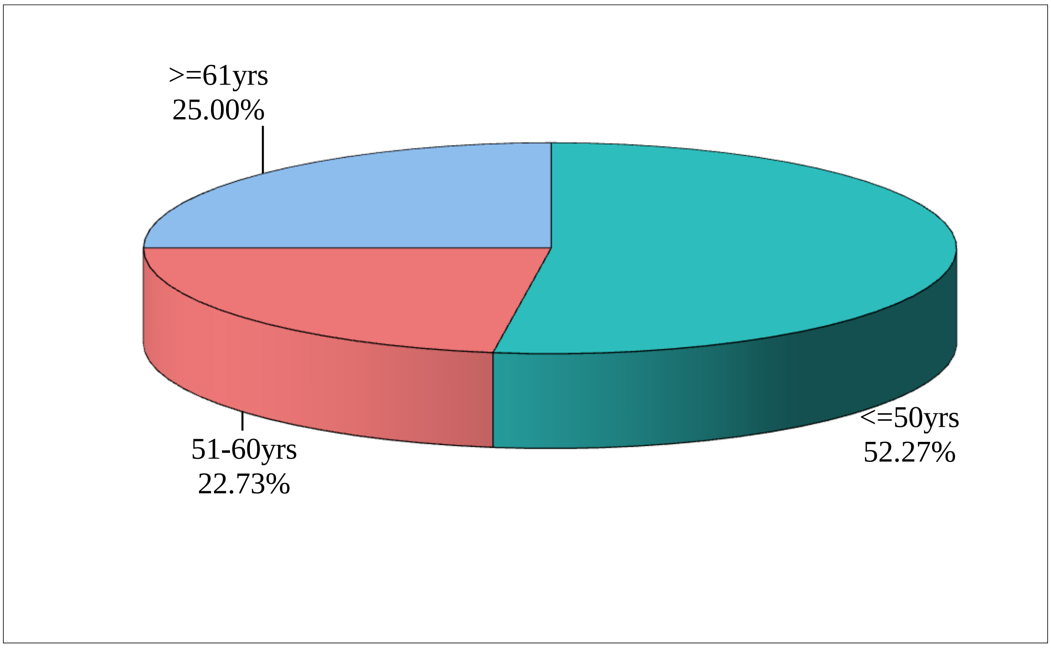
RESULTS

In the present study total of 44 cases of oral and oropharyngeal squamous cell carcinoma were studied for Ki-67 expression and its correlation with histological grading. Out of 44 cases, 20 were resection specimens and 24 were biopsy.

In this study age of patients ranged from 30 years to 75 years of age with a mean age of 57.70±10.08yrs. The youngest patient was 30 years old and the oldest was 72 years of age. Patients less than 50 years old accounted for 52.27% of cases. [TABLE 1, Graph 1]

Table 1: Age-wise distribution

Age groups	Number of cases (n=44)	%
<=50yrs	23	52.27
51-60yrs	10	22.73
>=61yrs	11	25.00
Total	44	100.00
Mean	51.70	
SD	10.08	



Graph 1: Age-wise distribution

In our study, Thirty-five males and nine females were involved. Twenty-six males and 7 females had oral SCC, and 9 males and 2 females had oropharyngeal SCC. Our study revealed a male prevalence in both OSCC (74.29%) and oropharyngeal (79.55%) squamous cell carcinoma.[Table 2 & 3 ; Graph 2]

Table 2: Gender-wise distribution

Gender	Number of cases (n=44)	%
Male	35	79.55
Female	9	20.45
Total	44	100.00

Graph 2 : Gender-wise distribution

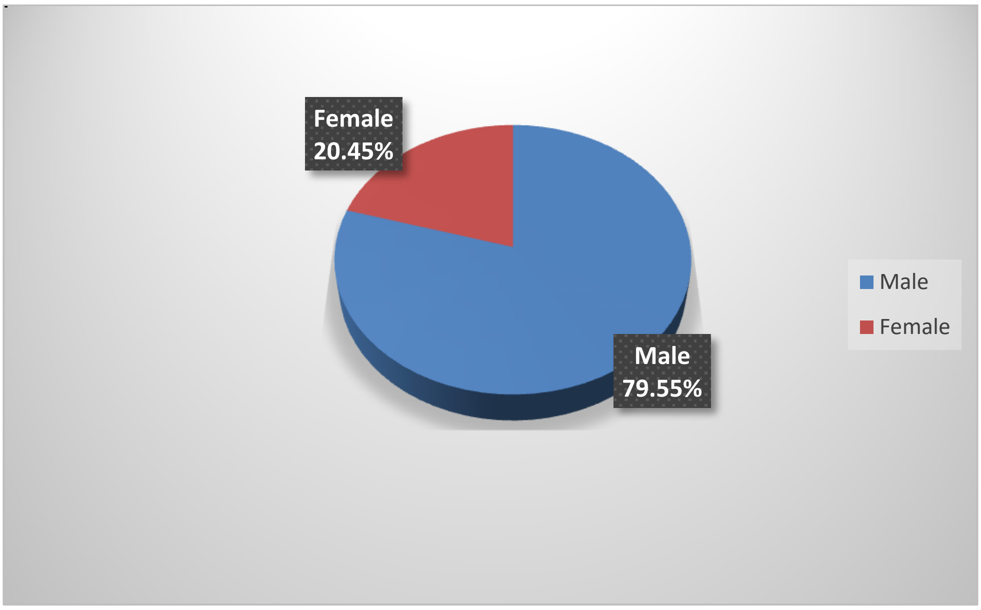


Table 3: Distribution of cases according to gender in oral and oropharyngeal SCC

	ORAL SCC		OROPHARYNGEAL SCC		
SEX	NUMBER	PERCENTAGE %	NUMBER	PERCENTAGE %	TOTAL
MALE	26	78.78	9	81.81	35
FEMALE	7	21.21	2	18.18	9
TOTAL	33	100	11	100	44

Out of 44 cases of oral and oropharyngeal squamous cell carcinoma, 33 cases had OSCC, and 11 cases had oropharyngeal squamous cell carcinoma [Table 4]

Table 4: Distribution of cases according to oral and oropharyngeal squamous cell carcinoma

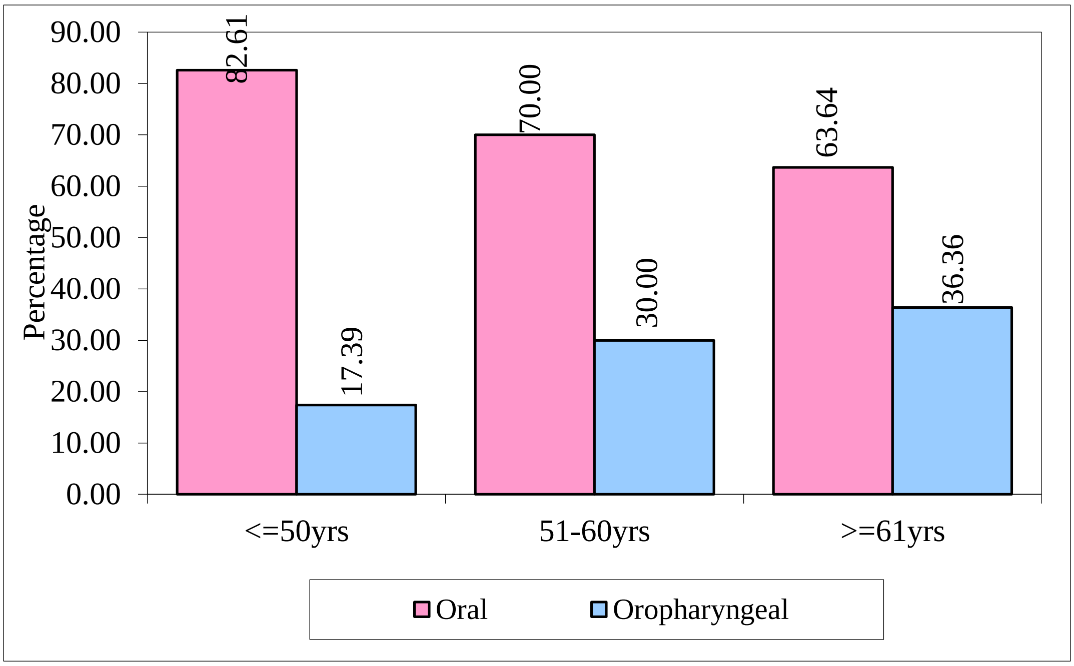
TYPE OF CARCINOMA	**NUMBER(n=44)**	**PERCENTAGE**
ORAL SCC	33	75%
OROPHARYNGEAL SCC	11	25%
Total	**44**	**100**

Nineteen patients with oral squamous cell carcinoma were older than fifty years, while the remaining seven were older than fifty years. Conversely, there were the same number of patients in both age groups who had oropharyngeal squamous cell carcinoma. However, the chia square test results showed no statistically significant relationship between age and type of cancer. [Table 5 , Graph 3]

Table 5: Association between age and types of cancer

Age groups	Oral	%	Oropharyngeal	%	Total	%	Chi-square	p-value
<=50yrs	19	82.61	4	17.39	23	52.27	1.6010	0.4490
51-60yrs	7	70.00	3	30.00	10	22.73		
>=61yrs	7	63.64	4	36.36	11	25.00		
Total	33	75.00	11	25.00	44	100.00		

Graph 3 : Association between age and types of cancer

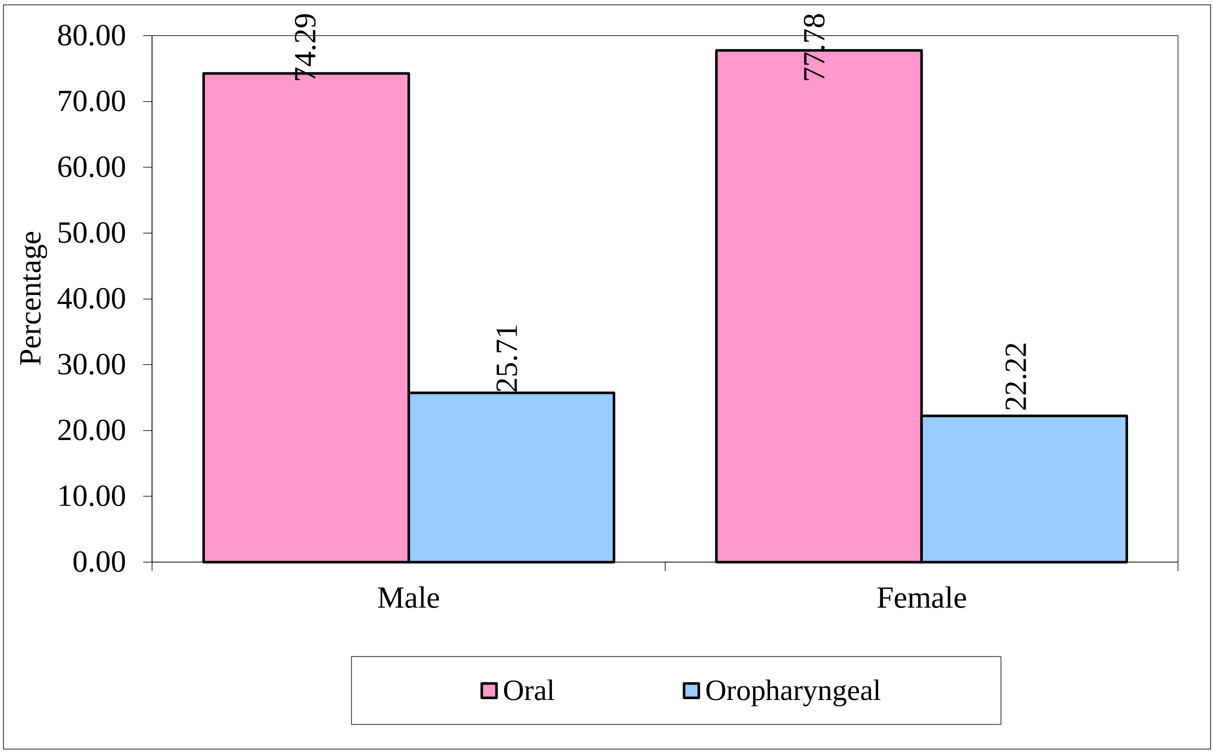


In both oral and oropharyngeal SCC Male preponderance was observed but the association between gender and type of cancer was not significant statistically.[Table 6, Graph 4]

Table 6: Association between gender and types of cancer

Gender	Oral	%	Oropharyngeal	%	Total	%	Chi-square	p-value
Male	26	74.29	9	25.71	35	79.55	0.0470	0.8290
Female	7	77.78	2	22.22	9	20.45		
Total	33	75.00	11	25.00	44	100.00		

Graph 4: Association between gender and types of cancer



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Buccal mucosa accounted for the maximum number of cases 19(44%) followed by tongue 8 (18.18%), GB sulcus, lips, and alveolus in decreasing order in oral SCC while tonsillar pillar 4 cases (9.09%) were seen to be involved by oropharyngeal SCC followed by Soft palate, posterior pharyngeal wall, uvula and base of the tongue(Table 7)

Table 7: Site-wise distribution of cases

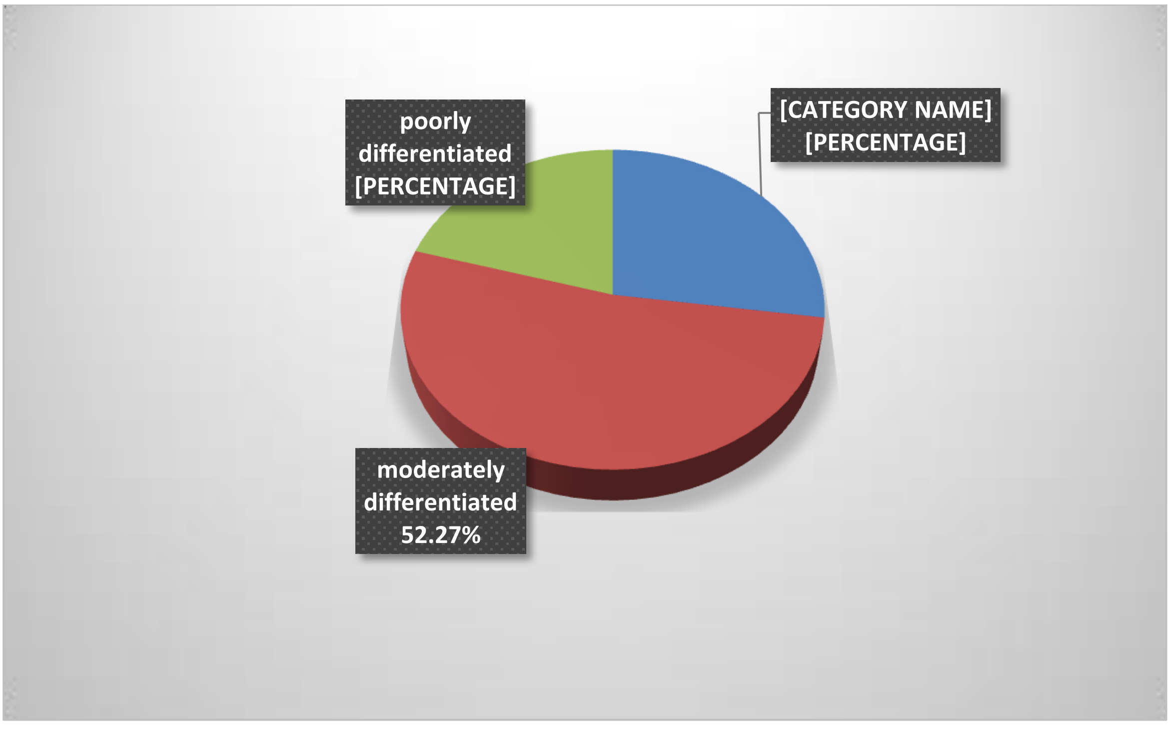
ORAL & OROPHARYNGEAL SCC CASES		
SITE OF THE LESION	**NUMBER OF CASES (n=44)**	**PERCENTAGE %**
Buccal mucosa	19	44
Tongue	8	18.18
GB sulcus	3	6.81
Lip	1	2.27
Alveolus	2	4.5
Tonsillar Pillar	4	9.09
Soft palate	3	6.81
Posterior pharyngeal wall	2	4.54
Uvula	1	2.27
Base of tongue	1	2.27

Out of 44 cases, 12 cases (27.27%) were well-differentiated carcinoma, 23(52.2%) were moderately differentiated carcinoma and 9 (20.45%) were poorly differentiated carcinoma. The most common histological grade seen was moderately differentiated followed by well differentiated and poorly differentiated. (Table 8, Graph 5)

Table 8 : Histological grade-wise distribution

Histological grades	Number	%
Well-differentiated	12	27.27
Moderately differentiated	23	52.27
Poorly differentiated	9	20.45
Total	44	100.0

Graph 5 : Histological grades wise distribution

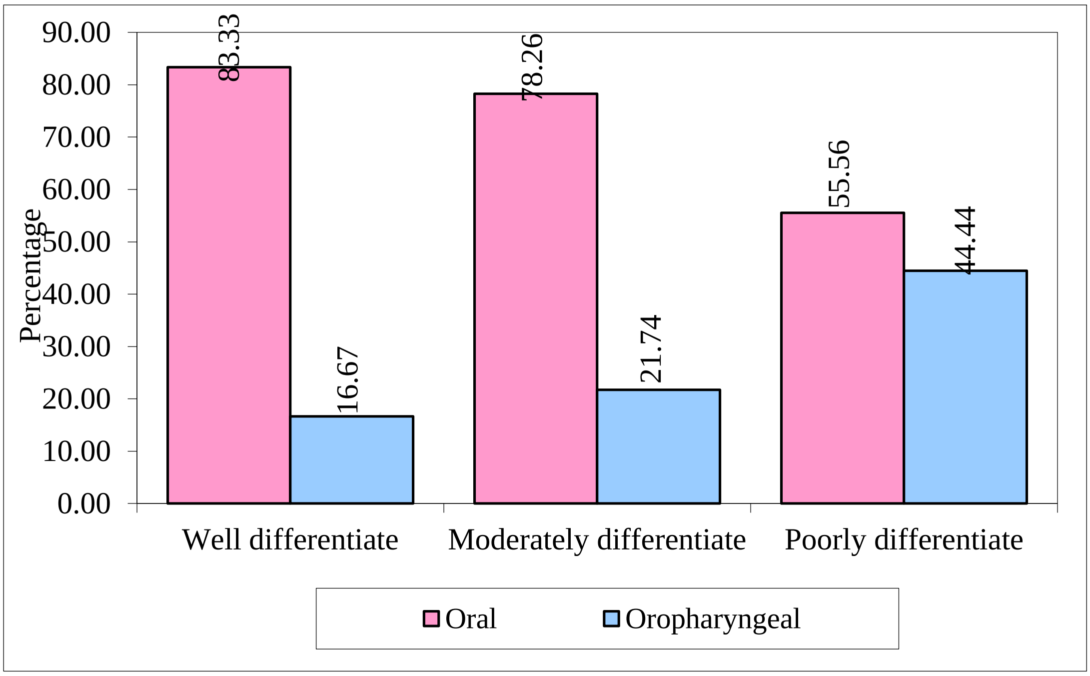


Moderately differentiated squamous cell carcinoma is most common histological type seen in oral and oropharyngeal SCC. However, there was no statistically significant relationship between the type of cancer and histological grades done by chia square test. [Table 9, Graph 6]

Table 9: Association between Histological grades and types of cancer

Histological grades	Oral	%	Oropharyngeal	%	Total	%	Chi-square	p-value
Well-differentiated	10	83.33	2	16.67	12	27.27	2.3900	0.3030
Moderately differentiated	18	78.26	5	21.74	23	52.27		
Poorly differentiated	5	55.56	4	44.44	9	20.45		
Total	33	75.00	11	25.00	44	100.00		

Graph 6: Association between Histological grades and types of cancer



Ki-67 expression was seen higher in oropharyngeal squamous cell carcinoma compared to OSCC. The association and comparison were done by chi-square test and independent t-test respectively but were not statistically significant. [Table 10,11;Graph 7,8]

Table 10: Association between expression of KI-67 and types of cancer

Expression of KI-67	Oral	%	Oropharyngeal	%	Total	%	Chi-square	p-value
1+	17	80.95	4	19.05	21	47.73	2.3900	0.3030
2+	10	71.43	4	28.57	14	31.82		
3+	6	66.67	3	33.33	9	20.45		
Total	33	75.00	11	25.00	44	100.00		

Graph 7 : Association between expression of KI-67 and types of cancer

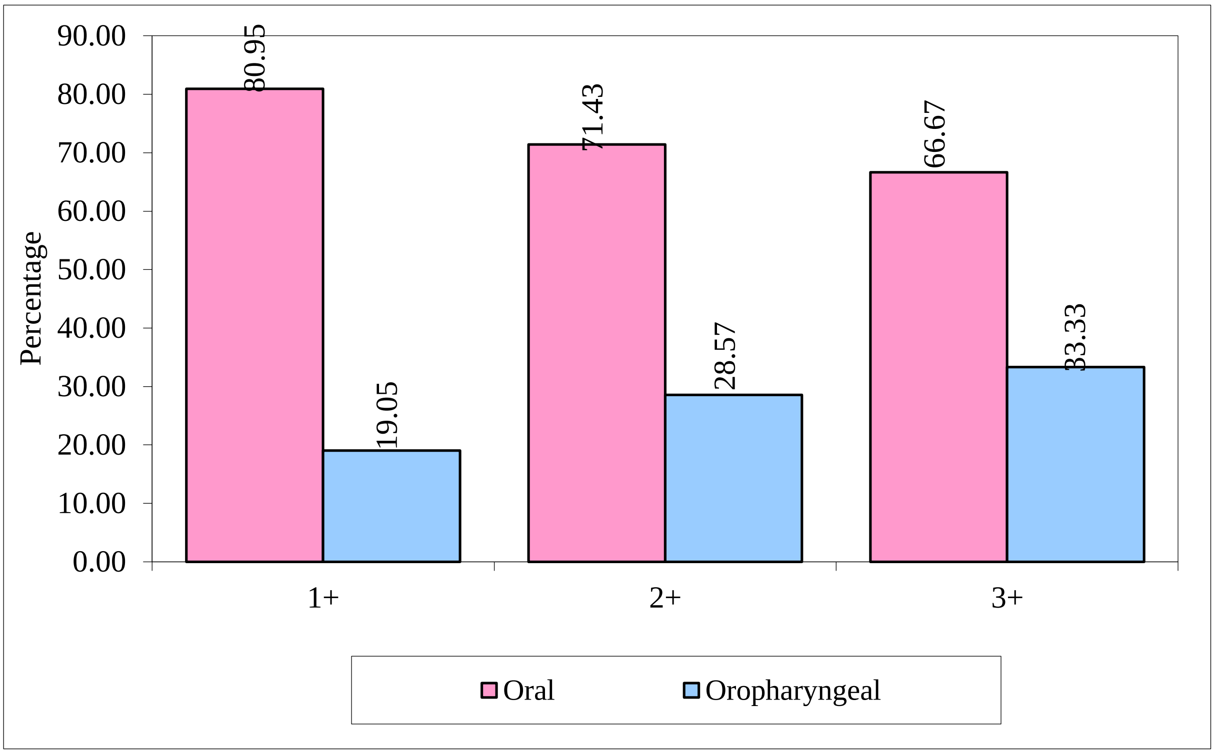
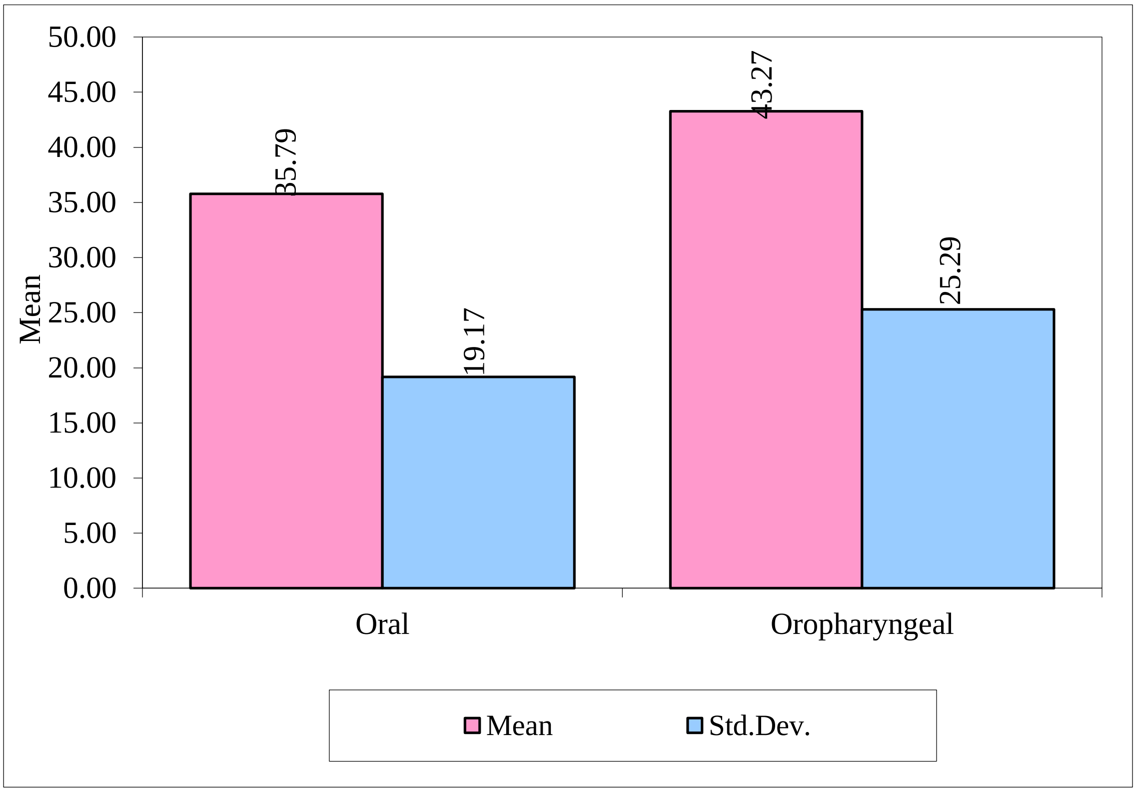


Table 11 Comparison of types of cancer with mean percentage of expression of KI-67 by independent t-test

Types of cancer	Mean	Std.Dev.	Q25	Median	Q75
Oral	35.79	19.17	25.00	35.00	45.00
Oropharyngeal	43.27	25.29	15.00	36.00	55.00
Total	37.66	20.81	20.00	35.00	47.50
t-value	-1.0341				
P-value	0.3070				

Graph 8 : Comparison of types of cancer with mean percentage of expression of KI-67



Twelve of the 21 individuals with a 1+Ki-67 score in our study were younger than 50, and six cases were older than 61. Of the nine cases with 3+ Ki-67 staining, 4 were less than 50 years old, and just 1 case was older than 61. Four of the fourteen cases (seven of which were older than 61 years old and four of which were younger than 50) had Ki-67 values of 2+. The age group between 51 and 60 years old had the highest mean Ki-67 (45.50±27.13), whereas those under 50 had a relatively lower mean Ki-67 (34.61±20.05). However, the chi-square test or one-way ANOVA revealed no statistically significant relationship between age and Ki-67 [TABLE 12 & 13; Graph 9 &10]

Table 12 - Association between age and expression of KI-67

Age groups	1+	%	2+	%	3+	%	Total	%	Chi-square	p-value
<=50yrs	12	52.17	7	30.43	4	17.39	23	52.27	3.6240	0.4590
51-60yrs	3	30.00	3	30.00	4	40.00	10	22.73		
>=61yrs	6	54.55	4	36.36	1	9.09	11	25.00		
Total	21	47.73	14	31.82	9	20.45	44	100.00		

Graph 9 : Association between age and expression of KI-67

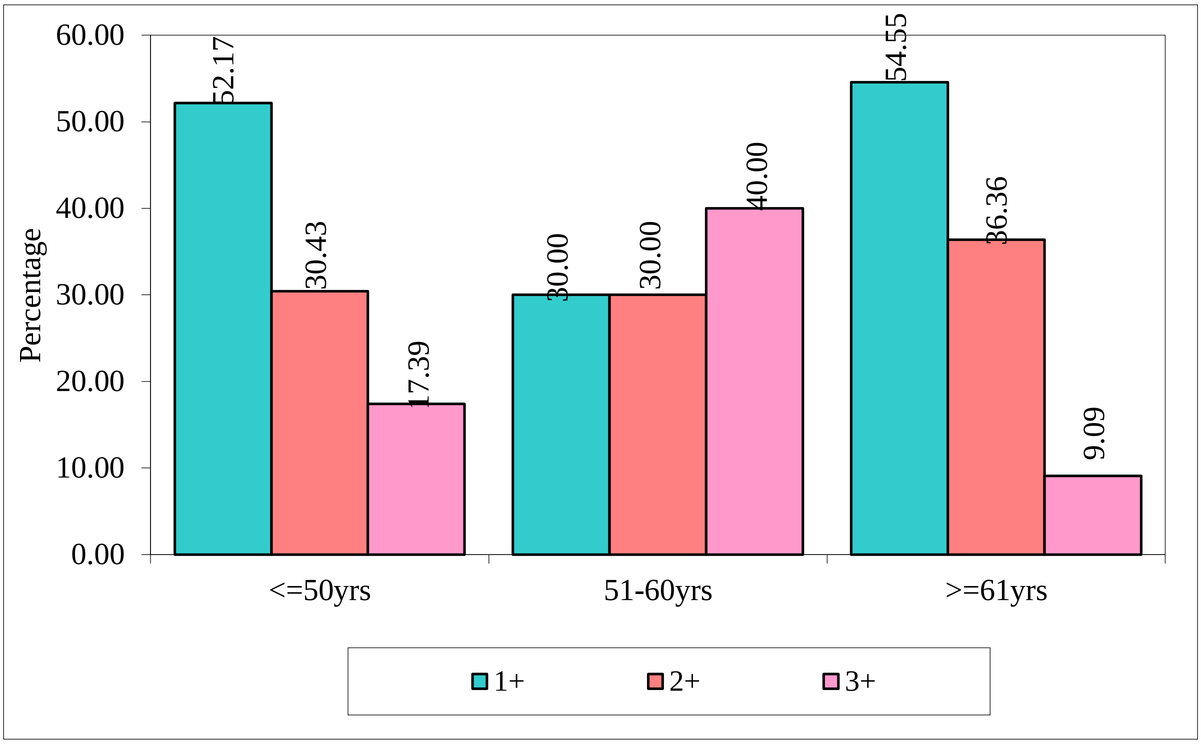
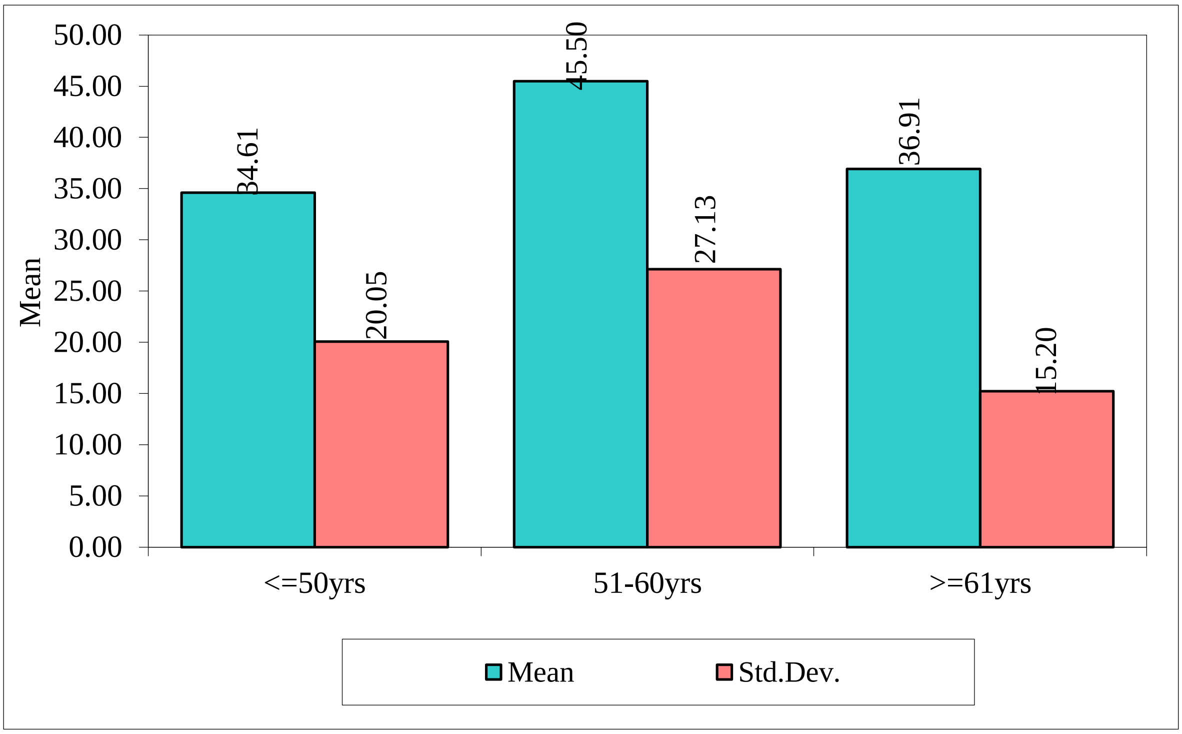


Table 13 - Comparison of age with mean percentage of expression of KI-67 by one-way ANOVA

Age groups	Mean	Std.Dev.	Q25	Median	Q75
<=50yrs	34.61	20.05	15.00	35.00	45.00
51-60yrs	**45.50**	27.13	15.00	45.00	55.00
>=61yrs	36.91	15.20	25.00	35.00	50.00
Total	37.66	20.81	20.00	35.00	47.50
F-value	0.9627				
P-value	0.3903				

Graph 10 : Comparison between age groups and expression of Ki-67



Out of 35 males in the current study, 16 had a 1+Ki-67 score while 8 had a Ki-67 score of 3. In females among 9 cases, only 1 had Ki-67 3+and 5 had 1+Ki-67 score. Mean Ki-67 was slightly higher in females compared to males. However, there was no statistical association between Ki-67 and gender done by the chi-square test and independent t-test. [TABLE 14 & 15; Graph 11&12]

Table 14: Association between Gender and expression of KI-67

Gender	1+	%	2+	%	3+	%	Total	%	Chi-square	p-value
Male	16	45.71	11	31.43	8	22.86	35	79.55	0.6360	0.7270
Female	5	55.56	3	33.33	1	11.11	9	20.45		
Total	21	47.73	14	31.82	9	20.45	44	100.00		

Graph 11 Association between gender and expression of KI-67

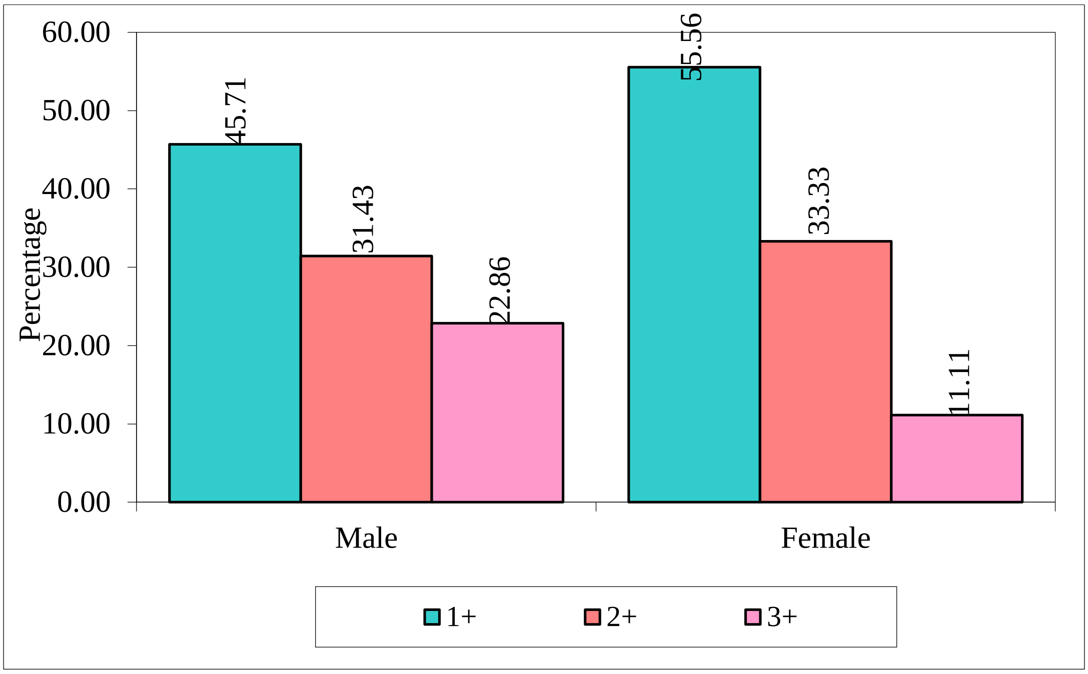
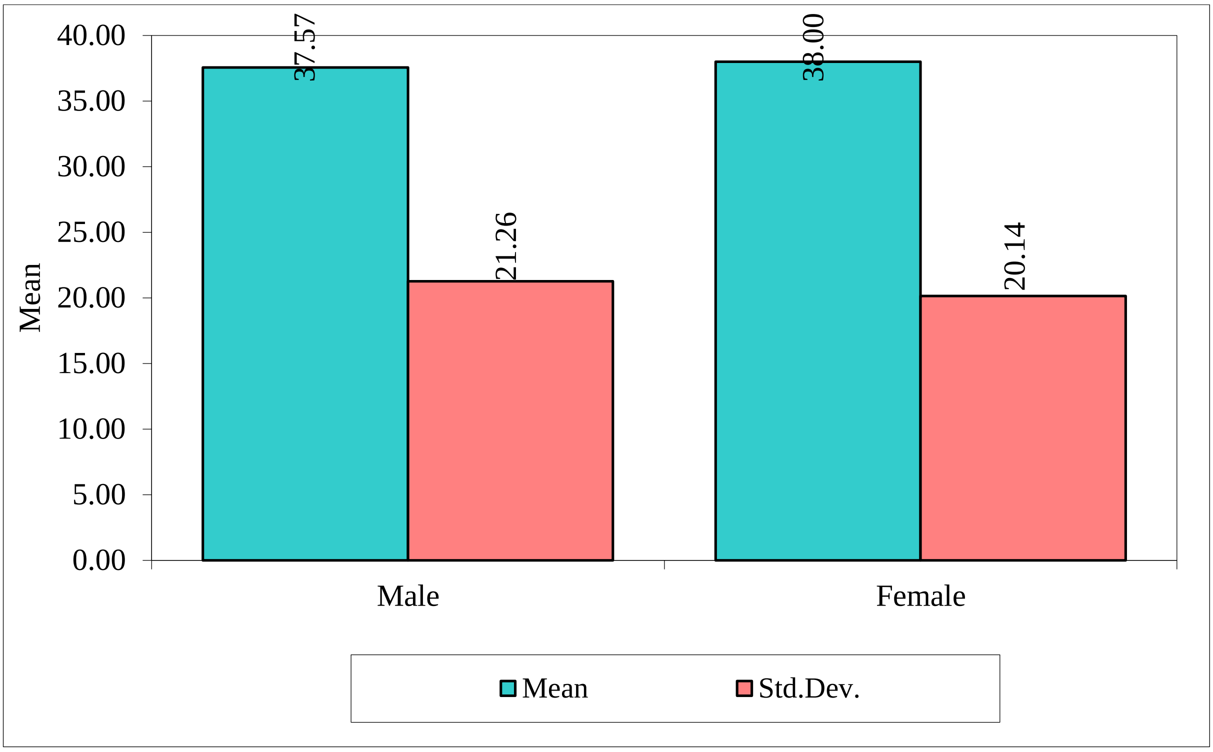


Table 15 - : Comparison of gender with mean percentage of expression of KI-67 by independent t-test

Gender	Mean Ki-67	Std.Dev.	Q25	Median	Q75
Male	37.57	21.26	15.00	35.00	55.00
Female	38.00	20.14	25.00	35.00	36.00
Total	37.66	20.81	20.00	35.00	47.50
t-value	-0.0545				
P-value	0.9568				

Graph 12 : Comparison of males and females with mean percentage of expression of KI-67



All well-differentiated carcinoma in our analysis had 1+Ki-67 staining, moderately differentiated ca have a maximum number of cases (13) with 2+Ki-67 staining followed by 1+Ki-67 staining in 7 cases and 3+Ki-67 staining in 3 cases. In poorly differentiated carcinoma the maximum cases (6) had a score of 3+staining followed by 1+in 2 cases and 2+ in 1 case. In our study mean Ki-67 was seen higher in poorly differentiated carcinoma (61.67=25.12) calculated with one-way ANOVA. There was an association and correlation between the Ki-67 score and histological grades done by chi-square test and Karl Pearson correlation coefficient p value<0.001.[TABLE 16,17, Graph 13]

The comparison between histological grades and the mean percentage of Ki-67 expression was statistically significant done by one-way ANOVA [Table 18, Graph 14]

Table 16: Association between Histological grades and expression of KI-67

Histological grades	1+	%	2+	%	3+	%	Total	%	Chi-square	p-value
Well differentiate	12	100.0	0	0.00	0	0.00	12	27.27	31.4490	0.0001*
Moderately differentiate	7	30.43	13	56.52	3	13.04	23	52.27		
Poorly differentiate	2	22.22	1	11.11	6	66.67	9	20.45		
Total	21	47.73	14	31.82	9	20.45	44	100.0		

*p<0.05

Graph 13 : Association between Histological grades and expression of KI-67

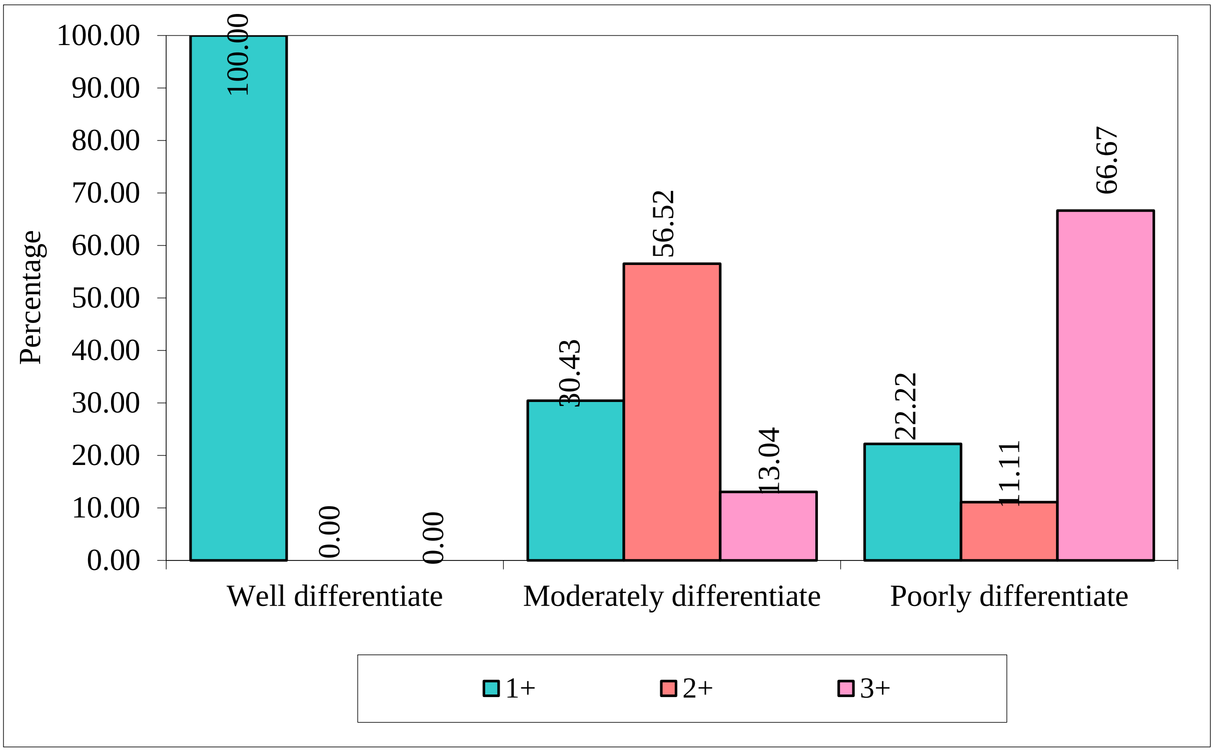


Table 17 : Correlation between Histological grades and expression of KI-67 by Karl Pearson's correlation coefficient

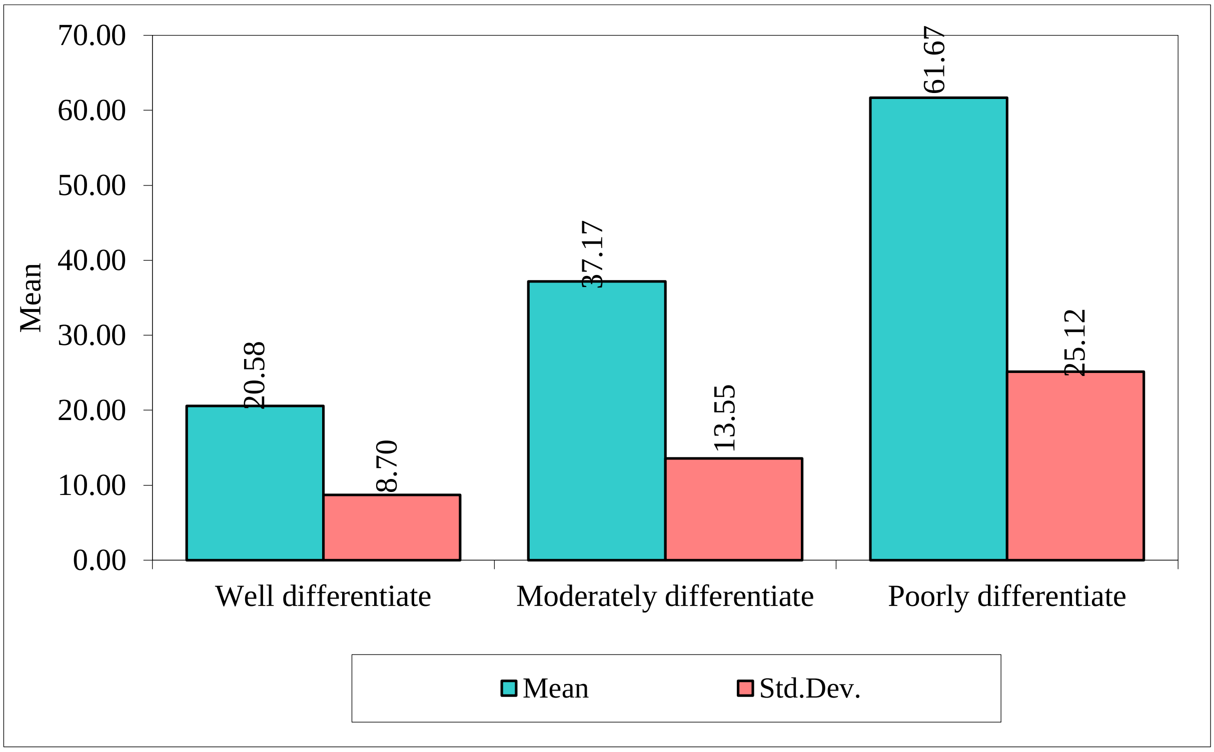
Variables	Correlation between Histological grades			
	N	Spearman R	t-value	p-value
Expression of KI-67	44	0.6416	5.4214	0.0001*

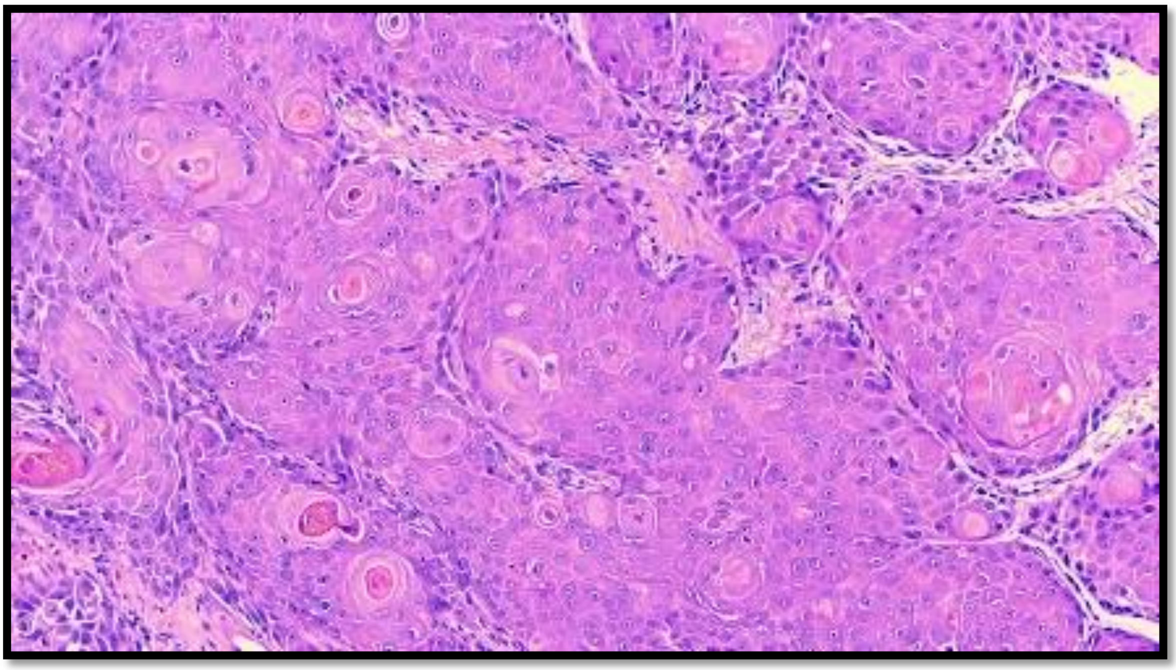
Table 18: Comparison of histological grades with mean percentage of expression of KI-67 by one-way ANOVA

Histological grades	Mean	Std.Dev.	Q25	Median	Q75
Well differentiate	20.58	8.70	15.00	15.00	27.50
Moderately differentiate	37.17	13.55	25.00	35.00	45.00
Poorly differentiate	61.67	25.12	55.00	55.00	85.00
Total	37.66	20.81	20.00	35.00	47.50
F-value	17.9540				
P-value	0.0001*				

*p<0.05

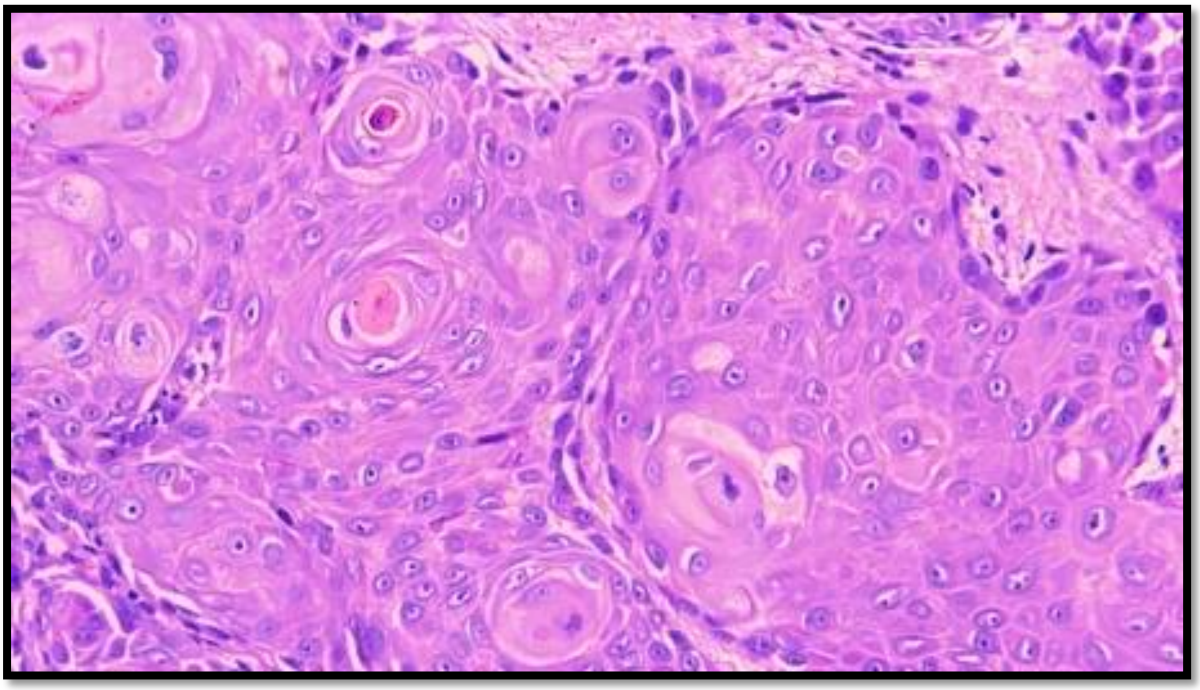
Graph 14: Comparison of histological grade with mean percentage of expression of KI-67



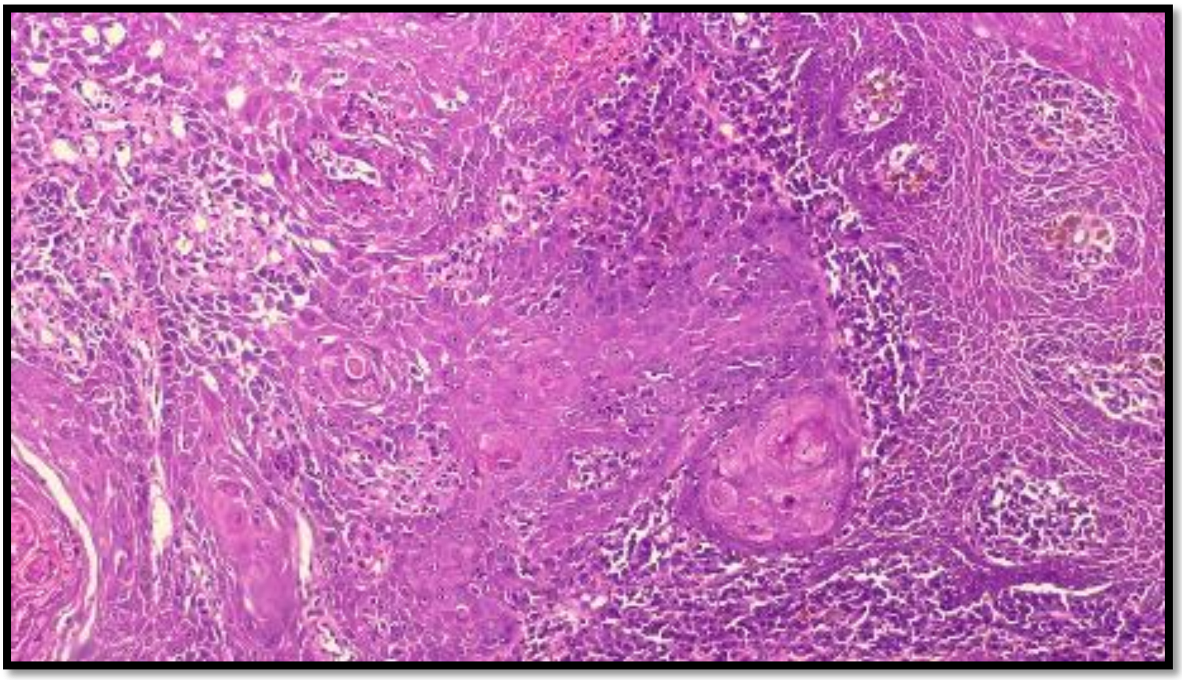


Photomicrograph 1: Shows well-differentiated oral SCC with keratin pearls

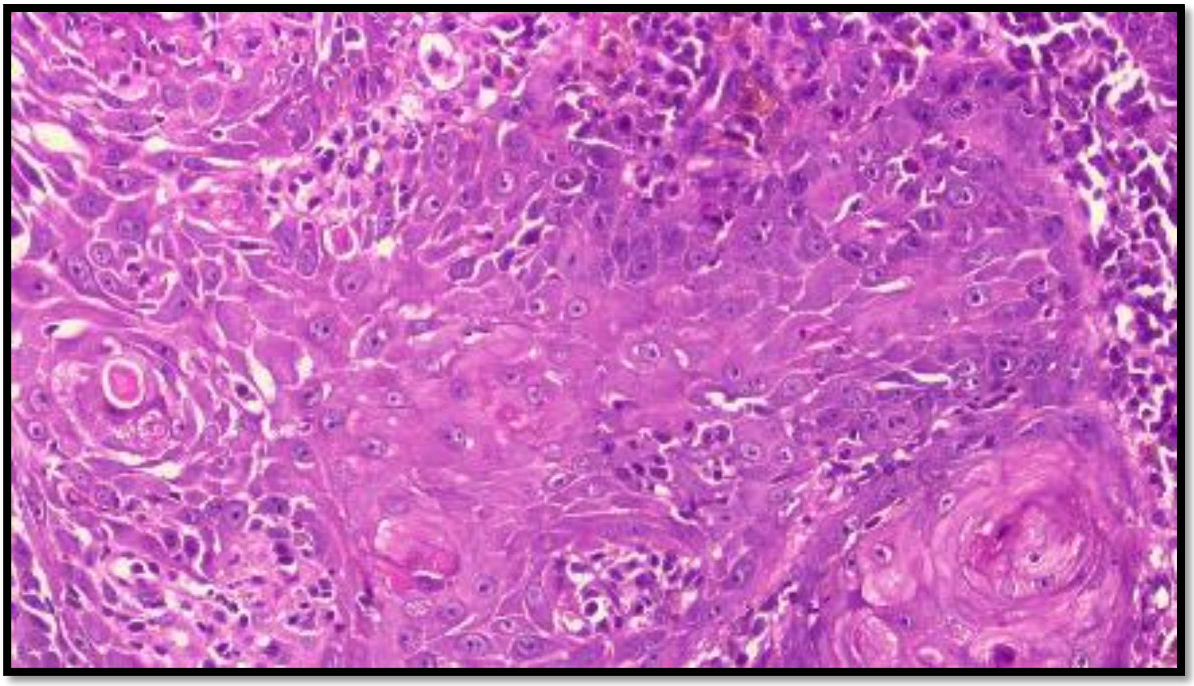
(H&E, x100)



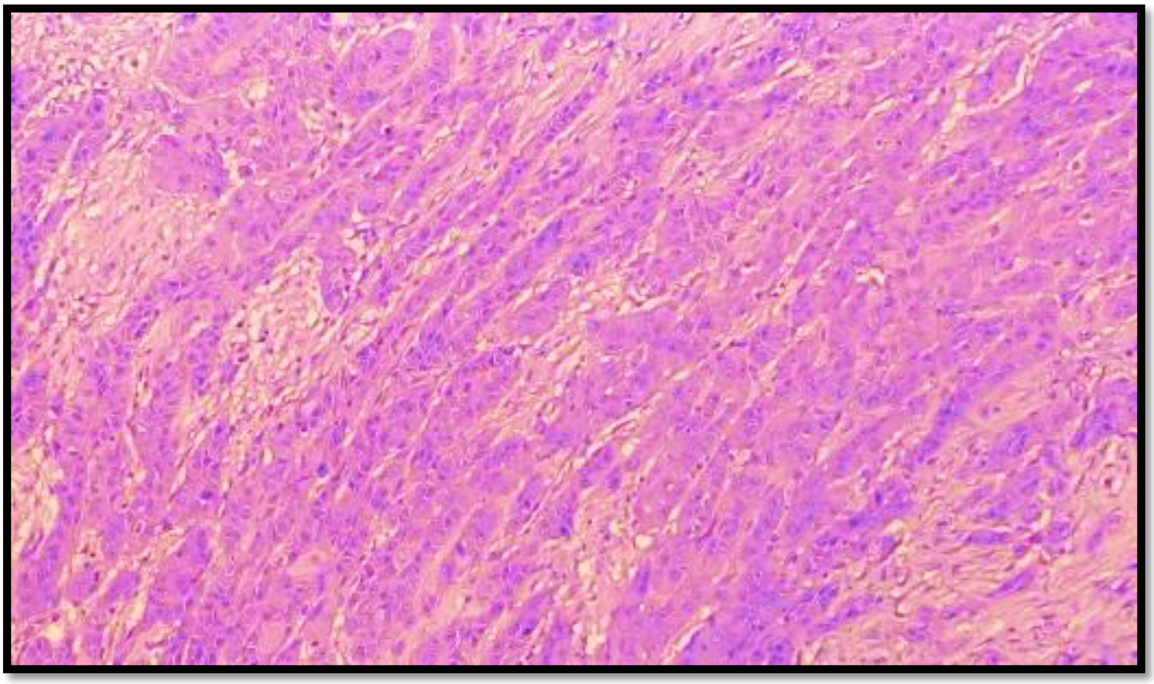
Photomicrograph 2: Shows well differentiated oral SCC (H&E, X200)



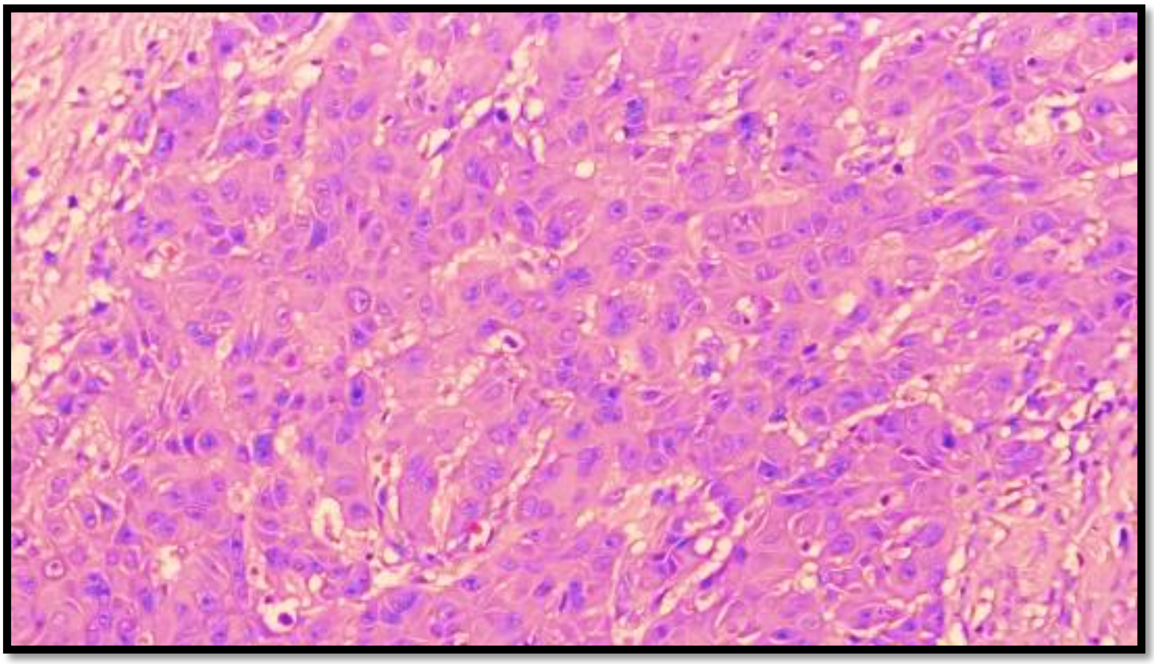
Photomicrograph 3: Shows moderately differentiated oral SCC with few keratin pearls (H&E, x100)



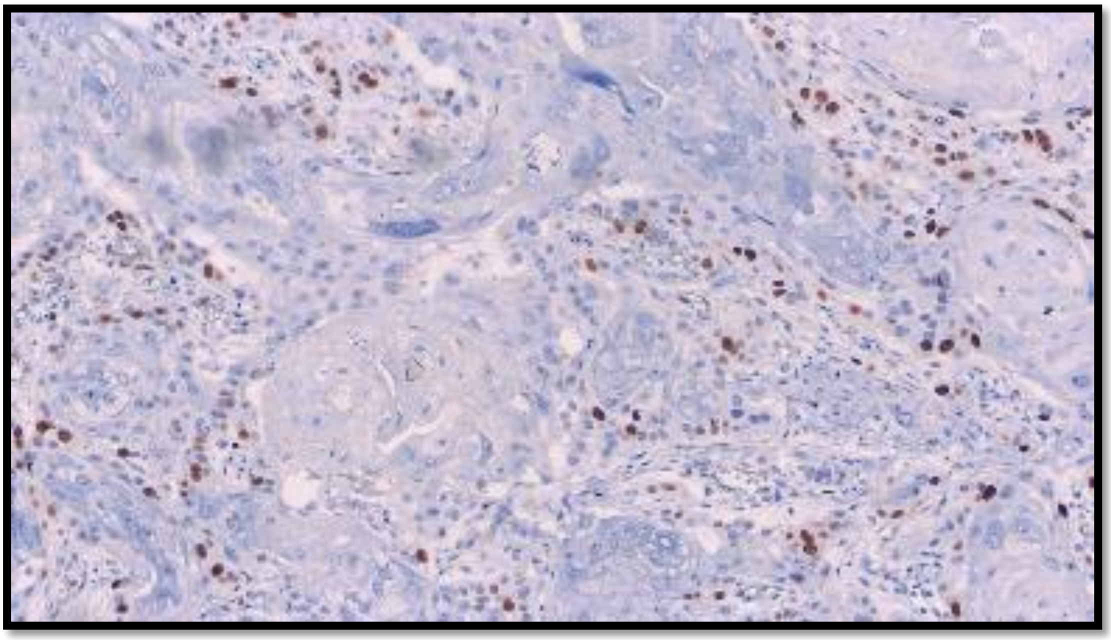
Photomicrograph 4: Shows moderately differentiated oral SCC (H&E, x200)



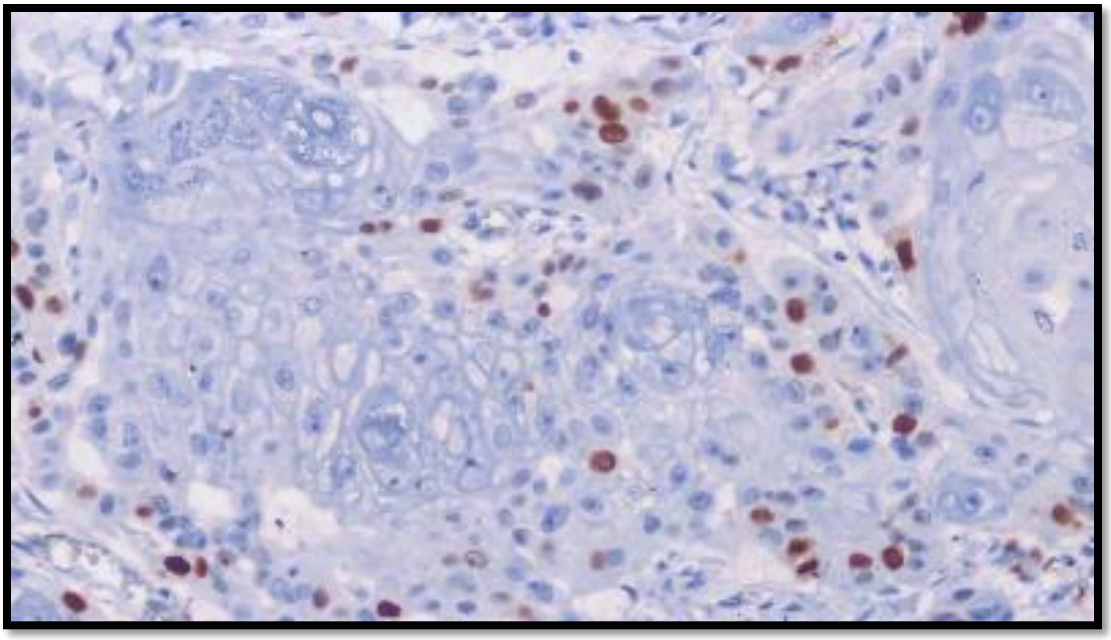
Photomicrograph 5: Shows poorly differentiated oral SCC (H&E, x100)



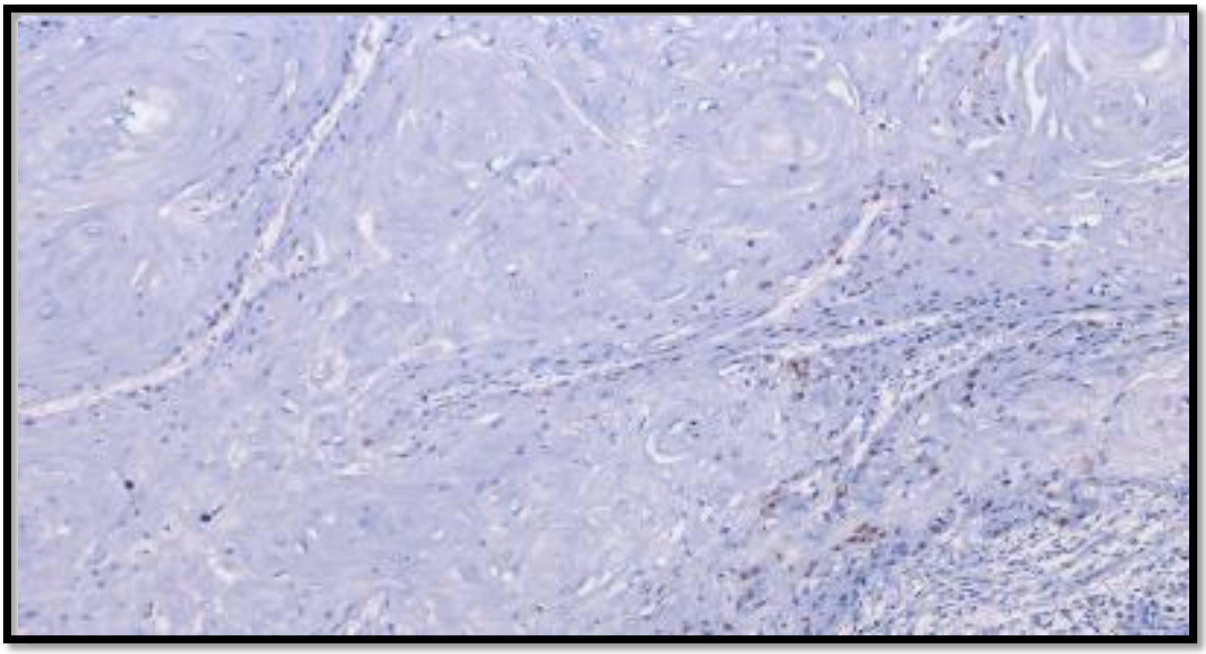
Photomicrograph 6: Shows poorly differentiated oral SCC (H&E, x200)



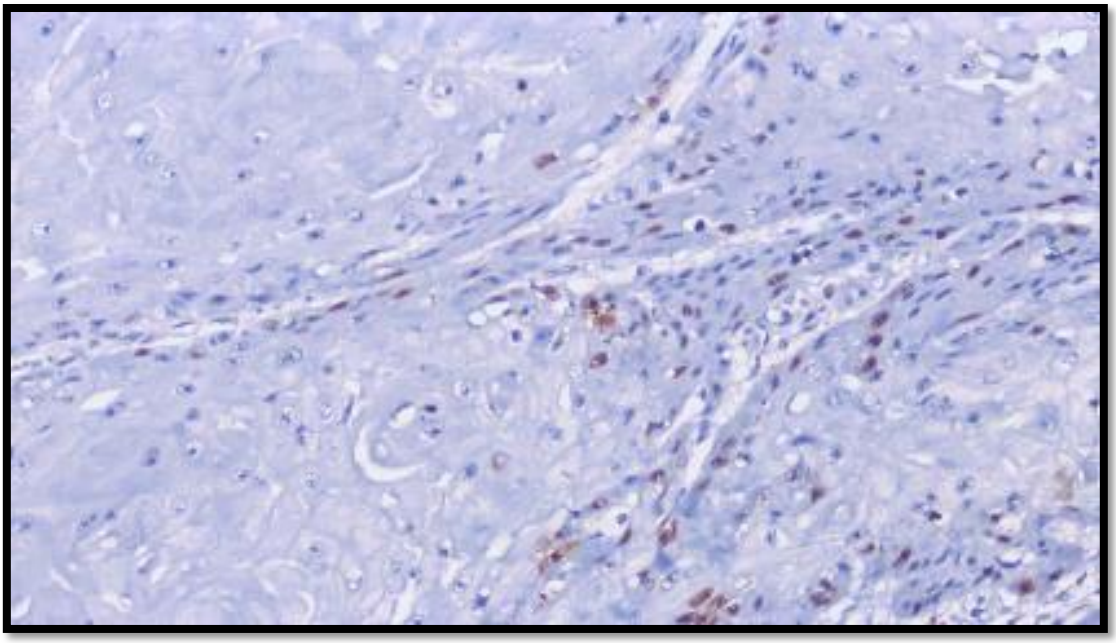
Photomicrograph 7: Shows 1+ staining of Ki67 in well differentiated oral SCC (IHC, x100)



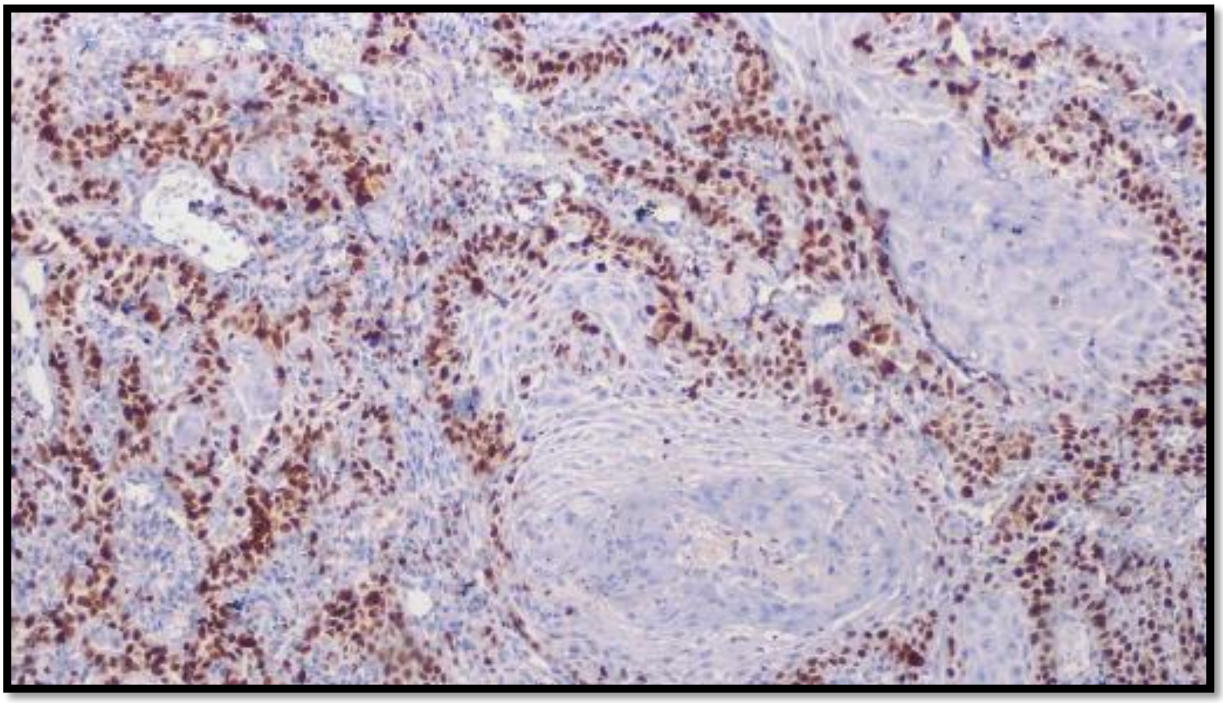
Photomicrograph 8: Shows 1+ staining of Ki67 in well differentiated oral SCC (IHC, x200)



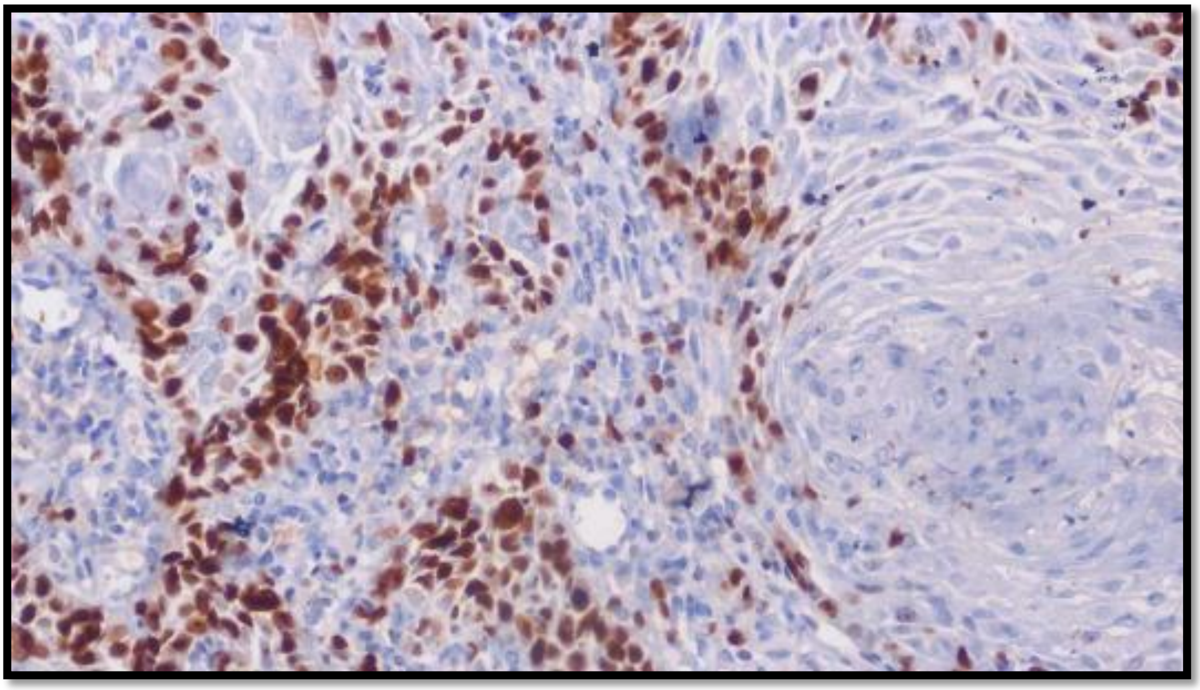
Photomicrograph 9: Shows 1+ staining of Ki67 in moderately differentiated oral SCC (IHC, x100)



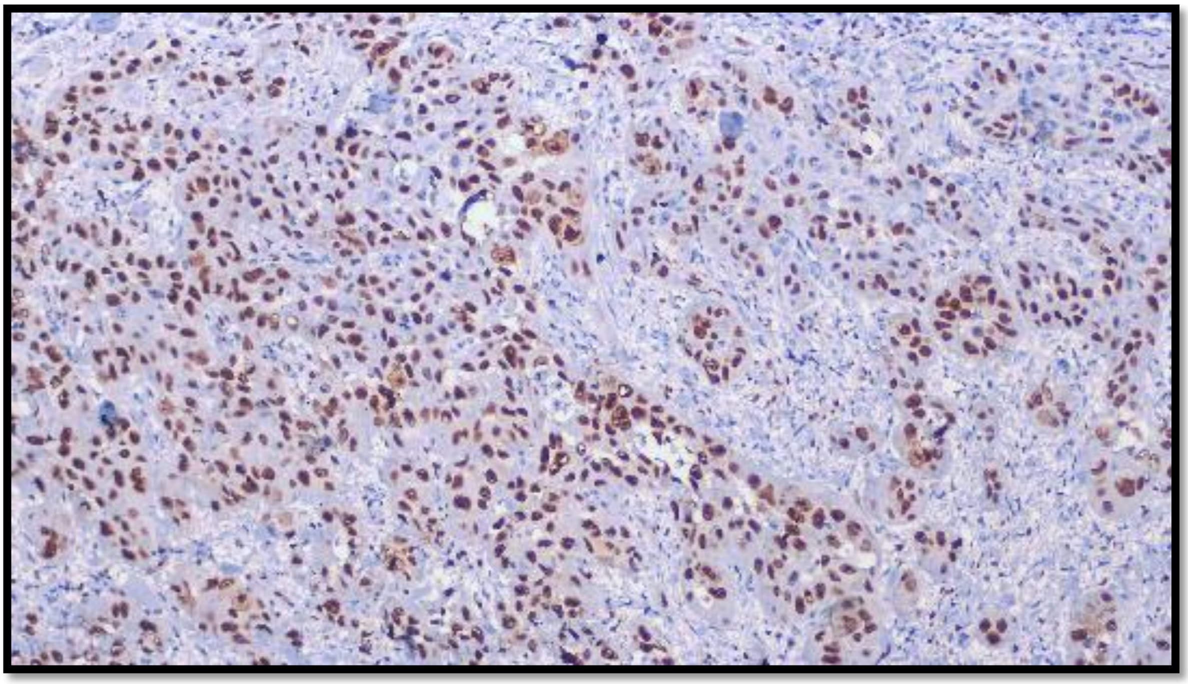
Photomicrograph 10: Shows 1+ staining of Ki67 in moderately differentiated oral SCC (IHC, x200)



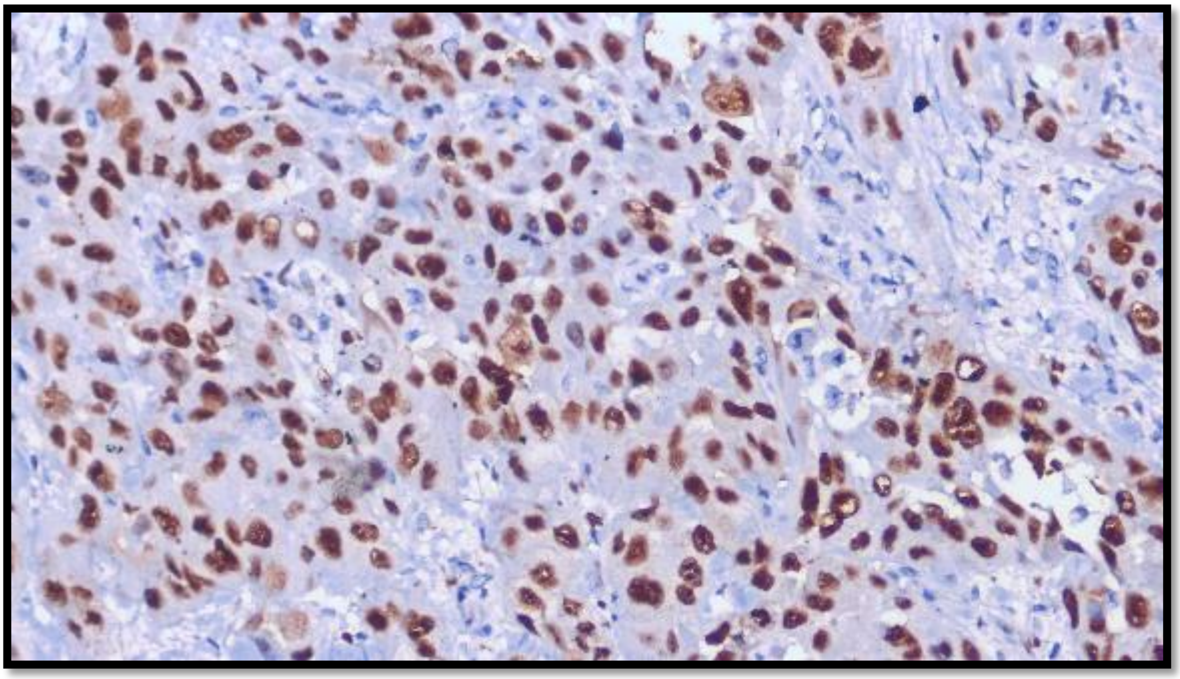
Photomicrograph 11: Shows 2+ staining of Ki67 in moderately differentiated oral SCC (IHC, x100)



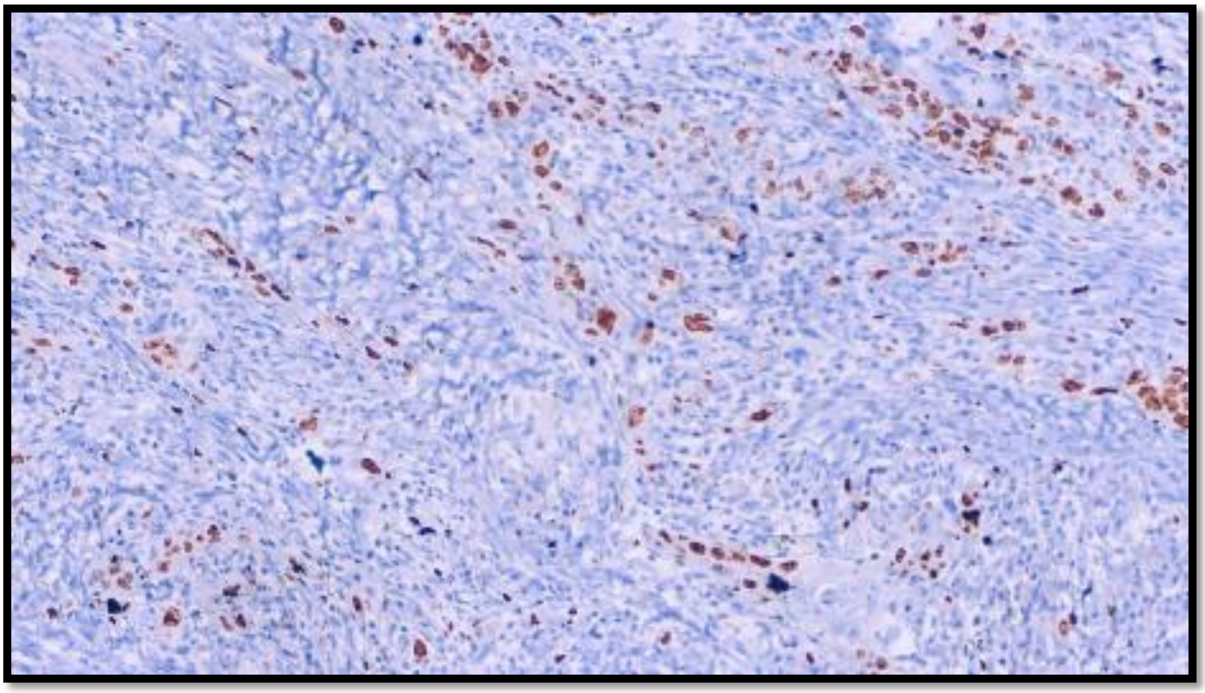
Photomicrograph 12: Shows 2+ staining of Ki67 in moderately differentiated oral SCC (IHC, x200)



Photomicrograph 13: Shows 3+ staining of Ki67 in moderately differentiated oral SCC (IHC, x100)

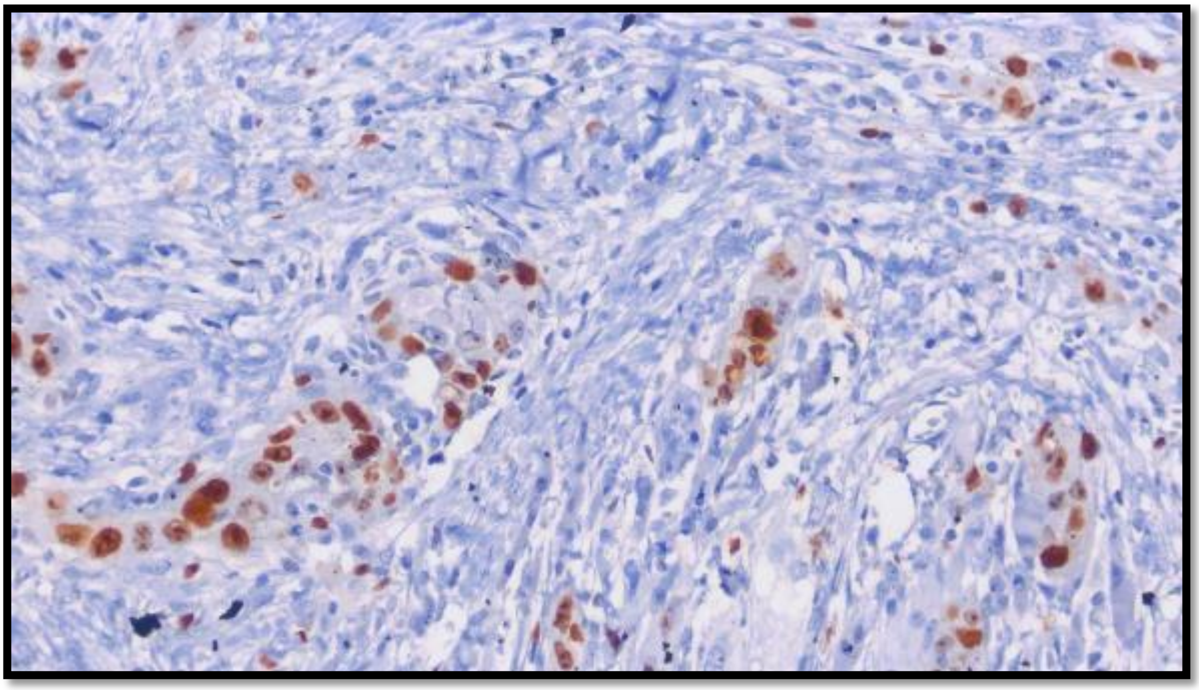


Photomicrograph 14: Shows 3+ Staining of Ki67 in moderately differentiated oral SCC (IHC, x200)



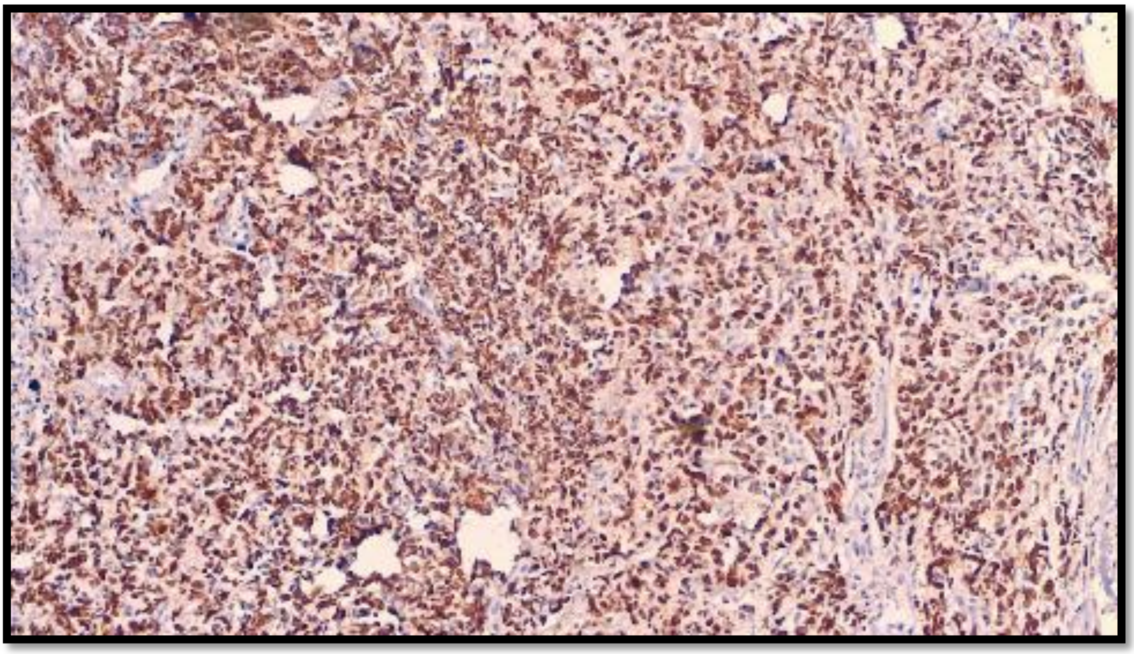
Photomicrograph 15: shows 1+ Staining of Ki67 in poorly differentiated oral SCC

(IHC, x100)



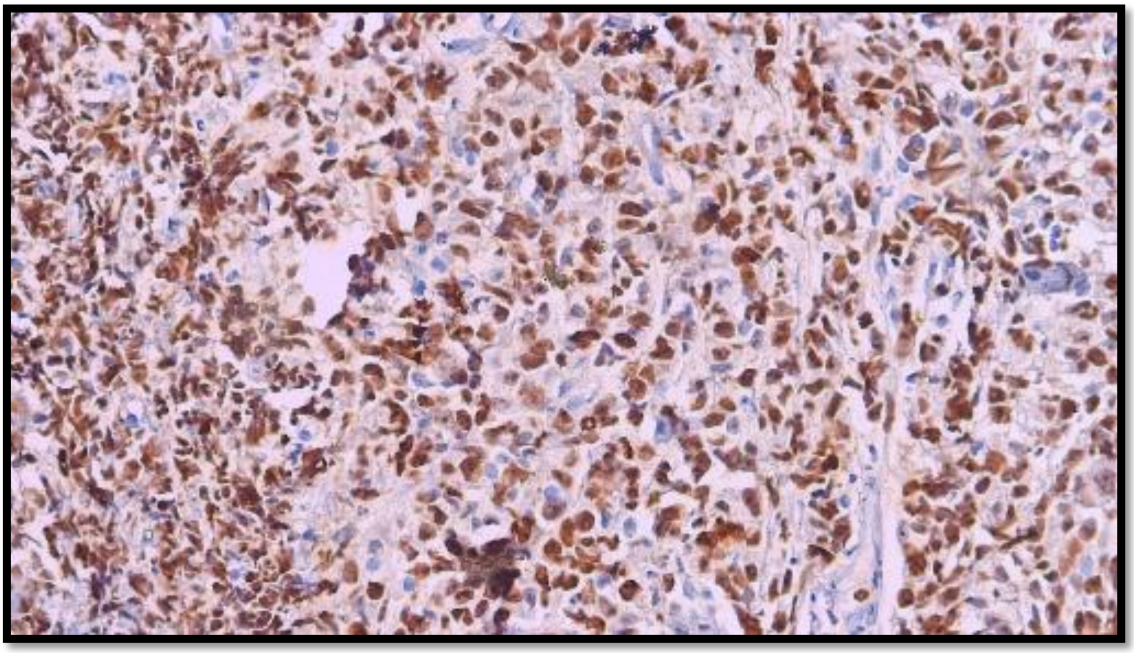
Photomicrograph 16: shows 1+ Staining of Ki67 in poorly differentiated oral SCC

(IHC, x200)



Photomicrograph 17: shows 3+ Staining of Ki67 in poorly differentiated oral SCC

(IHC, x100)



Photomicrograph 18: Shows 3+ Staining of Ki67 in poorly differentiated oral SCC

(IHC, x200)

DISCUSSION

OSCC is the 6th most frequent cancer globally accounting for around 377,000 cases annually.70% of incidences are been detected in males. Globally, oropharyngeal cancer is 16th in frequency. [1]

Johannes Gerdes and colleagues first reported the monoclonal antibody Ki-67 in 1983, speculating that it could be utilized as a marker for actively proliferating cells.[11] Numerous studies have examined the significance of the Ki-67 protein as a prognostic marker.[133] This study was used to evaluate the expression of the Ki-67 in oral squamous cell carcinomas (OSCC) and oropharyngeal squamous cell carcinomas.

1. Age distribution

In the current study, the age of patients ranged from 30-72 years with a mean age of 51.70 ± 10, which is similar to the study by Sharma N et al.[111] The most common age of presentation was 41-60 yrs in other studies. [134-136]. In the present study, the age group with the maximum number of patients was 41-50 years.[Table 20] The findings were similar to those of studies done by Sharma N et al. and Krishna A. et al., whereas a study done by**.** Kumar H et al. found the maximum number of cases **at** 30 to 60 years of age.[111,136,112], [Table 19,20]

Table no 19 . : Comparing mean age for OSCC of present study with other studies

Studies	Mean age
Sharma N et al[111]	51±15.5yrs
Omer S et al[12]	64.24±12.10yrs
Agrawal T et al[4]	48.4±14.14
Deng Z etal [137]	61.8yrs
Present study	51.70±10yrs

Table 20 – Distribution of oral and oropharyngeal Oral and oropharyngeal SCC according to age

	ORAL SCC and oropharyngeal SCC	
Age in years	Number	Percentage %
21-30	1	2.27
31-40	4	9.09
41-50	17	38.63
51-60	10	22.72
61-70	11	25
71-80	1	2.27
TOTAL	44	100

2. Sex distribution

Our study showed the maximum number of cases in males (79.55%). Females comprised only 20.45% of patents. Thus, the male-to-female ratio was 3.8:1. Males are affected more than females because of the habit of tobacco chewing and drinking alcohol. In a study done by Kumar H et al. on 38 patients with oral SCC, males (81.57%) were more than females (18.42%).[112] Research done by Gonzalez-Moles MA et al. also found male preponderance.[135] However, a study done by Ampur et al. showed more female (54.8%) cases than males (45.2%).[138] The reason for this difference was due to other factors like smoking, alcohol, tobacco chewing, oral sex, and education.

3. Oral and oropharyngeal SCC

In our study, 75% (33 cases) were oral SCC and 25% (11 cases) were oropharyngeal SCC. In the research conducted by Rivera C, et al. on the Chilean population, 16 cases were of oral SCC and 21 cases of oropharyngeal SCC.[139] **This may be due to racial differences.**

4. Site wise distribution

In the present study, the buccal mucosa was most common site involved in oral SCC. Similar findings were reported by Krishna A. et al, Ullah E et al., and Sudhakaran et al.[136,141,140] However, Bai X X et al.[142] found that the tongue was the most frequently involved site, and the floor of the mouth was the most frequently involved region in the study by Farhood Z et al. [143] [Table 21]

The oral anatomic site involvements vary from one region to another.

Table 21 – Comparison of distribution of sites of OSCC with other studies

Studies	Sites involved
Krishna A. et al[136]	buccal mucosa (35.5%) >alveolus (28%)> tongue (17%) >gingo-buccal cavity (10%)
Ullah E et al[141]	buccal mucosa (32.4%)>tongue (21.6%)> palate (10.8%) >lower lip (10.8%).
Sudhakaran et al[140]	buccal mucosa (73.3%) > lip(26.7%) >alveolus (13.3%) > tongue
Present study	Buccal mucosa>tongue>GB sulcus>alveolus>lip

4. Histological grades

In the present study, out of 44 cases of squamous cell carcinoma, the maximum number of cases were moderately differentiated (52.27%), followed by well differentiated (27.27%), and poorly differentiated (20.25%). The study conducted by Sharma N et al., Abdelbary RM. et al., and Kumar H et al. also stated moderately differentiated SCC as the most common type, which is in favor of the present study.[111,115,112] [Table22]

Table 22 - COMPARISON OF GRADES OF OSCC WITH OTHER AUTHORS' STUDIES

HISTOLOGICAL GRADE	SHARMA N et al[111]	Abdelbary RM et al[115]	Kumar H et al[112]	PRESENT STUDY
Well Differentiated	32%	25%	26.31%	27.27%
Moderately differentiated	62%	65%	55.26%	52.27%
Poorly Differentiated	6%	10%	18.42%	20.25%

In our study, both oral and oropharyngeal SCC showed the maximum cases of moderately differentiated SCC; however, in oral SCC, well-differentiated SCC was more common than poorly differentiated carcinoma, while in oropharyngeal SCC, poorly differentiated carcinoma had more cases than well-differentiated carcinoma.

The study conducted by Rivera C al. also reported a greater number of poorly differentiated CA cases than well-differentiated carcinoma in Oropharyngeal SCC. Oral SCC showed an equal number of cases of well-differentiated and poorly-differentiated carcinoma.[139]

According to a study by Brian M. et al., the oropharynx's squamous epithelium is derived from the endoderm and has a higher tendency to form aggressive, poorly differentiated carcinomas. In contrast, the squamous epithelium of the oral cavity derives from the ectoderm and tends to give rise to more differentiated and less aggressive lesions.[144]

5.Ki-67 Expression in oral and oropharyngeal SCC

In the present study, Ki-67 was 1+ in 17 cases, 2+ in 10 cases, and 3+ in 6 cases in oral SCC. In oropharyngeal SCC, an equal number of cases (4) had Ki-67 1+, Ki-67 2+, and 3 cases showed a 3+Ki-67 score. The mean Ki-67 was higher in oropharyngeal SCC compared to oral SCC.

The study conducted by Gonzalez-Moles MA et al. on oral SCC divided Ki-67 as 1+(1-25%), 2+(6-50%), 3+(51-75%), and 4+(76-100%). They found 13 cases with 1+, 12 with 2+, 23 cases with 3+, and 17 cases to have 4+ scores.[135]

6. Comparison of age and gender with Ki-67

The mean Ki-67 index was slightly lower in males (37.27) compared to females (38); however, it was not statistically significant association between Ki-67 expression and gender. Similar findings were seen in studies done by Sharma N et al. and Lime JJ et al.[111,145]

In the present study, the mean ki 67 index was seen as the maximum in the age group of 51–60 years compared to other groups. However, there was no significant association between Ki-67 and age. Studies done by Sharma N et al., Omer et al., and Lim JJ et al. also revealed no association between age and Ki-67 expression.[111,12,145]

7. Histological grade and Ki-67

Positive Ki-67 staining was seen in all the cases. It was observed that Ki-67 LI was 20.58±8.70 in well-differentiated SCC, which increased to 37.17±13.55 in moderately diff SCC and 67.67±25.12 in poorly differentiated SCC. There was a statistically significant difference in Ki-67 staining between the various histological grades of OSCC in the present study.[Tabel 23]

Table 23 - COMPARISON OF KI-67 LI WITH OTHER STUDIES

KI-67 Labelling index	Kumar H et al[112]	Priyanka Yadav[114]	Present study
Well-differentiated	28.52±21.25	12.2%	20.58 ±8.70
Moderately differentiated	42.85±18.2	41.09%	37.17±13.55
Poorly differentiated	68.57±17.6	75%	67..67±25.12

In the above studies by Kumar H et al., Yadav P et al. also observed a positive association between histological grade and Ki-67. A study by . Sharma S et al, Agrawal T et al., and Krishna A et al. also showed a significant correlation between histological grade and Ki-67 expression.[5,4,114] Poorly differentiated SCC had the greatest mean ki 67 staining, while well-differentiated SCC showed the lowest staining. In our study of well-differentiated carcinoma, the Ki-67 staining was seen mainly at the periphery of the tumor cell nest compared to poorly differentiated carcinoma, which showed diffuse Ki-67 staining. Similar results were found in a study by Takkem A et al., which found that the tumor island's central cells in well-differentiated SCC lacked Ki-67 expression because the central cells are highly differentiated and capable of keratinization, while the peripheral layer contains less differentiated cells.[133]

8.Ki-67 and type of cancer

The mean Ki-67 was higher in oropharyngeal SCC compared to oral SCC in the present study. However, the association between KI-67 and the type of cancer was not statistically significant.

SUMMARY

- This is a cross-sectional study conducted at KLE'S DR. PRABHAKAR KORE CHARITABLE HOSPITAL and KLE'S DR. PRABHAKAR KORE HOSPITAL and MEDICAL RESEARCH CENTER BELAGAVI by collecting data and blocks of oral and oropharyngeal SCC.
- 44 cases of SCC of oral and oropharynx were studied from 1st January 2023 to 1ST January 2024.
- Out of 44 cases 20 were resection specimens and 24 were biopsy.
- All the cases were subjected to IHC for ki67 expression, the results of which were correlated with age, sex, type of cancer, and histological grading.
- The peak incidence of oral and oropharyngeal SCC was seen in the age group of 41-50 years of age with a mean age of 51.70±10.08 yrs.
- Of 44 oral and oropharyngeal SCC cases male patients accounted for 79.55% and females were 20.45%.
- The most common site involved in oral SCC was buccal mucosa and it was tonsillar pillars in oropharyngeal SCC.
- Out of 44 cases of oral and oropharyngeal SCC histological grade with highest frequency was moderately differentiated (52.27%), followed by well differentiated (27.27%)and poorly differentiated (20.25%) cases.
- In oral squamous cell carcinoma well-differentiated carcinoma was more common than poorly diff ca while in oropharyngeal SCC poorly diff carcinoma had a higher number of cases.
- Ki67 expression was 1+ in 17 cases, 2+in 10 cases and 3+ in 6 cases in oral SCC . Oropharyngeal SCC had an equal number of cases(4) with Ki67 1+,2+, and 3 cases had Ki67 3+ scores.

- Mean Ki 67 index was higher in females and males but there was no statistically significant.
- In the current study, the age group of 51 to 60 years old had the highest mean Ki-67 index.
- The study showed significant correlation between ki67 expression and borders histological staging (p value<0.0001)
- The mean Ki67 was higher in oropharyngeal SCC compared to oral SCC but it was not statistically significant.

CONCLUSION

In our study, oral and oropharyngeal SCC were seen predominantly in the male gender. The most common site is buccal mucosa, and this is due to the prevalence of the habit of tobacco chewing in various forms in this region of India. Ki-67 expression was also significantly higher in poorly differentiated OSCC as compared to well-differentiated tumors, indicating that Ki-67 expression increases with decreasing tissue differentiation (p-value <0.0001). No significant correlation between Ki-67 and age, sex, or type of cancer was seen.

Since Ki-67 is a proliferative marker, it can be useful as a prognostic marker and further help in evaluating the treatment of patients with OSCC.

LIMITATION

1. In our study, staging was not carried out.

2. There was no HPV testing conducted.

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ANNEXURE - I- INFORMED CONSENT FORM

STUDY OF KI67 EXPRESSION IN ORAL AND OROPHARYNGEAL SQUAMOUS CELL CARCINOMA

Principal Investigator: BN0121004

Purpose of the study: .You are being asked to enroll in this study as you are eligible for participation in this study. If you are diagnosed with oral carcinoma, you will be included in this study.

Procedure: During this study, you will be asked questions regarding history and background and you are supposed to answer to the best of your knowledge. If you agree to enroll yourself in this study, you will be interviewed regarding your present, past and family history and your clinical manifestations.

Risks and benefits: There are no risks involved in taking part in this study. You will/will not have nor get any benefits by participating in this study. The data gathered will help the population at large.

Alternatives: Taking part in this study is voluntary. You may choose not to take part in this study or if you decide to take part now, you can later change your mind and withdraw from the study. The study doctor may terminate your participation in this study anytime.

Privacy and confidentiality: All information collected about you during the course of this study will be kept confidential to the extent permitted by law. The code numbers will identify you in this research record. Information from this study will be published but your identity will be confidential in any publication. No information about you or information provided by you during research will be disclosed to other without your written permission except:

1. In emergency to protect your rights and welfare.
2. If required by law.

Financial incentives for participation: You will not be paid / offered any gift/incentives for participating in this study.

Authorization to publish results: The results of this study would be forwarded to the KAHER, Belagavi as a part of requirement towards the completion of MD degree, review and publishing.

Questions: In case you have any questions related to the study in future you can contact:

1. If you have any queries about your rights as a study subject, you may call Dr. Harsha Hegde, Chairman of J.N. Medical College, Institutional Ethical Committee & scientist D, ICMR, National Institute of Traditional Medicine, Phone No-9480422500, at J.N. Medical College, Belagavi.

CONSENT STATEMENT

I voluntarily agree to take part in this study by signing below. I may withdraw at any time. I am not giving up any legal rights by signing this form. My signature below indicates that I have read or it has been read to me this entire consent form and have had all my questions answered.

Name of the participant: (Signature/thumbprint)

Name of the witness: (Signature/thumbprint)

Name of the investigator: (Signature)

Date:

ANNEXURES II – PROFORMA

PROFORMA FOR ORAL CARCINOMA

PATIENT HISTORY

Name: Age: Sex: IPno.:

History:

Clinical diagnosis with stage:

Type of sample: Biopsy/Complete excision/Any other procedureGross:

Size:

Extension:

Lymph node involvement: No. of Lymph nodes involved: Groups of lymph node:

Microscopy:

1. Histopathological diagnosis and grade:
2. IHC staining:

- Pattern of staining:
- Percentage of tumour cells stained by Ki 67 :
- Score based on staining percentage of tumor cells:

ANNEXURE- III

HEMATOXYLIN AND EOSIN STAINING PROTOCOL

1. Deparaffinize in Xylene I and II and III changes. (III change use warmed xylene) (5 minutes in each)
2. Rehydrate using
    a. Absolute ethanol 100% (5 minutes)
    b. Absolute Ethanol 100% (5 minutes)
3. Rinse in distilled water (5 minutes)
4. Rinse in running tap water (5 minutes)
5. Stain in Harris's haematoxylin by progressive method (2 minutes) Fresh and filtered
6. Rinse in running tap water (20 minutes)
7. Decolorize in 1% acid alcohol (1 second)
8. Rinse well in tap water (5 minutes)
9. Immerse in hot water bath, 55˚C for blueing (3 seconds)
10. Rinse in tap water (5 minutes)
11. Counterstain in Eosin (15 seconds)
12. Dehydrate with absolute alcohol 100% (2-4 dips)
13. Clear in xylene I and II (5 minutes)
14. Mount with DPX.

Stock solution – Eosin:

Stock – 1% aqueous Eosin – YStock – 1% aqueous Phloxin B

Working Solution – Eosin:

100ml stock Eosin

10 ml stock Phloxin B780 ml 95% Ethanol

4 ml glacial acetic acid

Working Solution – Hematoxylin

Harris Hematoxylin, 1 litre

Working solution – 0.25% Acid Alcohol

95% Ethanol, 2578 ml

dH2O, 950 ml

HCl, 9ml

Result: Nuclei – blue, cytoplasm – pink, RBCs – red.

Reference: Bancroft D, Laylon C. The haematoxylin and eosin, In: Kim SS Ed, Bancroft's Theory and practice of histopathological techniques. &th Ed., China, Churchill Livingstone; 2013: p173-187.

ANNEXURE- IV

IMMUNOHISTOCHEMICAL STAINING PROTOCOL FOR Ki67

1. Cut the sections at approximately 3-4 µm thickness in poly L Lysine coatedslides.
2. Bake the sections at 37 degree celsius overnight. Before test bake it at 60degree celsius for 1 hour
3. Deparaffinise steps-

i. Xylene I- 10 minutes

ii. Xylene II- 10 minutes

iii. Absolute alcohol I- 10 minutes

iv. Absolute alcohol II- 10 minutes

v. Rinse in water- 5 minutes

vi. Rinse in distilled water- 1 minute

2. Antigen retrieval by (TRIS buffer +EDTA)- Buffer solution

(Required amount of buffer is prepared and cook the slides in pressure cooker for 3 whistles)

3. Cooling of sections to room temperature for 15 minutes.
4. Wash with wash buffer 2 times with gap of 30 seconds each
5. Treatment with 3% hydrogen peroxide for 8-10 minutes to block endogenous peroxidase.
6. Wash with water buffer 3 times with a gap of 30 seconds
7. Treatment with primary monoclonal antibody for p16 protein (biogenex) for 45 to 60 minutes in closed chamber at room temperature

8. Wash with wash buffer 3 times with gap of 30 seconds each
9. Treatment with polymer Horseradish peroxidase (HRP) for 25 to 30 minutes in closed chamber at room temperature.
10. Wash with wash buffer 3 times with gap of 30 seconds each
11. Treatment with Diaminobenzidine (DAB) substrate (secondary antibody) for 10 minutes to give brown colour to antigens.
12. Wash with water for 2 minutes
13. Wash with distilled water for 1 minute.
14. Counter stain with Harris haematoxylin for 3 minutes.
15. Blueing in warm water- 1minute
16. Clearing with xylene for two minutes. Dry the slides and mount with DPX

PREPARATION OF REAGENTS

1. Antigen retrieval Buffer

TRIS EDTA Buffer- pH: 8.5 to 9.0

Preparation:

TRIS Base- 1.21 gram

EDTA (atomic number: 372)- 0.37 gramDissolve in 1000ml of water

2. Wash buffer

TRIS BUFFERED SALINE (TBS)- pH: 7.2 to 7.6

Preparation:

TRIS Base- 8.6 gramNaCl- 9.6 gram

Dissolve in 1000ml of water.

Adjust pH by using concentrated HCl

17. Treatment with polymer Horseradish peroxidase (HRP) for 25 to 30 minutesin closed chamber at room temperature.
18. Wash with wash buffer 3 times with gap of 30 seconds each
19. Treatment with Diaminobenzidine (DAB) substrate (secondary antibody) for10 minutes to give brown colour to antigens.
20. Wash with water for 2 minutes
21. Wash with distilled water for 1 minute.
22. Counter stain with Harris haematoxylin for 3 minutes.
23. Blueing in warm water- 1minute
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PREPARATION OF REAGENTS

3. Antigen retrieval Buffer

TRIS EDTA Buffer- pH: 8.5 to 9.0

Preparation:

TRIS Base- 1.21 gram

EDTA (atomic number: 372)- 0.37 gramDissolve in 1000ml of water

4. Wash buffer

TRIS BUFFERED SALINE (TBS)- pH: 7.2 to 7.6

Preparation:

TRIS Base- 8.6 gramNaCl- 9.6 gram

Dissolve in 1000ml of water.

Adjust pH by using concentrated HCl

ANNEXURE V

WHO classification of tumors of the oral cavity and mobile tongue[31]

Epithelial tumors and lesions

Squamous cell carcinoma

Oral epithelial dysplasia

Low grade

High grade

Proliferative verrucous leukoplakia

Papilloma's

Squamous cell papilloma

Condyloma acuminatum

Verruca Vulgaris

Multifocal epithelial hyperplasia

Tumors of uncertain histogenesis

Congenital granular cell epulis

Ectomesenchymal chondromyxoid tumor

Soft tissue and neural tumors

Granular cell tumor

Rhabdomyoma

Lymphangioma

Hemangioma

Schwannoma

Neurofibroma

Kaposi sarcoma

Myofibroblastic sarcoma

Oral mucosa melanoma

Salivary type tumors

Mucoepidermoid carcinoma

Pleomorphic adenoma

Haematolymphoid tumours

CD30-positive T-cell lymphoproliferative disorder

Plasmablastic lymphoma

Langerhans cell histiocytosis

Extramedullary myeloid sarcoma

WHO classification of tumors of the oropharynx (base of tongue, tonsils, adenoids)[31]

Squamous cell carcinoma

Squamous cell carcinoma. HPV-positive

Squamous cell carcinoma, HPV-negative

Salivary gland tumors

Pleomorphic adenoma

Adenoid cystic carcinoma

Polymorphous adenocarcinoma

Haematolymphoid tumours

Hodgkin lymphoma,

nodular lymphocyte predominant

Classical Hodgkin lymphoma

Nodular sclerosis

classical Hodgkin lymphoma

Mixed cellularity classical Hodgkin lymphoma

Lymphocyte-rich classical Hodgkin lymphoma

Lymphocyte-depleted classical Hodgkin lymphoma

Burkitt lymphoma

Follicular lymphoma

Mantle cell lympl1oma T-lymphoblastic leukemia/lymphoma

Follicular dendritic cell sarcoma

ANNEXURE VI

The TNM staging of the Oral squamous cell carcinoma

TNM STAGING 2017

Tx	Primary tumor cannot be assessed
Tis	Carcinoma in situ
T1	Tumor< 2 cm, < 5 mm depth of invasion (DOI)
T2	Tumor < 2 cm, DOI > 5 mm and < 10 mm or tum or > 2 cm but < 4 cm, and < 10 mm DOI
T3	Tumor>4 cm or any tumor> 10 mm DOI
T4	moderately advanced or very advanced local disease
T4a	Moderately advanced local disease (lip) Tumor invades through cortical bone or involves the inferior alveolar nerve, floor of the mouth, or skin of the face (i.e., chin or nose) (oral cavity) Tumor invades adjacent structures only (e.g., through cortical bone of the mandible or maxilla, or involves the maxillary sinus or skin of the face)
T4b	Very advanced local disease Tumor invades masticator space, pterygoid plates, or skull base and/or encase the internal carotid artery

N Category	N Criteria
NX	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	metastasis in a single ipsilateral lymph node, 3 cm or smaller in greatest dimension and E N E (-)
N2	Metastasis in a single ipsilateral lymph node. 3 cm or smaller in greatest dimension and ENE(+); or larger than 3 cm but not larger than 6 cm in greatest dimension and E N E (-); or metastases in multiple ipsilateral lymph nodes, none larger than 6 cm in greatest dimension and E N E (-); or in bilateral or contralateral lymph nodes, none larger than 6 cm in greatest dimension, E N E (-)
N2a	metastasis in a single ipsilateral or contralateral node 3 cm or smaller in greatest dimension and ENE(+); or a single ipsilateral node larger than 3 cm but not larger than 6 cm in greatest dimension and ENE (-)
N2b	metastasis in multiple ipsilateral nodes, none larger than 6 cm in greatest dimension and ENE (-)
N2c	metastasis in bilateral or contralateral lymph nodes, none larger than 6 cm in greatest dimension and E N E (-)
N3	metastasis in a lymph node larger than 6 cm in greatest dimension and EN E (-); or in a single ipsilateral node larger than 3 cm in greatest dimension and ENE(+); or multiple ipsilateral, contralateral or bilateral nodes any with ENE(+)
N3a	metastasis in a lymph node larger than 6 cm in greatest dimension and E N E (-)
N3b	metastasis in a single ipsilateral node larger than 3 cm in greatest dimension and ENE(+); or multiple ipsilateral, contralateral, or bilateral nodes any with ENE(+)

M Category	M Criteria
M0	No distant metastasis
M1	Distant metastasis

T	N	M	stage group
T1	N0	MO	I
T1	N0	M0	II
T3	N0	M0	III
T 1,2,3	N	M0	III
T4a	N0,1	M0	IVA
T1,2,3,4a	N2	M0	IVA
Any T	N3	M0	IVB
T4b	Any N	M0	IVB
Any T	Any N	M 1	IVC

Oropharynx (p16-)

T	T Category T Criteria
TX	Primary tumor cannot be assessed
Tis	Carcinoma in situ
T1	Tumor 2 cm or smaller in greatest dimension
T2	Tumor larger than 2 cm but not larger than 4 cm in greatest dimension
T3	Tumor larger than 4 cm in greatest dimension or extension to the lingual surface of epiglottis
T4	moderately advanced or very advanced local disease T4a Moderately advanced local disease Tum or invades the larynx, extrinsic muscle of tongue, medial pterygoid, hard palate, or mandible* T4b Very advanced local disease Tumor invades lateral pterygoid muscle, pterygoid plates, lateral nasopharynx, or skull base or encases carotid artery

N Category	N Criteria
NX	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	metastasis in a single ipsilateral lymph. node, 3 cm or smaller in greatest dimension and E N E (-)
N2	Metastasis in a single ipsilateral node larger than 3 cm but not larger than 6 cm in greatest dimension and E N E (-); or metastases in multiple ipsilateral lymph nodes, none larger than 6 cm in greatest dimension and E N E (-); or in bilateral or contralateral lymph nodes, none larger than 6 cm in greatest dimension and E N E (-)

N2a	Metastasis in a single ipsilateral node larger than 3 cm but not larger than 6 cm in greatest dimension and ENE (-)
N2b	Metastasis in multiple ipsilateral nodes, none larger than 6 cm in greatest dimension and ENE (-)
N2c	Metastasis in bilateral or contralateral lymph nodes, none larger than 6 cm in greatest dimension and ENE (-)
N3	metastasis in a lymph node larger than 6 cm in greatest dimension and ENE (-); or metastasis in any node(s) and clinically overt ENE(+)
N3a	metastasis in a lymph node larger than 6 cm in greatest dimension and E N E (-)
N3b	metastasis in any node(s) and clinically overt ENE(+)

Category	M Criteria
M0	No distant metastasis
M1	Distant metastasis

TNM	stage group
Tis NO MO	0
TI NO MO	I
T2 NO MO	II
T3 NO MO / T1,T2,T3 NI MO	III
T4a N0,1 MO/ Tl,T2,T3,T4a N2 MO	IVA
Any T N3 MO / T4b Any N MO	IVB
Any T Any N M I	IVC

ANNEXURE- VII- KEY TO MASTER CHART

M	Male
F	Female
BM	Buccal Mucosa
(R)	Right
(L)	Left
WLE	Wide local excision
PMMC	Pectoralis major myocutaneous flap
MRND	Modified radical neck dissection
H&E	Hematoxylin and Eosin
PD	Poorly differentiated
MD	Moderately differentiated
WD	Well differentiated
%	Percentage
TP	Tonsillar Pillars
SP	Soft Palate
PPW	Posterior pharyngeal wall
BOT	Base of Tongue

ANNEXURE- VIII - MASTER CHART

3x SERIAL NO	HP NO	AGE	SEX	Tumor site	TYPE OF SPECIMEN	H&Ediagnosis	KI67 POSITIVE RATE	Score	Average	Type of cancer
1	1108/23	61	M	Alveolus	WLE R lower alveolus+ Hemimandebulectomy +MRND	MD	60-70%	65%	3+	Oral
2	2054/23	72	F	BM	biopsy BM	MD	45-55%	50%	2+	Oral
3	5080/22	45	M	GBS	biopsy left GBS sulcus + floor of mouth	WD	10-20%	15%	1+	Oral
4	5026/22	49	F	LIPS	biopsy R upper lip	WD	10-20%	15%	1+	Oral
5	535/23	48	M	BM	biopsy bm	WD	10-20%	15%	1+	Oral
6	5134/22	40	M	TONGUE	L Hemiglosectomy+ MRND	MD	30-40%	35%	2+	Oral
7	5196/22	65	M	GBS	R Hemimandibulectomy +MRND+PMMC Recons GBS	PD	20-30%	25%	1+	Oral
8	477/23	43	M	TONGUE	WLE of tongue	PD	30-40%	25%	2+	Oral
9	5077/22	60	M	BM	R bite resection +MRND - R BM	MD	50-60%	55%	3+	Oral
10	4967/22	60	M	BM	biopsy BM	WD	10-20%	15%	1+	Oral
11	2089/23	42	M	alveolus	biopsy from lower alveolus	PD	80-90%	85%	3+	Oral
12	486/23	55	F	BM	biopsy BM	WD	10-20%	15%	1+	Oral
13	481/23	50	M	GBS	WLE + Hemimandebulectomy+MRND	MD	20-30%	25%	1+	Oral
14	1119/23	50	F	BM	WLE of L BM +Mrginal mandibulectomy	MD	30-40%	35%	2+	Oral
15	4910/22	42	M	BM	WLE+Rt MRND+Collagean graft reconstruction	MD	40-50%	45%	2+	Oral
16	5076/22	59	M	BM	WLE R BM + hemimandubulectomy+MRND	MD	40-50%	45%	2+	Oral
17	536/23	42	M	TP	Bite resection L BM+MRND(tumor - tumors from anterior tonsillar pillar)	PD	50-60%	55%	3+	Oropharyngeal
18	170/23	54	M	Tongue	biopsy tongue	MD	40-50%	45%	2+	Oral
19	2213/23	40	F	BM	biopsy BM LEFT	WD	10%	10%	1+	Oral
20	3644/23	54	M	BM	WLE right BM + Hemimandibulectomy	PD	50-60%	55%	3+	Oral
21	2884/23	45	M	BM	biopsy BM	MD	40-50%	45%	2+	Oral
22	2846/23	30	M	BM	Right composite bite resection + segemental mandibulectomy BM	MD	50-60%	55%	3+	Oral
23	2987/23	45	M	BM	bm biopsy	MD	40-50%	45%	2+	Oral
24	2202/23	65	F	Tongue	WLE of ca of floor of mouth+lateral tongue right	MD	20-30%	25%	1+	Oral
25	3059/23	40	F	Tongue	commando + PMMC BM	WD	20-30%	25%	1+	Oral
26	3106/23	32	M	Tongue	Hemiglossectomy	MD	20-30%	25%	1+	Oral
27	3217/23	58	F	BM	Bite resection +MRND+PMMC flap BM	PD	80-90%	85%	3+	Oral
28	3273/23	61	M	Tongue	glossectomy+pmmc	MD	20-30%	25%	1+	Oral
29	3271/23	35	M	BM	biopsy BM	MD	30-40%	35%	2+	Oral
30	2841/23	46	M	BM	commando left +PMMC flap bm	MD	35-45%	40%	2+	Oral
31	4877/22	50	M	SP	biopsy from rmt and soft palate	PD	75-85%	80%	3+	Oropharyngeal
32	867/23	66	M	TP	biopsy from right tosillar pillar	PD	50-60%	55%	2+	Oropharyngeal
33	1126/23	55	M	SP	biopsy from soft palate	MD	30-40%	35%	2+	Oropharyngeal
34	1135/23	50	M	PPW	Biopsy oropharynx	WD	10-20%	15%	1+	Oropharyngeal
35	1614/23	61	M	BOT	biopsy RMT	MD	40-50%	45%	2+	Oropharyngeal
36	1852/23	65	F	PPW	Oropharynx biopsy	MD	30-40%	35%	2+	Oropharyngeal
37	2349/23	60	M	TP	biopsy from tonsillar pillar	PD	85-95%	90%	3+	Oropharyngeal
38	1398/23	63	F	SP	Biopsy from SP	WD	10%	10%	1+	Oropharyngeal
39	4153/23	51	M	TP	tonsils biopsy	MD	10-20%	15%	1+	Oropharyngeal
40	3274/23	50	M	Uvula	uvula biopsy	MD	10-20%	15%	1+	Oropharyngeal
41	3242/23	65	M	BM	left BM	WD	35-45%	30%	2+	Oral
42	2523/23	43	M	Tongue	tongue ca	WD	10-20%	15%	1+	Oral
43	390/23	62	M	Alveolus	Alveolus	MD	10-20%	15%	1+	Oral
44	2918/32	46	M	BM	BM resection	WD	10-20%	15%	1+	Oral