EVOLUTION OF KI-67 EXPRESSION IN EPITHELIAL DISPLASIA AND SQUAMOUS CELL CARCINOMA OF ORAL CAVITY

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ABSTRACT

BACKGROUND:

Oral malignancy is one of the most common malignancies with squamous cell carcinoma being the commonest oral malignancy type. Proliferative index activity helps to evaluate the cancer growth rate and aggressiveness of the carcinoma, which helps to decide the outcome and plan the choice of treatment. Immunohistochemistry marker Ki-67 is one of the proliferative markers which helps in predicting the survival of the patient. Hence the current study is aimed to examine the expression of Ki-67 in oral epithelial dysplasia (OED) and oral squamous cell carcinoma (OSCC).

OBJECTIVES:

To evaluate the expression of Ki-67 in oral epithelial dysplasia and oral squamous cell carcinoma and to correlate the Ki-67 expression in various grades of OED and OSCC.

MATERIAL AND METHODS:

A prospective observational study was done on 64 samples of clinically diagnosed cases of epithelial dysplasia and malignancy of the oral cavity received in the Histopathology section. For each case, two sections of 4 μm were prepared. One section was stained for routine Haematoxylin and eosin (H&E) stain, and another section was used for IHC staining of Ki-67. Expression of Ki-67 was noted in all the cases of OED and OSCC. In OED, expression of Ki-67 was noted in the epithelial layers. In OSCC, the Ki67 scoring and labelling index (LI) was calculated, and scoring was done.

RESULTS:

Total 64 cases were studied, out of which 19 were OED, and 45 were OSCC. In OED, 12 cases were Mild OED, 1 and 3 were moderate and severe OED

respectively. In moderate and severe OED, Ki67 expression was seen in the basal, parabasal, and spinous layers. Out of 45 cases of OSCC, 13 were Well differentiated OSCC, 31 were Moderately differentiated and 1 was Poorly differentiated OSCC. Ki67 expression was highest in poorly differentiated OSCC and the lowest in well differentiated OSCC.

CONCLUSION:

Ki67 expression correlates with grading of OED and OSCC, thus it can be considered as a proliferative marker and predictor of survival of the patient.

KEYWORDS:

Oral Epithelial dysplasia, Ki67, Oral Squamous cell carcinoma**,** Proliferative marker.

INTRODUCTION

Oral malignancy is one of the most common malignancies reported in India. Squamous cell carcinoma (SCC) is the commonest oral malignancy type accounting for 95% of all malignant tumors detected in the oral cavity. [1,2]

Treatment for oral malignancy is decided based on the features such as lymph node involvement, tumor size, and distant metastases. However, in some cases, these criteria could not justify the poor prognosis in patients with carcinoma diagnosed early. Conventional diagnostic modalities such as imaging and routine histopathological examination are insufficient to predict the behavior of malignant tumors in all cases. Hence molecular marker studies are done to assess the aggressiveness of the malignant tumors. [2]

The proliferative index activity in oral squamous cell carcinoma helps to evaluate the cancer growth rate and aggressiveness of the tumor on which the outcome and the choice of treatment depend. The most common immunohistochemistry marker (IHC) for cell proliferation study is Ki67. [1]

The anti-Ki-67 monoclonal antibody is to determine the cell proliferation of squamous epithelial cells in various dysplastic lesions and squamous cell carcinoma of the oral cavity. Ki-67 can also be used as a marker of proliferation in the early diagnosis of pre-malignant conditions that are more prone to developing malignancy. [1]

Ki-67 protein has been widely studied in various malignancies such as carcinoma breast, cervix, non-Hodgkin's lymphoma, and carcinoma of the large intestine as a prognostic marker. It was concluded in these studies that Ki-67 can be considered one of the markers for predicting the survival of the patient and the recurrence of the tumor. In these studies, it was also mentioned that Ki-67 protein could be considered as a potential therapeutic target. In these studies, it was also noted

that strategies that inactivate Ki-67 protein could be considered for the anti-proliferative approach to the tumor. These findings may help in the applicability of the inactivation of Ki67 in the treatment of cancer. In very few studies, evaluation of Ki67 expression in Oral Epithelial Dysplasia (OED) and Oral Squamous cell carcinoma (OSCC) has been done to predict the aggressiveness of the tumor. [1,2]

Hence, the present study was done to evaluate the expression of proliferative IHC marker Ki-67 in OED and OSCC of the oral cavity and the association of Ki-67 expression in various grades of OED and OSCC.

AIMS AND OBJECTIVES OF THE STUDY

1. To evaluate the immunohistochemistry expression of Ki-67 in epithelial dysplasia and squamous cell carcinoma of oral cavity.
2. To correlate the association of Ki-67 expression in various grades of epithelial dysplasia and squamous cell carcinoma of oral cavity.

REVIEW OF LITERATURE

Oral Cavity:

The oral cavity and oropharynx are the uppermost part of the digestive tract. The oral cavity and oropharynx are distinctive for having a wide range of tissues in a little space. The oral cavity comprises of vestibule that lies anterior to teeth and the oral cavity proper that lies posterior to teeth. The oral cavity is bounded by the lips on the anterior side and laterally by the cheeks. It is surrounded by the hard palate above, the mucous membrane that covers the upper surface of the tongue below, and the muscles that attach to the inside of the mandible, including the geniohyoid, mylohyoid, and digastric muscles. [3,4]

The oral cavity proper is subdivided into various anatomical subsites. It includes the tongue, hard palate, buccal mucosa, retromolar trigone, lips, and sheets of mylohyoid, digastric, and geniohyoid muscles attached to the inner surface of the mandible. The retromolar trigone is a small mucosal area in the oral cavity behind posterior molars on the mandibular ramus. It is present between the oral cavity, oropharynx, and nasopharynx. This junction point plays a major role in the spread of malignant tumors of the oral cavity. [5]

Oral mucosa begins from the vermillion border of lips and extends up to pharyngeal mucosa. The oral mucosa is lined by non-keratinized stratified squamous epithelium. Subepithelial tissue shows fibrous lamina and a few minor salivary glands. The mucosa overlying the tongue is lined by stratified squamous epithelium posteriorly and keratinized stratified squamous epithelium anteriorly, as the anterior aspect is subjected to more friction during mastication. [3] Lymphatic drainage of the oral cavity occurs through submental, submandibular, and parotid groups of lymph nodes. These lymph nodes drain into the right and left deep cervical lymph nodes. [6]

Examination of the oral cavity:

The oral examination has historically received less attention, especially in the bachelor's course of the medical curriculum. Systematic evaluation of the oral cavity gives important information about various health and disease conditions. Hence, a complete and detailed oral examination of the oral cavity with medical and dental history can provide valuable information about the health and well-being of the individuals. [4]

Oral Cavity Cancers:

Cell cycle:

The cell cycle is a process in which a living cell copies all of its parts and splits into two daughter cells. Each daughter cell receives all the materials and information needed to continue the process of cell division. [7]

The cell cycle is divided into four phases: the pre-synthetic growth phase, also called as G1, the DNA synthesis phase, called as S phase. Pre-mitotic growth phase called as G2, and the mitotic phase called as M phase. [8] At any point in time, the cells of the body may either be in a cell cycle or remain quiescent. When the cells are not actively proliferating, that phase is called a quiescent or G0 state. Cells can enter the G1 phase from the G0 quiescent cell pool and undergo the further process of the cell cycle. Some cells which are continuously replicating enter the G1 phase after completing a round of mitosis. DNA replication occurs in the S phase, followed by G2 and M phase. [8] Body tissue like epithelial lining, intestinal mucosa, and hematopoietic progenitor cells are continuously replicating. They are constantly transitioning from mitosis (M phase) to the G1 phase, which is the precursor to further cell division. On the contrary, cells like hepatocytes replicate infrequently and are generally in the quiescent phase. The cell cycle contains surveillance systems that are

set up to detect DNA or chromosomal damage. The quality of cells passing from one phase to another phase of the cell cycle is regulated at cell cycle checkpoints. These quality control checkpoints ensure that cells with genetic imperfections do not replicate. DNA integrity has been continuously monitored by the G1-S checkpoint before irreversibly committing cellular resources to DNA replication. The G2-M restriction checkpoint makes sure that there is correct genetic replication before the actual cell division. When cells having DNA anomalies are detected, checkpoint activation slows down the process of the cell cycle and activates DNA repair mechanisms. Cells either go through apoptosis or enter a non-replicative condition known as senescence if the genetic abnormality is too severe to be fixed. [8]

Cell division:

Cell division is a meticulously planned process. It is essential for cell growth and renewal of dead and damaged cells of the body, and thus it is necessary for maintaining steady-state tissue homeostasis. The core of cell proliferation is the accurate replication of DNA in correlation with the genesis of all other components of a cell. Equal distribution of cellular RNA, DNA, and other constituents like cytoplasm and cell organelles to daughter cells happens by mitosis. [7,9]

The cell cycle comprises several clearly defined stages of cell division. Types of cell division are meiosis and mitosis. The process of meiosis occurs during the gametes' formation, reducing the reproductive cell's chromosome number to half. In the process of mitosis, the nucleus of a eukaryotic cell splits in half, causing the parent cells to divide into two daughter cells with an equal number of chromosomes. [9] Cell cycle disruption is the primary indicator of cancer development. Increased cell proliferation is regarded as an early marker of disordered growth. Cancer arises from a cascade of uncontrolled cellular events called atypia, in which nuclear and cellular

morphometric changes occur due to excessive changes in DNA synthesis, which are accompanied by proliferation and death. The defining feature of malignant tumors is excessive cell proliferation that is brought in by an increase in abnormal mitosis. [10] Carcinomas of the oral cavity can arise from a pre-malignant dysplastic lesion that presents clinically as leukoplakia, erythroplakia, or a combination of the two, or they can arise de novo. [11]

In comparison to the normal mucosa of the oral cavity, epithelial dysplasia and SCC exhibit higher mitotic activity, which is significant evidence of accelerated cell turnover. [10]

Oral Squamous cell carcinoma:

Oral malignancy is one of the most common malignancies reported in India. Oral Squamous cell carcinoma (OSCC) is the commonest oral malignancy type accounting for 95% of all malignant tumors detected in the oral cavity. [7,9] OSCC is considered the 8th most prevalent cancer in males and the 5th most prevalent cancer in females. [9]

Oral cavity cancer has an incidence of approximately 4.3 out of 100 thousand cases. The oral cavity cancer has observed a 5-year overall survival rate of 56%. [12] Oral squamous cell carcinomas (OSCC) solely represent 66% of oral and oropharyngeal SCC. It is mainly linked to various risk factors that includes, heavy consumption of alcohol, smoking of tobacco, and areca nut use. Persistent high-risk human papillomavirus (HPV) infection is responsible only for 3–5% of OSCCs. Other potential and emerging risk factors for OSCC include a low-antioxidant diet, a low socio-economic status, local and systemic immunosuppression, oral potentially malignant disorders (OPMDs), inheritable cancer syndromes such as Bloom syndrome, dyskeratosis congenita, Cowden syndrome, familial genetic alterations,

etc. UV-light exposure is also considered an emerging risk factor, especially for carcinoma of the lip. [13] Tobacco chewing and smoking is a robust and modifiable risk factor for various diseases like pulmonary disease, cardiovascular disease, and cancer. Smokers have a lifetime chance of developing tobacco-related cancer that results in early death amongst 1 in 3 tobacco chewers. Lung cancer and cancers of the oropharynx, larynx, and esophagus are all tobacco-related malignant tumors. [14]

Malignant tumors of the oral cavity most commonly affect the anterior two-thirds of the tongue, followed by the floor of the mouth, buccal mucosa, retromolar trigone, hard palate, and gingiva. The tongue is the subsite within the oral cavity responsible for the highest number of OSCC. [11] This might be due to the rich lymphatic network in the tongue. Moreover, tongue muscles are not encapsulated by fascia or bone, which may lead to easy spread of a tumor. [11]

Grading of oral OSCCs is based on histopathological features such as nuclear pleomorphism, degree of keratinization, mitotic activity, and cellular atypia. [11] Broders was the first one who introduced the histopathological grading for squamous cell carcinoma. This classification was based on the differences in differentiation between tumors.[15] WHO Classification of Head and Neck Tumors was published in 2017, which supports a simple grading system based on the Broders criteria. It classifies OSCCs as well-differentiated OSCCs, moderately differentiated OSCCs, and poorly differentiated OSCCs.[16,17]

Progression of oral epithelial dysplasia to carcinoma:

The exact method by which the progression of any dysplastic tissue occurs into malignant tissue is not clearly understood. However, various studies observed that alterations in DNA synthesis result in the impairment of cellular events, leading to cellular and nuclear morphological changes. These atypical morphological changes

may be due to alterations in proliferation and apoptosis. One of the commonest histopathologically detected precursor lesions of SCC is epithelial dysplasia. Epithelial dysplasia of the oral cavity clinically presents as red or white mucosal patches called as "erythoplakia and leukoplakia." [18,19] It has been observed in various studies that only a small percentage of epithelial dysplasia progress till the end stage of carcinoma, whereas other dysplastic conditions either disappear entirely over a period of time or remain as dysplastic lesions for many years. [19,20] Some studies are also done on the long-term monitoring of leukoplakia and erythroplakia of the oral cavity and how analysis of DNA content can be useful as a marker for better prognosis. Many molecular studies have been done to determine the predictive prognostic markers which are responsible for conversion of oral epithelial dysplasia (OED) to carcinoma. These molecular aberrations consist of alteration in microRNA expression, loss of heterozygosity (LOH) at specific chromosomal loci, promoter methylation of the p16 tumor suppressor gene, and p53 tumor suppressor protein. Molecular markers for these aberrations have not yet been widely used to assess the risk of progression of OED. [19]

Dysplasia and pre-malignant conditions:

The terminology 'dysplasia' was introduced in 1958 to describe exfoliated cells in the uterine cervical lesions. [21,22] Dysplasia is a Greek terminology in which dys means bad and plasis means form. [22] In dysplasia, abnormal and atypical proliferation is noted in the lining epithelium. Earlier, epithelial dysplasia, epithelial atypia, and dyskeratosis were used interchangeably. In 1977, the term epithelial dysplasia was used for the lesions in which part of the thickness of the epithelium is replaced by the cells showing varying degrees of cellular atypia. Later in 1981, the degree of dysplasia was determined as a measure of tissue and cellular deviation from the

normal epithelial structure. [23] Kumar *et al.* [21] in 1992 defined dysplasia as a disturbance in the maturational sequence of the stratified squamous epithelium and also further mentioned disturbance in the kinetics of the proliferative cell compartment with cytological changes. Pre-malignant disorders of the oral cavity, which were previously known as potentially malignant disorders of the oral cavity, are a group of conditions that have been defined as clinical presentations which carry a risk of development of cancer in the oral cavity, either in a clinically definable precursor lesion or in clinically normal mucosa by the WHO in 2017. [24] In potentially cancerous mouth lesions of leukoplakia and erythroplakia with dysplasia, early morphological alterations are noted. Controlled proteolysis is associated with a series of molecular alterations that lead to the transition from healthy looking lining epithelium to a premalignant condition of dysplasia or carcinoma in situ, to invasion, and ultimately metastasis. Additionally, it involves interactions between tumor cells and the extracellular matrix (ECM), which are regulated by numerous cell surface chemicals. [25] A red, granular, pebbly plaque is referred to as an erythroplakia. Out of all the oral pre-malignant lesions, erythroplakia is the least common. At the time of the initial biopsy, more than 90% of these lesions display OED, carcinoma-in-situ (CIS), or SCC. [24, 26-28] Long-term use of areca nut in various preparations causes another pre-malignant condition known as submucous fibrosis. It clinically presents as ‑marble-like pallor and palpable dense bands in the mucosa.‖ Only those lesions that have developed at an early stage gets diagnosed with submucosal fibrosis, whereas lesions developed at a later stage usually get diagnosed with OED. The relative risk for transformation from pre-malignant to malignant lesions is 6.19. [29,30] Leukoplakia is defined as a white plaque of questionable risk having excluded other (known) diseases or conditions with no elevated risk of cancer. It carries a prevalence

of 1–4% of the population. [18] Homogeneous leukoplakia is generally ―demarcated and fissured.‖ In contrast, non-homogenous leukoplakia is sub-typed as ―verrucous/nodular or erythro-leukoplakia‖, and these non-homogenous leukoplakia lesions have a higher tendency of transforming into malignancy. [24] In any case of epithelial dysplasia assessing the grade of dysplasia is very important. It has been done based on architectural and cytological changes. Assessment of these changes is done by subjective assessment method, which may lead to significant inter- and intra-observer disparity in the grading of dysplastic lesions. In 1977 criteria for diagnosis of Epithelial Dysplasia were described based on architectural changes and cytological changes. [23,24]

Architectural changes include irregular epithelial stratification, loss of polarity of basal cells, increased number of mitotic figures, abnormally superficial mitoses, drop-shaped rete ridges, keratin pearls present within the rete ridges, loss of epithelial cell cohesiveness and premature keratinization in single cells. Cytologic changes include alteration and variation in nuclear size, abnormal variation in nuclear shape, abnormal variation in cell size and cell shape, atypical mitotic figures, increased number and size of nucleoli, increased nuclear: cytoplasmic ratio, and hyperchromasia. Grading of Oral epithelial dysplasia mentioned in 2005 is regarded as the ―gold standard‖ which is used routinely to report epithelial dysplasia. Additionally, it aids in the prediction of malignant transformation of any oral conditions that could develop into cancer. [24] OED is divided into three grades such as mild, moderate, and severe. ―**Mild epithelial dysplasia"** is represented by architectural changes limited only to the lower third of the epithelium, along with cytological atypia. ―**Moderate epithelial dysplasia"** is represented by architectural changes limited to the middle third of the epithelium, accompanied by a higher degree

of cytological atypia.‖ **Severe epithelial dysplasia"** is represented by disturbances in the architecture of the epithelium and cytological atypia crossing more than 2/3rds of the epithelium. [24,17]

Ki67:

The Ki-67 antigen is a large basic nuclear protein. The molecular weights of Ki-67 are 345 kD and 395 kD. The gene of Ki67 is located on chromosome 10q25-ter. When various attempts were made to raise the monoclonal type of antibodies to antigens specific for –Hodgkin and Reed-Steinberg cells‖, that was the time when the Ki-67 antibody got derived first. Ki-67 was found to be unique from other antibodies as it only reacted with the proliferating cells, such as cells present in the crypts of small intestine and cortical thymocytes. No reaction was noted in terminally differentiated cells or resting cells such as neurons and liver cells. [31] Ki-67 is a nuclear protein which is expressed in the –G- and M-phases‖ of actively dividing cells. It is a proliferation marker that strongly correlates with the presence and severity of epithelial dysplasia. Furthermore, it offers essential knowledge regarding the prognosis of OSCC and the extent of aggressiveness. [25,36]

Increased cell proliferation will transform the normal oral epithelium from dysplasia to malignancy at a significantly higher rate. With the help of various proliferative markers, it has helped in detecting the hyperactive nature of the lining epithelium. The only proliferative layer considered for the squamous epithelium of the oral cavity is the the basal layer. Therefore, any indication of the proliferative activity in the squamous lining epithelium above the basal layer should be taken as an alarming sign and should be addressed as soon as possible. [32] Uncontrolled proliferation of cells is known to be associated with one of the essential mechanisms of oncogenesis. When any proliferative activity is noted in the tumors, Ki67 is one of

the major indicators of mitoses. Ki-67 expression is considered a measure of tumor cell proliferation. It is one of the indicators for the invasive activity of cancers related to the degree of malignant neoplastic cells and tumor invasion potential. [25,39,41] Expression of Ki-67 protein increases with decreasing tissue differentiation of OED and OSCC. [1,25,33,37]

In cases of OSCC, various authors also studied the Ki-67 labelling index (LI) as a marker of cell proliferation. These authors mentioned that Ki67 LI increases with decreasing tissue differentiation of OED and OSCC. This marker can predict pre-malignant or malignant conditions of the oral cavity. [33,34,38]

MATERIAL AND METHODS

Source of data

The study was done on biopsy specimens of clinically suspected cases of epithelial dysplasia and malignancy of the oral cavity received in the Histopathology section of the Department of Pathology, BLDE (Deemed to Be University) Shri B M Patil Medical College Hospital & Research Centre, Vijayapura.

Study period: 1st December 2020 – 31st July 2022.

Study Design: Descriptive cross-sectional study.

Methods of collection of data.

All the specimens of clinically suspected cases of epithelial dysplasia and malignancy of the oral cavity received in the Histopathology section were processed as per the standard format of tissue processing, and paraffin blocks were prepared. For each case, two sections of 4 μm were prepared. One section was stained by routine Haematoxylin and eosin (H&E) stain, and another section was used for IHC staining of Ki-67. Cases diagnosed with epithelial dysplasia and malignancy on histopathology were included in the study. Immunohistochemical (IHC) Ki-67 was performed on all the slides of selected cases. IHC staining was done as mentioned below:

1. Sections were cut at 4 μm thickness & placed on pre-coated slides.
2. Sections with pre-coated slides were incubated at 60°C for 1 hour in an incubator.
3. Deparaffinization was done by keeping the slides in an incubator at 60°C for 30 minutes.
4. Sections were kept in citrate buffer in the microwave for antigen retrieval.
5. 3% hydrogen peroxide was added to the slide and kept for 10 min.

6. The sections were washed in 0.05mM solution of Tris-buffered saline (TBS) at the pH of 7.4.
7. For primary antibodies, antibodies that were used were diluted monoclonal antibodies especially against Ki-67. Incubation of these sections were incubated with primary antibodies at 37°C for 1 hour.
8. Washing was done in 0.05mM solution of Tris-buffered saline.
9. Conjugation of the secondary antibody was done with peroxidase-labelled dextran polymers. Incubation of the sections were done with this antibody at room temperature for 30 minutes.
10. Rinsing was done with TBS.
11. Sections were treated then with 0.5 mg/ml of 3, 3'-diaminobenzidine solution that contained hydrogen peroxide of 0.001%.
12. Counterstaining was done with Mayer's haematoxylin for 3 min
13. Sections were dehydrated in ethanol, cleared in xylene, and then the mounting of slides was done.

Appropriate positive and negative control were used.

Ki-67 stained slides were scanned under 400x magnification for visual counting using a microscope. In cases of OED, the expression of Ki-67 in each nucleus was studied according to the Ki67 positivity seen in the epithelial layers. When Ki67 expression was noted just above the basement membrane, it was mentioned as Ki67 positivity in the basal layer. When Ki67 expression was noted within the two layers above the basement membrane, it was mentioned as Ki67 positivity in the basal and parabasal layer. When Ki67 expression was noted in one more upper layer above the parabasal layer, it was mentioned as Ki67 positivity in basal, parabasal, and spinous layer positivity. [32]

In cases of OSCC, the Ki-67 labelling index (LI) was done as per the study done by Chandrakanta et al. [34] When intranuclear staining for Ki-67 was observed in each tumor cell, Ki-67 antigen expression was considered positive for that specific slide. Regardless of the staining intensity, all the Ki-67stained nuclei were counted as positive. All sections with invasive tumor areas and areas having higher density of Ki-67 labelled tumor positive cells were located by screening the IHC sections at 100x magnification. Each section was counted thoroughly to ensure that there were at least 1000 Ki67-labelled tumor positive cells. The number of nuclei which were positively stained by Ki-67 was calculated as ‒the percentage of the total number of detected neoplastic epithelial cells.‖ Ki-67 LI was calculated as the "number of Ki-67-positive cells multiplied by 100, divided by the total number of neoplastic epithelial cells observed." [34] The analysis of Ki-67 expression was done as per the study done by Gonzales-Moles *et al.* [45] as follows:

Score 1 (+) [1-25% of tumour cells stained by Ki-67],

Score 2 (++) [26% to 50% of tumour cells stained by Ki-67],

Score 3 (+++) [51-75% of tumour cells stained by Ki-67] and

Score 4 (++++) [76% to 100% of tumour cells stained by Ki-67]

Sample Size:

- As per the study done by Takkem A *et al.* [1], the anticipated Mean±SD of Ki -67 among OED was 36.83±19.56. Based on this finding, the formula used for the calculation of sample size was,
- $\mathbf{n = \frac{z^2 S^2}{d^2}}$

Where Z= Z statistic at α level of significance

d^2= Absolute error

S= Common standard deviation

- The required minimum sample size was 62, with a 95% level of confidence and precision 5.
- The total sample size in the present study was 64.

Statistical Method for Future Data Analysis:

The data obtained were entered into a Microsoft Excel sheet, and statistical analysis was done using a statistical package for the social sciences (Verson 20).

- Results were presented as Mean±SD and percentages.
- The statistically significant difference between OED and OSCC was found using the unpaired/ Mann Whitney U test and paired/ Wilcoxon Signed Rank test.
- A p-value of less than 0.05 was considered statistically significant. All statistical tests were performed two-tailed.

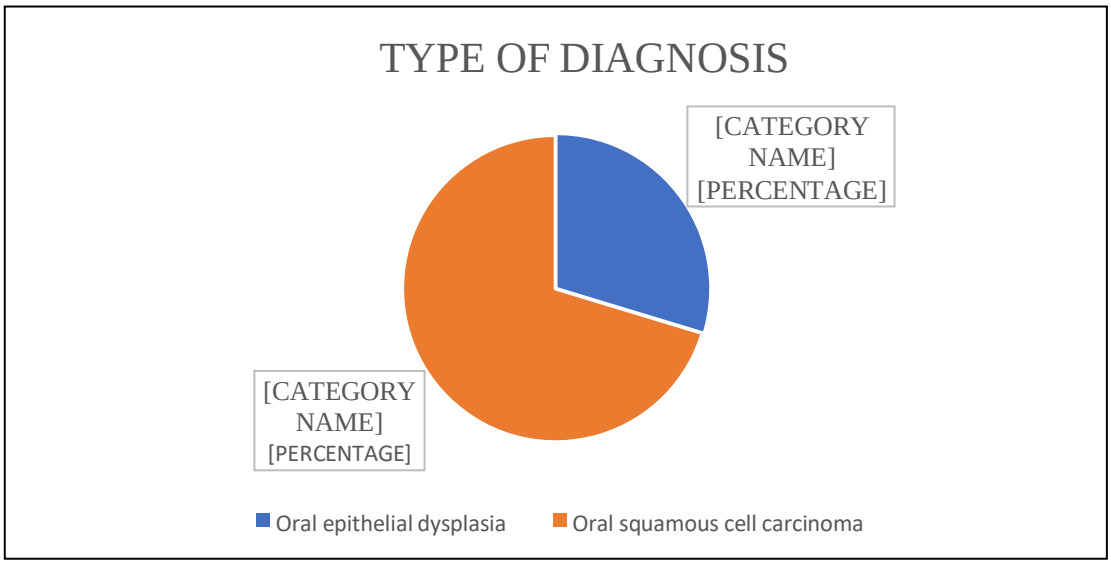
RESULTS

In the present study, total 64 cases of clinically suspected epithelial dysplasia and squamous cell carcinoma of the oral cavity were studied for Ki67 expression and its correlation with histopathological grading. Out of 64 cases, 60 cases were biopsies, and in 4 cases, resection specimens were received.

TABLE 1: DISTRIBUTION OF CASES ACCORDING TO THE HISTOPATHOLOGICAL DIAGNOSIS (n=64)

TYPE OF DIAGNOSIS	NUMBER	PERCENTAGE
Oral epithelial dysplasia	19	30
Oral squamous cell carcinoma	45	70
Total	**64**	**100**

FIGURE 1: PIE CHART REPRESENTING THE TYPE OF DIAGNOSIS

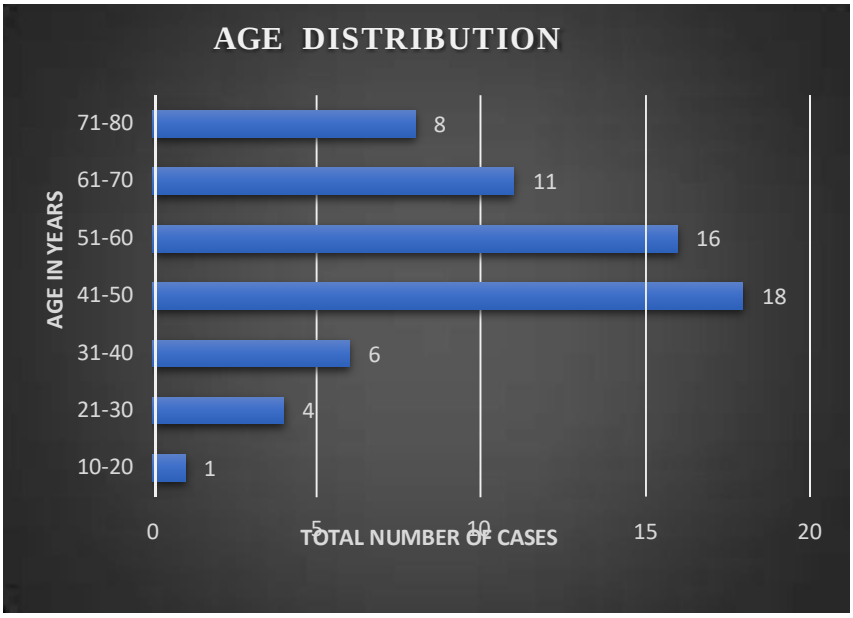


Out of 64 cases of dysplastic and malignant lesions of the oral cavity, 45 cases were histopathologically diagnosed as OSCC, and 19 cases were diagnosed as OED.

TABLE 2: DISTRIBUTION OF CASES ACCORDING TO AGE AND SEX(n=64)

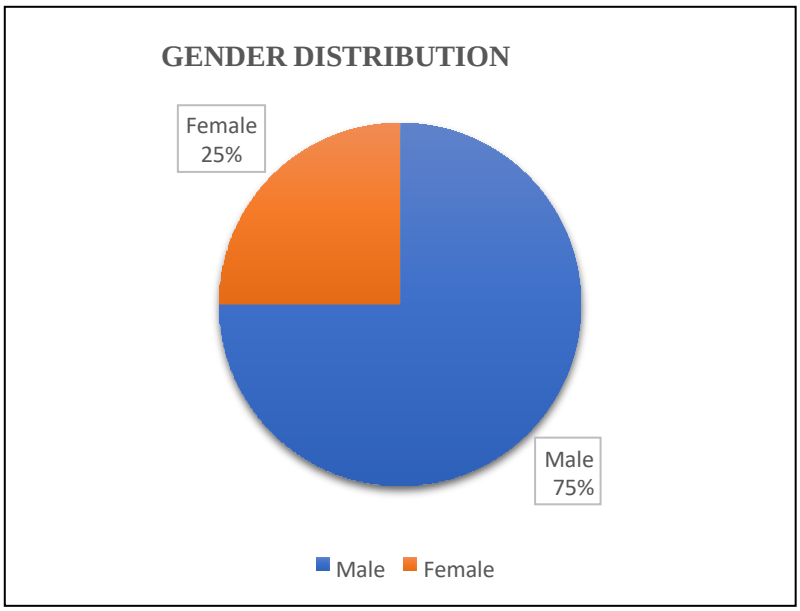
AGE IN YEARS	MALE		FEMALE		TOTAL	
	NUMBER	PERCENTAGE	NUMBER	PERCENTAGE	NUMBER	PERCENTAGE
10-20	1	2.0%	0	0%	1	1.5%
21-30	3	6.2%	1	6.2%	4	6.2%
31-40	6	12.5%	0	0%	6	9.3%
41-50	13	27.0%	5	31.2%	18	28%
51-60	14	29.1%	2	12.5%	16	25%
61-70	6	12.5%	5	31.2%	11	17%
71-80	5	10.4%	3	18.7%	8	13%
Total	**48**	**75%**	**16**	**25%**	**64**	**100%**

FIGURE 2: BAR DIAGRAM SHOWING THE DISTRIBUTION OF CASES WITH RESPECT TO AGE.



The maximum number of the cases were in the age group 41-50 years, amounting to 28%, followed by 51-60 years, amounting to 25%

FIGURE 3: PIE CHART SHOWING THE DISTRIBUTION OF CASES WITH RESPECT TO GENDER.

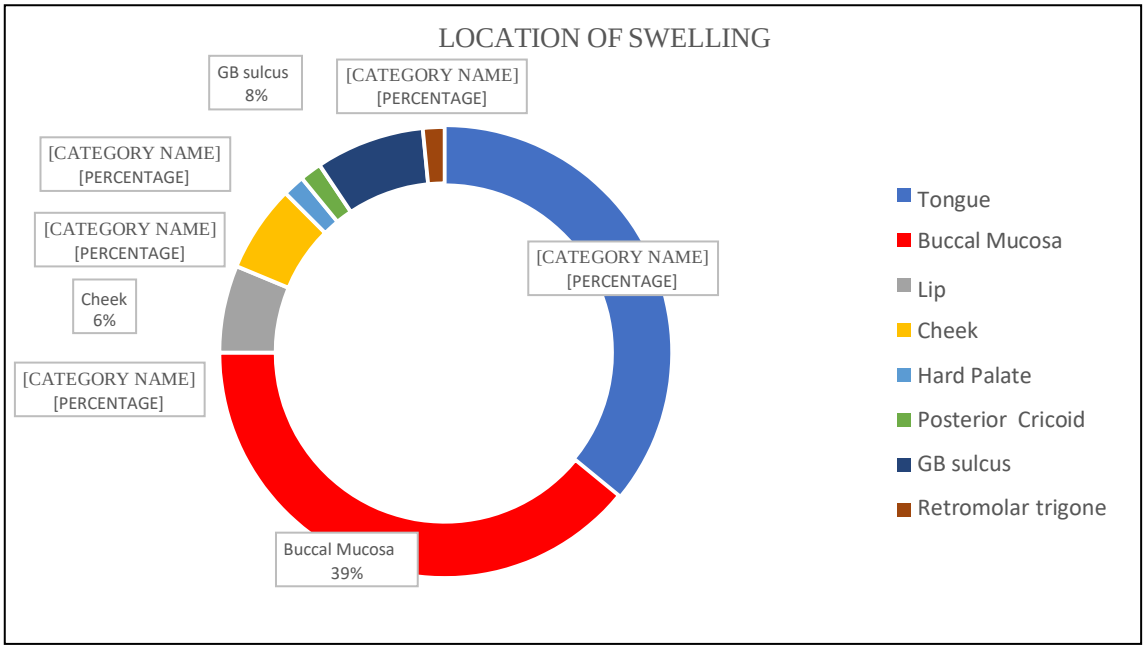


Male preponderance was noted in the present study amounting to 75% of cases

TABLE 3: SITE WISE DISTRIBUTION OF CASES (n=64)

SITE OF THE LESION	NUMBER OF CASES	PERCENTAGE OF CASES
Buccal mucosa	25	39%
Tongue	23	36%
GB sulcus	5	7%
Lip	4	6%
Cheek	4	6%
Hard palate	1	2%
Posterior cricoid	1	2%
Total	**64**	**100%**

FIGURE 4: PIE CHART REPRESENTING THE SITE-WISE DISTRIBUTION OF CASES

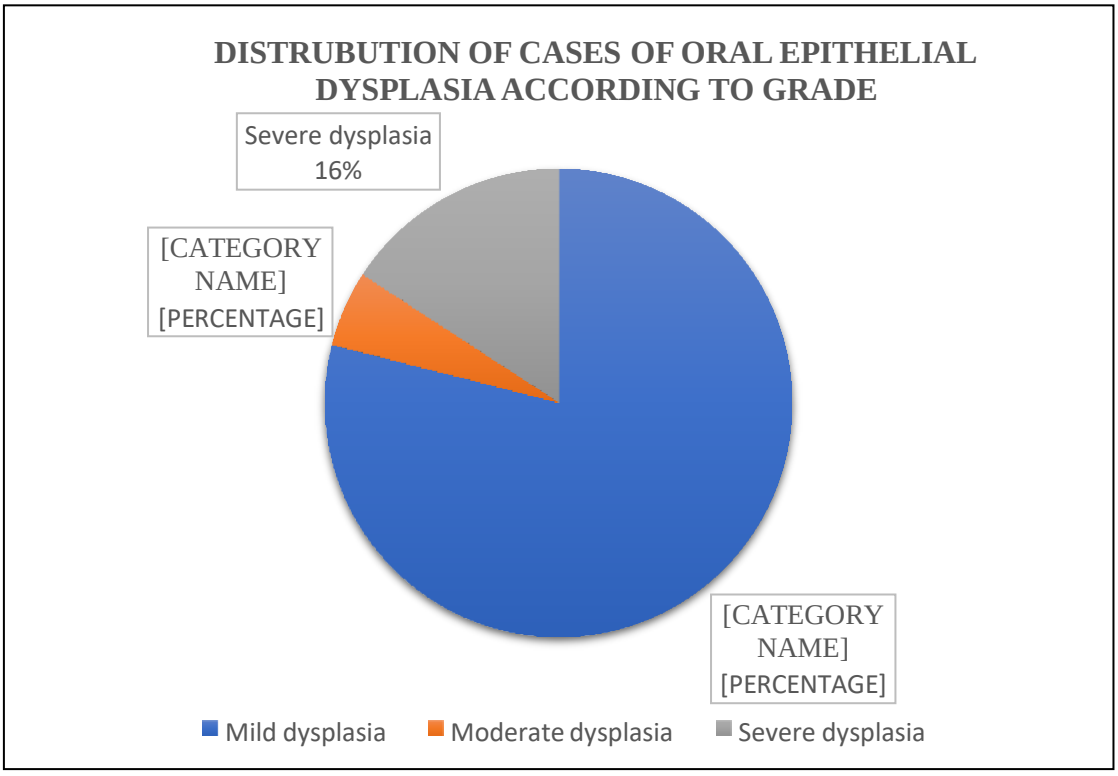


Buccal mucosa was the most common site of involvement, amounting to 39%, followed by the tongue, amounting to 36%

TABLE 4: DISTRIBUTION OF CASES OF ORAL EPITHELIAL DYSPLASIA AS PER THE GRADE (n=19)

DYSPLASIA	NUMBER	PERCENTAGE
Mild Dysplasia	15	79
Moderate Dysplasia	1	5
Severe Dysplasia	3	16
Total	**19**	**100**

FIGURE 5: PIE CHART REPRESENTING DISTRIBUTION OF CASES OF ORAL EPITHELIAL DYSPLASIA ACCORDING TO GRADE

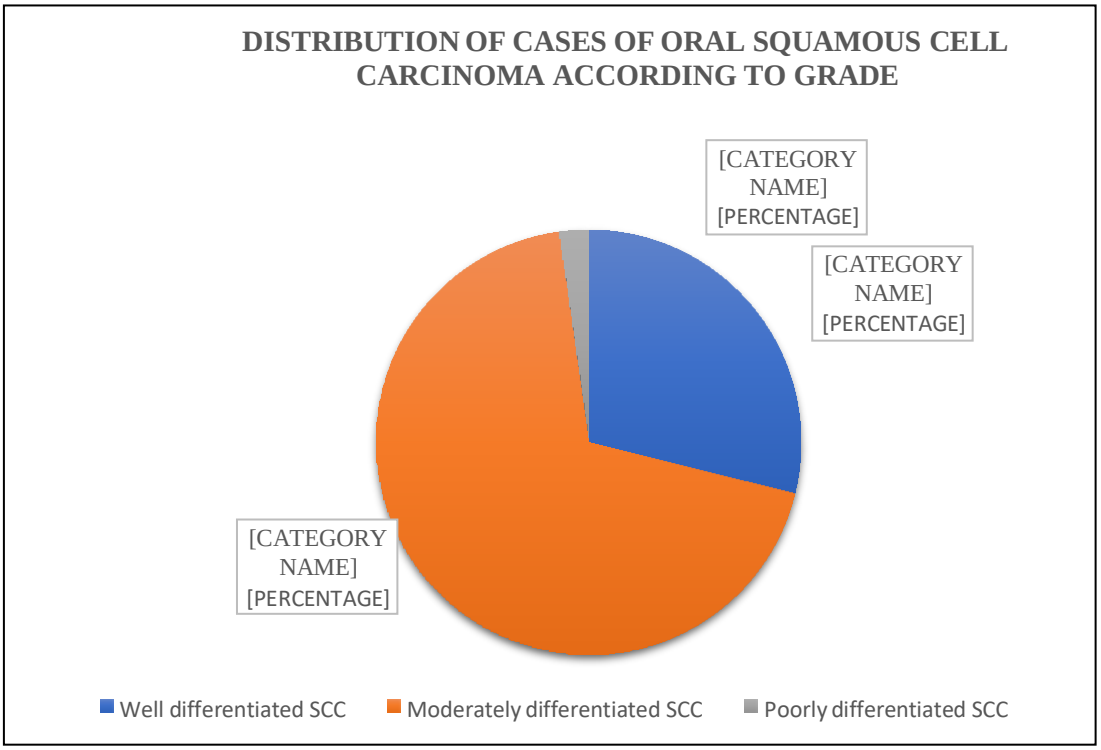


In OED, maximum number of cases were diagnosed as Mild dysplasia, amounting to 79%.

TABLE 5: DISTRIBUTION OF CASES OF ORAL SQUAMOUS CELL CARCINOMA (OSCC) AS PER THE GRADE (n=45)

SQUAMOUS CELL CARCINOMA	NUMBER	PERCENTAGE
Well differentiated OSCC (WD OSCC)	13	29
Moderately differentiated OSCC (MD OSCC)	31	69
Poorly differentiated OSCC (PD OSCC)	1	2
Total number of cases	**45**	**100**

FIGURE 6: PIE CHART REPRESENTING DISTRIBUTION OF CASES OF ORAL SQUAMOUS CELL CARCINOMA ACCORDING TO GRADE (n=45)



In OSCC, maximum numbers of cases were diagnosed as Moderately differentiated OSCC amounting to 69%

TABLE 6: DISTRIBUTION OF CASES OF ORAL EPITHELIAL DYSPLASIA AS PER THE AGE (n=19)

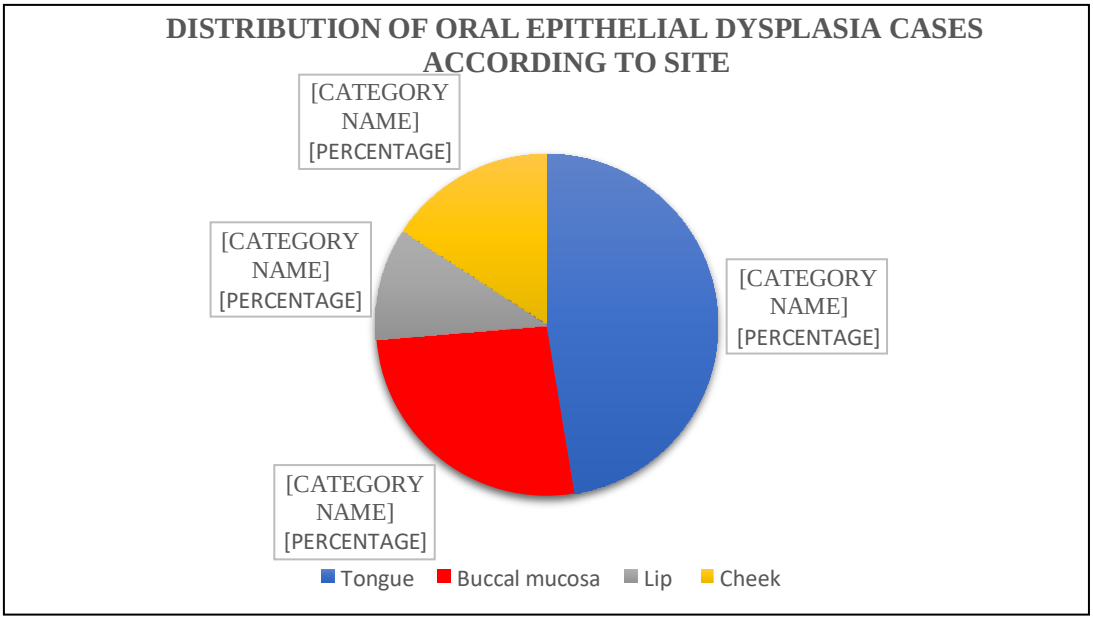
AGE IN YEARS	MILD DYSPLASIA		MODERATE DYSPLASIA		SEVERE DYSPLASIA		TOTAL	
	N	%	N	%	N	%	N	%
10-20	1	6.7%	0	0%	0	0%	1	5.2%
21-30	2	13.2%	0	0%	0	0%	2	10.5%
31-40	1	6.7%	1	100%	0	0%	2	10.5%
41-50	6	40%	0	0%	1	33.3%	7	37.1%
51-60	3	20%	0	0%	1	33.3%	4	21%
61-70	1	6.7%	0	0%	0	0%	1	5.2%
71-80	1	6.7%	0	0%	1	33.3%	2	10.5%
TOTAL	**15**	**79%**	**1**	**5.3%**	**3**	**15.7%**	**19**	**100%**

Maximum number of cases of OED was observed in the age group of 41-50 years, followed by 51-60 years. Maximum number of cases were diagnosed as Mild dysplasia, amounting to 79%. Severe dysplasia was noted in the elderly age group that is between 41 to 80 years. Mild dysplasia was noted in the younger age group that is between 10 to 39 years of age. In these cases, mild dysplasia was secondary to inflammation. Only one case of moderate dysplasia was noted in the present study in the age group of 31 to 40.

TABLE 7: DISTRIBUTION OF CASES OF ORAL EPITHELIAL DYSPLASIA AS PER THE SITE (n=19)

SITE	NUMBER	PERCENTAGE
Tongue	9	47
Buccal mucosa	5	26
Lip	2	11
Cheek	3	16
Total	**19**	**100**

FIGURE 7: PIE CHART REPRESENTING DISTRIBUTION OF CASES OF ORAL EPITHELIAL DYSPLASIA ACCORDING TO THE SITE



In OED, maximum number of cases were observed in the tongue, amounting to 47%

TABLE 8: DISTRIBUTION OF CASES OF ORAL SQUAMOUS CELL CARCINOMA AS PER THE AGE (n=45)

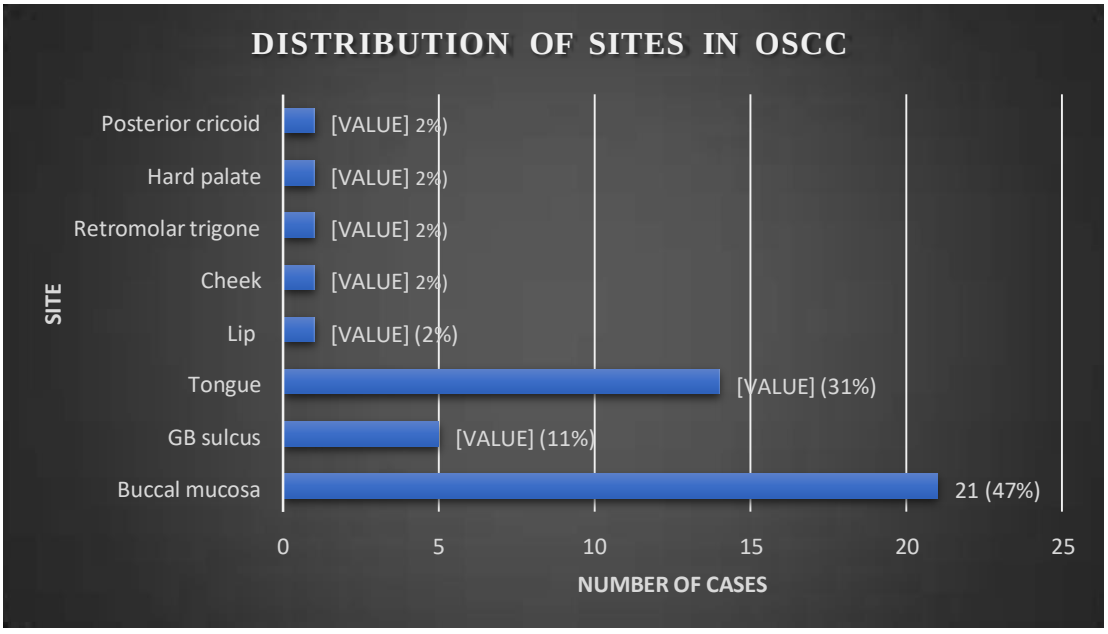
AGE IN YEARS	WD OSCC		MD OSCC		PD OSCC		TOTAL	
	N	%	N	%	N	%	N	%
21-30	0	0%	2	6.4%	0	0%	2	4.4%
31-40	2	15.4%	2	6.4%	0	0%	4	8.9%
41-50	5	38.5%	6	19.3%	0	0%	11	24.4%
51-60	3	23%%	10	32.2%	0	0%	13	28.9%
61-70	1	7.7%	7	22.7%	1	100%	9	20%
71-80	2	15.4%	4	13%	0	0%	6	13.4%
TOTAL	**13**	**28.9%**	**31**	**68.9%**	**1**	**2.2%**	**45**	**100%**

In OSCC, the youngest age of the patient was 28 years, and the eldest was 75 years. Maximum number of cases were diagnosed as MD OSCC and were observed in the age group of 51-60 years, amounting to 28.9%

TABLE 9: DISTRIBUTION OF CASES OF ORAL SQUAMOUS CELL CARCINOMA AS PER THE SITE (n=45)

SITE	NUMBER	PERCENTAGE
Buccal mucosa	21	47%
Tongue	14	31%
GB sulcus	5	11%
Cheek	1	2.2%
Lip	1	2.2%
Retromolar trigone	1	2.2%
Hard palate	1	2.2%
Posterior cricoid	1	2.2%
Total	**19**	**100**

FIGURE 8: BAR DIAGRAM REPRESENTING DISTRIBUTION OF CASES OF ORAL SQUAMOUS CELL CARCINOMA ACCORDING TO THE SITE

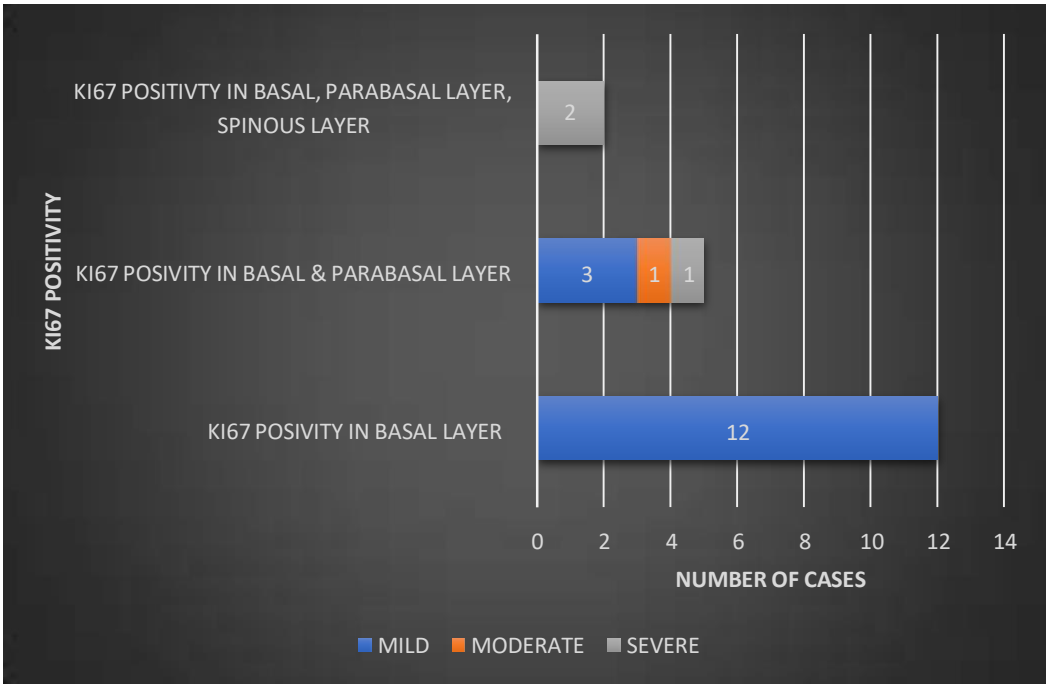


The commonest site of OSCC was buccal mucosa amounting to 47%.

TABLE 10: CORRELATION OF HISTOLOGICAL GRADING OF DYSPLASIA AND KI67 EXPRESSION IN OED (n=19)

HISTOLOGICAL GRADING	KI67 EXPRESSION								CHI SQUARE VALUE	P VALUE
	Basal layer		Basal and parabasal layer		Basal, parabasal, and spinous layer		Total			
	N	%	N	%	N	%	N	%		
Mild dysplasia (n=15)	12	80%	3	20%	0	0	15	100%	16.213	**0.003***
Moderate dysplasia (n=1)	0	0	1	100%	0	0	1	100%		
Severe dysplasia (n= 3)	0	0	1	33.3%	2	66.7%	3	100%		
Total	**12**	**63.1%**	**5**	**26.4%**	**2**	**10.5%**	**19**	**100%**		

FIGURE 9: BAR DIAGRAM REPRESENTING THE CORRELATION OF HISTOLOGICAL GRADING AND KI67 SCORE IN OED

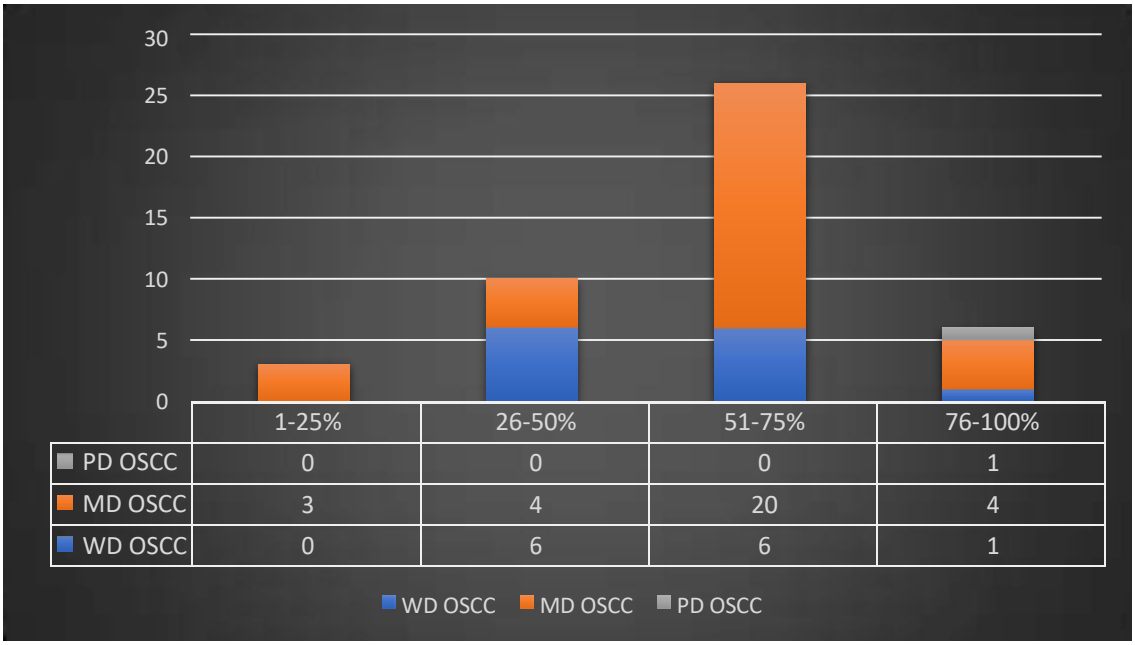


Out of 15 cases of mild OED, 12 cases showed Ki67 positivity in the basal layer amounting to 80%. All cases of moderate dysplasia and 1 case of severe dysplasia showed Ki67 positivity in the basal and parabasal layer, and 2 cases of severe dysplasia showed Ki67 positivity in the basal, parabasal, and spinous layer amounting to 66.7% Statistical analysis of the correlation of histopathological grading and Ki67 expression in cases of oral epithelial dysplasia showed a p-value of 0.003, suggesting a statistically significant correlation between histopathological grading and Ki67 expression.

TABLE 11: CORRELATION OF HISTOLOGICAL GRADING OF SCC AND KI67 SCORE IN OSCC (n=45):

HISTOLOGICAL GRADING	KI67 EXPRESSION										CHI SQUARE VALUE	P VALUE
	-Score 1 (1-25%)‖		-Score 2 (26-50%)‖		-Score 3 (51-75%)‖		-Score 4 (76-100%)‖		-Total‖			
	N	%	N	%	N	%	N	%	N	%		
Well differentiated OSCC (n=13)	0	0	6	46%	6	46%	1	8%	13	100%	68.382	**0.001***
Moderately differentiated OSCC (n=31)	3	9%	4	13%	20	6%	4	13%	31	100%		
Poorly differentiated OSCC (n= 1)	0	0	0	0	0	0	1	100%	1	100%		
Total	**3**	**6.7%**	**10**	**22.2%**	**26**	**58%**	**6**	**13.1%**	**45**	**100%**		

FIGURE 10: BAR DIAGRAM REPRESENTING THE CORRELATION OF HISTOLOGICAL GRADING AND KI67 SCORE IN OSCC



In WD OSCC, Score 4 was noted in 8% of cases. In MD OSCC, Score 4 was noted in 13% of cases. In PD OSCC, Score 4 was noted in 100% of cases. The highest Ki67 expression was noted in PD OSCC and the lowest in WD OSCC. Statistical analysis of the correlation of histopathological grading and Ki67 expression in cases of OSCC showed a p-value of 0.001, suggesting a statistically significant correlation between histopathological grading and Ki67 expression.

TABLE 12: CORRELATION BETWEEN KI67 EXPRESSION AND GENDER IN OED (n=19)

GENDER	KI67 EXPRESSION								CHI SQUARE VALUE	P VALUE
	Basal layer		Basal and parabasal layer		Basal, parabasal, and spinous layer		Total		1.104	0.576
	N	%	N	%	N	%	N	%		
Female (n=6)	4	66.7%	2	33.3%	0	0%	6	100%		
Male (n=13)	8	61.5%	3	23.1%	2	15.4%	13	100%		
Total (n= 19)	**12**	**63.2%**	**5**	**26.3%**	**2**	**10.5%**	**19**	**100%**		

In OED, Ki67 expression in the basal layer was higher in females as compared to males. However, the difference is not statistically significant.

TABLE 13: CORRELATION BETWEEN KI67 EXPRESSION AND GENDER IN OSCC (n=45)

GENDER	KI67 EXPRESSION										CHI SQUARE VALUE	P VALUE
	-Score 1 (1-25%)‖		-Score 2 (26-50%)‖		-Score 3 (51-75%)‖		-Score 4 (76-100%)‖		-Total‖		2.593	0.45
	N	%	N	%	N	%	N	%	N	%		
Female (n=10)	0	0	1	10%	8	80%	1	10%	10	100%		
Male (n=35)	3	8.6%	9	25.7%	18	51.4%	5	14.3%	35	100%		
Total	**3**	**6.7%**	**10**	**22.2%**	**26**	**58%**	**6**	**13.1%**	**45**	**100%**		

In OSCC, the Ki67 expression score was slightly higher in males than in females. However, the difference is not statistically significant.

TABLE 14: CORRELATION BETWEEN KI67 EXPRESSION AND AGE IN OED (n=19)

AGE IN YEARS	KI67 EXPRESSION								CHI SQUARE VALUE	P VALUE
	Basal layer		Basal and parabasal layer		Basal, parabasal, and spinous layer		Total			
	N	%	N	%	N	%	N	%		
10-20	1	100%	0	0	0	0	1	100%	6.261	0.395
21-30	2	100%	0	0	0	0	2	100%		
31-40	1	50%	1	50%	0	0	2	100%		
41-50	4	50%	3	37.5%	1	12.5%	8	100%		
51-60	3	100%	0	0	0	0	3	100%		
61-70	1	100%	0	0	0	0	1	100%		
71-80	0	0	2	100%	0	0	2	100%		
Total	**12**	**63.1%**	**6**	**31.6%**	**1**	**5.3%**	**19**	**100%**		

When the analysis was done for the correlation between age and Ki67 expression in OED, maximum number of cases were found in the age group 41-50 years, and Ki67 positivity was seen in the basal layer amounting to 63.1%. However, the difference is not statistically significant.

TABLE 15: CORRELATION BETWEEN KI67 EXPRESSION AND AGE IN OSCC (n=45)

AGE IN YEARS	KI67 EXPRESSION										CHI SQUARE VALUE	P VALUE
	Score 1 (1-25%)		Score 2 (26-50%)		Score 3 (51-75%)		Score 4 (76-100%)		Total			
	N	%	N	%	N	%	N	%	N	%		
21-30	0	0%	0	0	2	100%	0	0	2	100%		
31-40	0	0%	1	25%	2	50%	1	25%	4	100%	18.63	0.23
41-50	0	0%	2	18.1%	9	81.9%	0	0%	11	100%		
51-60	2	15.4%	3	23%	7	53.9%	1	7.7%	13	100%		
61-70	1	11.1%	3	33.3%	3	33.3%	2	22.3%	9	100%		
71-80	0	0	1	16.7%	4	66.6%	1	16.7%	6	100%		
Total	**3**	**6.7%**	**10**	**22.2%**	**26**	**58%**	**6**	**13.1%**	**45**	**100%**		

When the analysis was done for correlation between age and Ki67 expression in OSCC, maximum number of cases were found in the age group 51-60 years, and maximum number of cases showed Score 3 (51-75%) Ki67 positivity. However, the difference is not statistically significant.

TABLE 16: KI67 LABELLING INDEX (LI) IN VARIOUS GRADES OF OSCC (n=45)

SQUAMOUS CELL CARCINOMA	MEAN KI67 LI	STANDARD DEVIATION	KRUSKAL WALLIS VALUE	P VALUE
Well differentiated OSCC	54%	±10.01	6.7	**0.034***
Moderately differentiated OSCC	59%	±15.0		
Poorly differentiated OSCC	80%	±0		

Ki-67 LI was highest in PD OSCC. Statistical analysis of Ki-67 LI in various grades of OSCC showed a p-value of 0.03, suggesting a statistically significant correlation between Ki-67 LI and various grades of OSCC.

MICROPHOTOGRAPHS

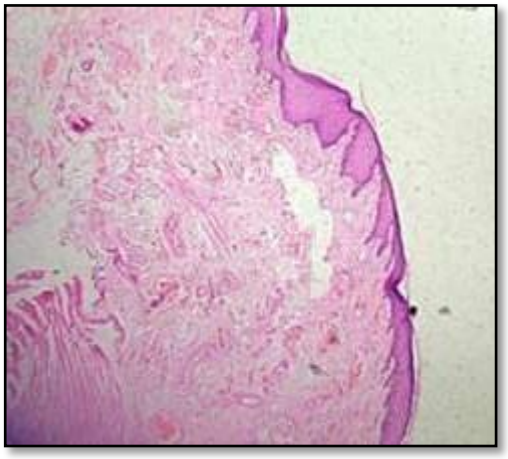


Figure 11: Photomicrograph showing mild dysplasia (H&E 100x)

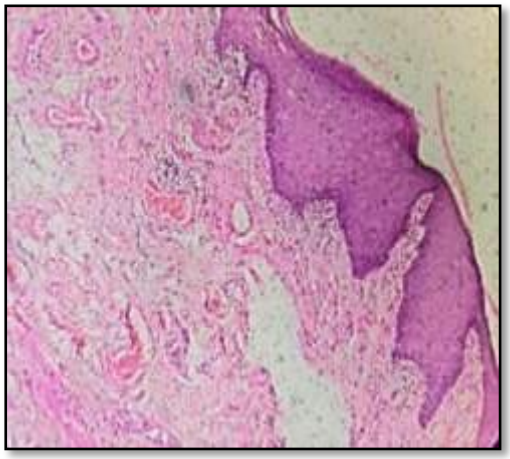


Figure 12: Photomicrograph showing mild dysplasia (H&E 400x)

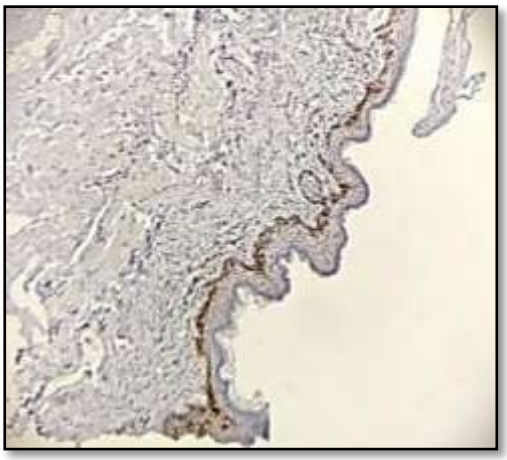


Figure 13: Photomicrograph showing Ki-67 expression in basal layer (IHC 100x)

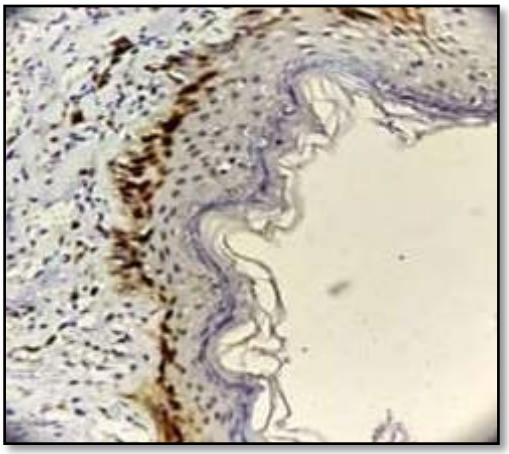


Figure 14: Photomicrograph showing Ki-67 expression in basal layer (IHC 400x)

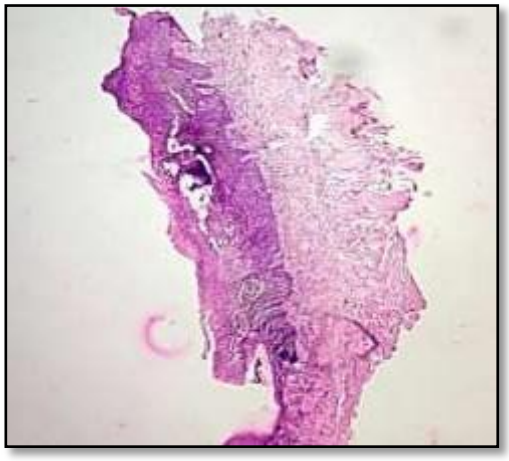


Figure 15: Photomicrograph showing moderate dysplasia (H&E 100x)

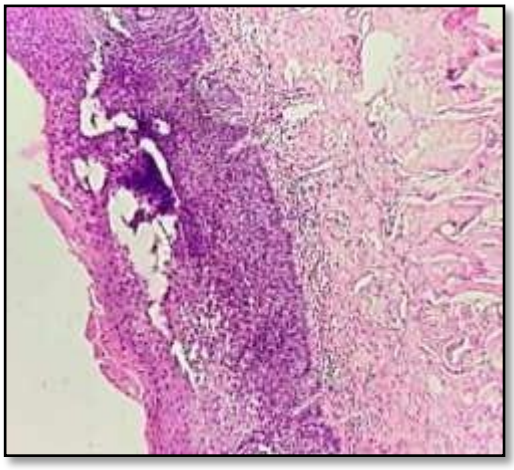


Figure 16: Photomicrograph showing moderate dysplasia (H&E 400x)

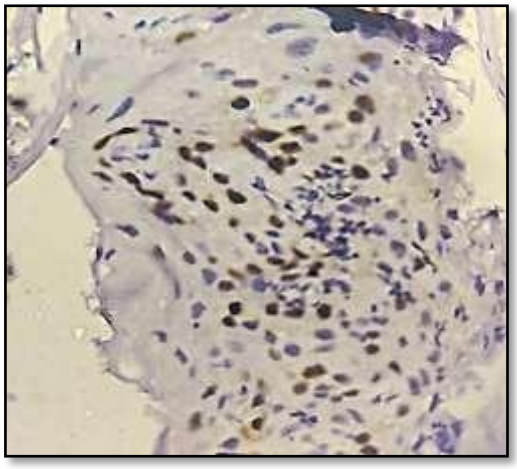


Figure 17: Photomicrograph showing Ki-67 expression in basal and parabasal layer (IHC 100x)

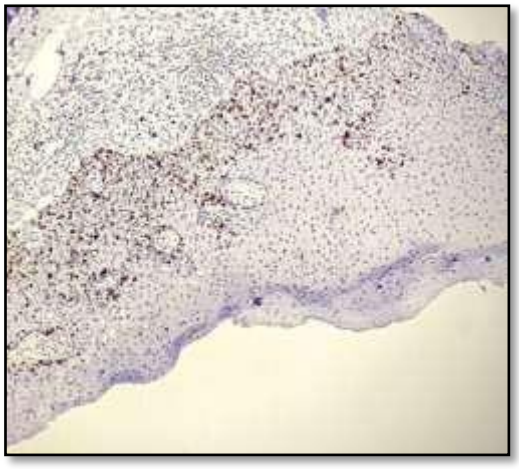


Figure 18: Photomicrograph showing Ki-67 expression in basal and parabasal Layer (IHC 400x)

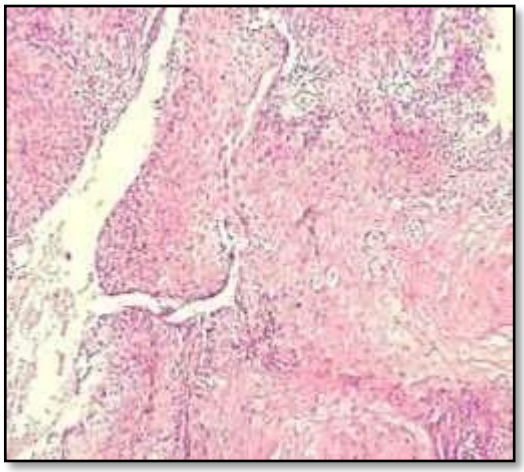


Figure 19: Photomicrograph showing severe dysplasia (H&E 100x)

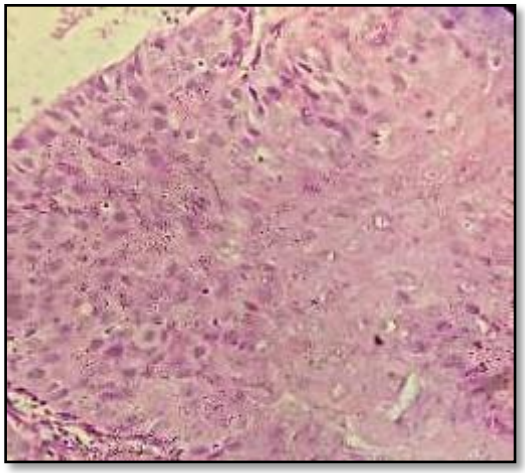


Figure 20: Photomicrograph showing severe dysplasia (H&E 400x)

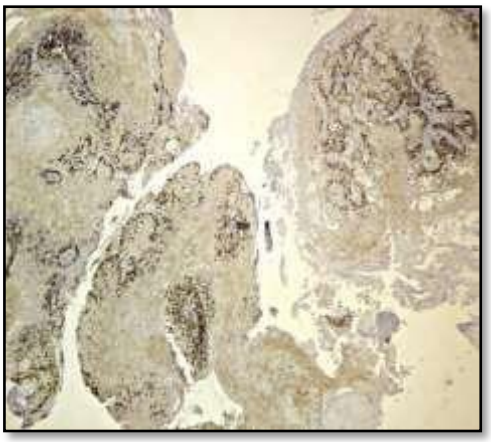


Figure 21: Photomicrograph showing Ki-67 expression in basal, parabasal and suprabasal layer (IHC 100x)

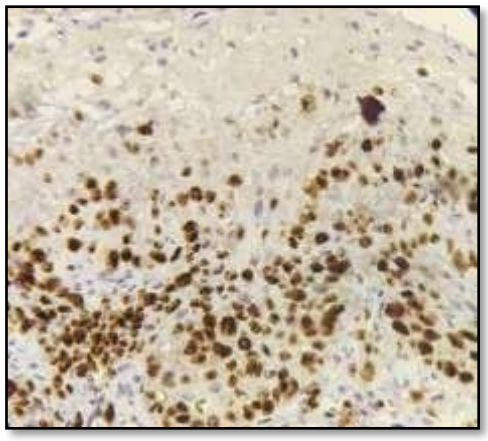


Figure 22: Photomicrograph showing Ki-67 expression in basal, parabasal and suprabasal layer (IHC 400x)

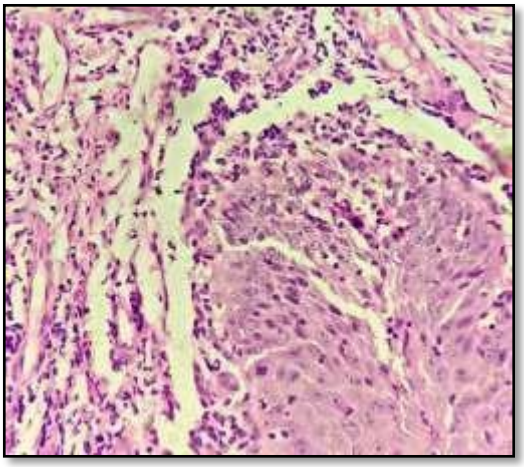


Figure 23: Photomicrograph showing severe dysplasia on histopathologyslide (H&E 100x)

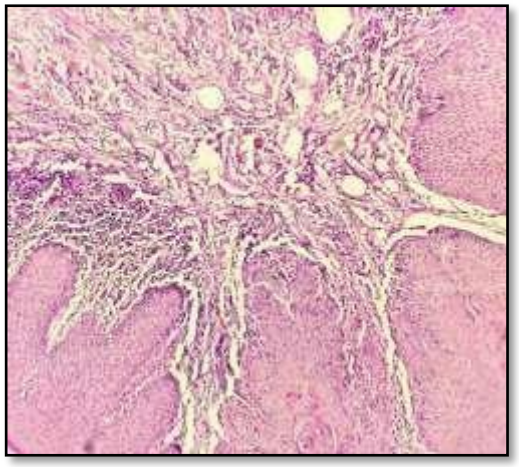


Figure 24: Photomicrograph showing severe dysplasia on histopathology slide (H&E 400x)

Figure 25: Photomicrograph showing severe dysplasia with a focus of Ki-67 positive tumor cells in the sub epithelium in the above case, hence diagnosed as Severe dysplasia with minimal invasion after IHC
(IHC 100x)

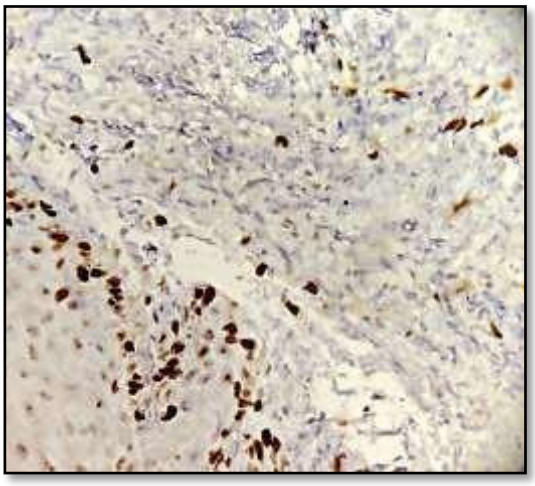


Figure 26: Photomicrograph showing severe dysplasia with a focus of Ki-67 positive tumor cells in the sub epithelium in the above case, hence diagnosed as Severe dysplasia with minimal invasion after IHC
(IHC 100x)

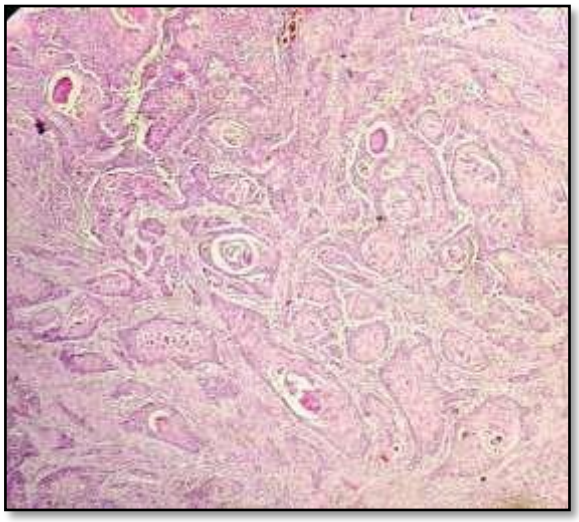


Figure 27: Photomicrograph showing WD OSCC
(H&E 100x)

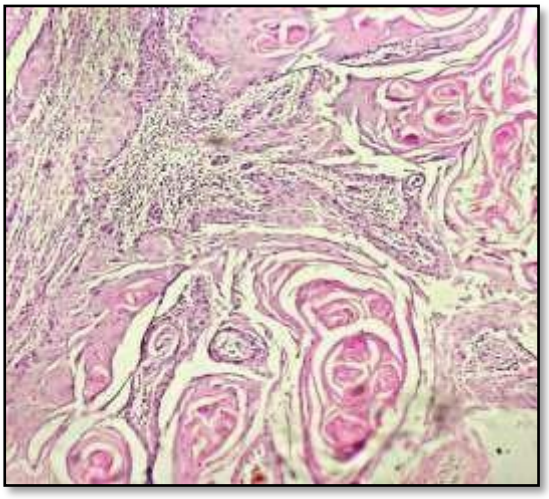


Figure 28: Photomicrograph showing keratin pearls in WD OSCC
(H&E 200x)

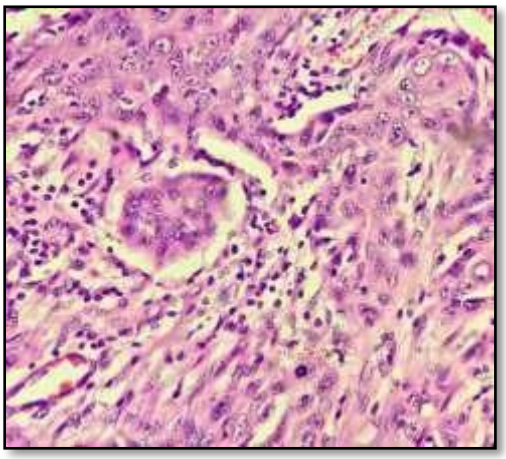


Figure 29: Photomicrograph showing tumor giant cells in WD OSCC
(H&E 400x)

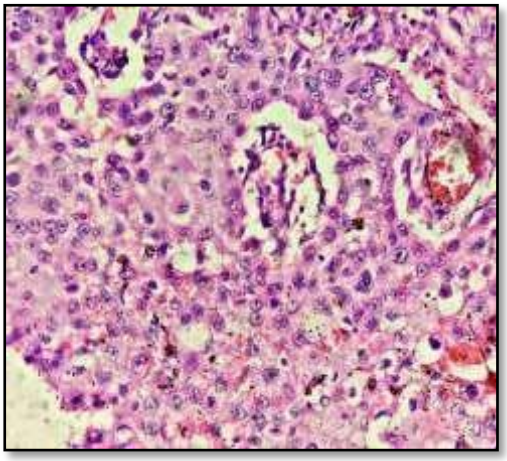


Figure 30: Photomicrograph showing individual cell morphology in WD OSCC (H&E 400x)

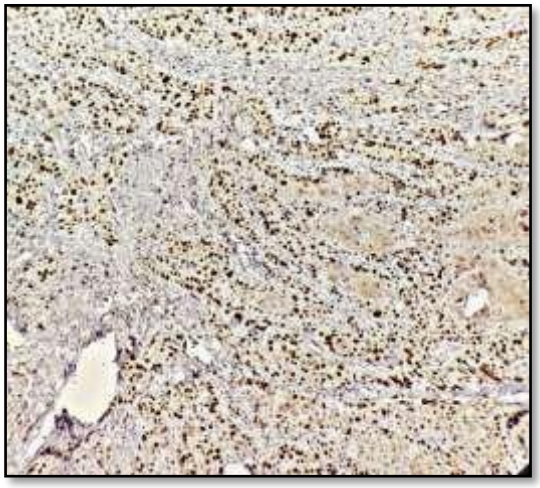


Figure 31: Photomicrograph showing Ki-67 expression in WD OSCC (IHC 100x)

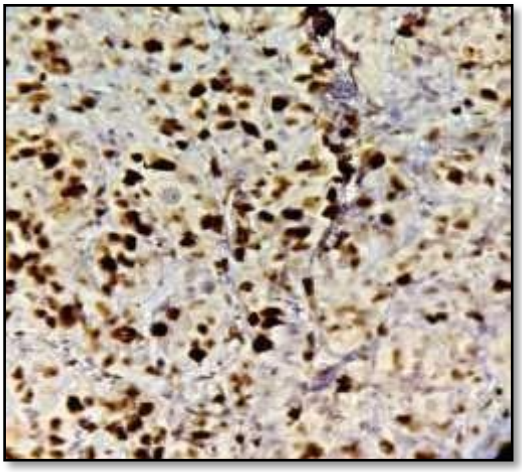


Figure 32: Photomicrograph showing 52% Ki-67 LI in WD OSCC (IHC 400x)

Figure 33: Photomicrograph showing Ki-67 expression in WD OSCC (IHC 100x)

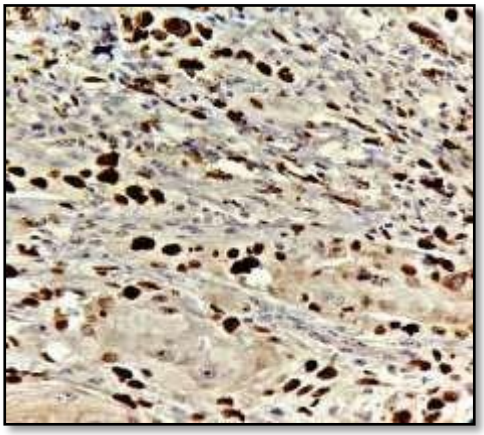


Figure 34: Photomicrograph showing 45% Ki-67 LI as 50% in WD OSCC (IHC 400x)

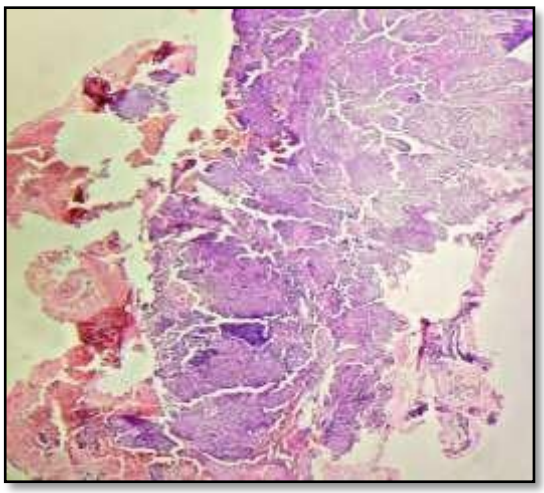


Figure 35: Photomicrograph showing MD OSCC
(H&E 100x)

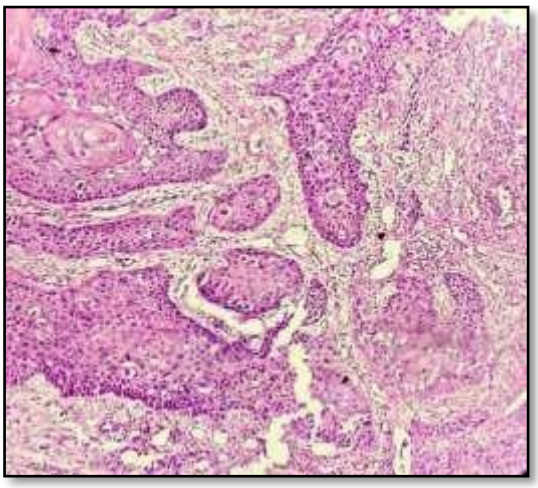


Figure 36: Photomicrograph showing MD OSCC
(H&E 100x)

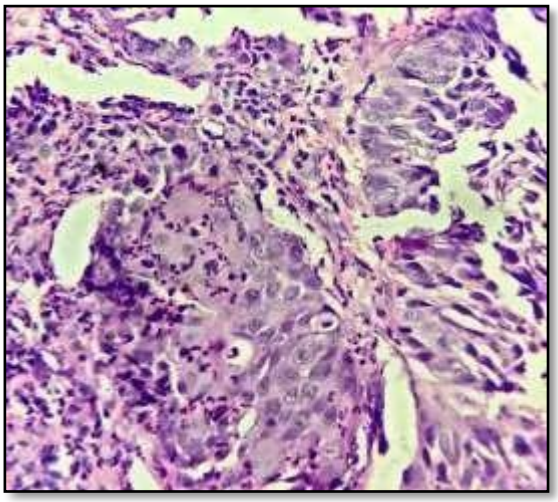


Figure 37: Photomicrograph showing individual cell morphology in MD OSCC. (H&E 400x)

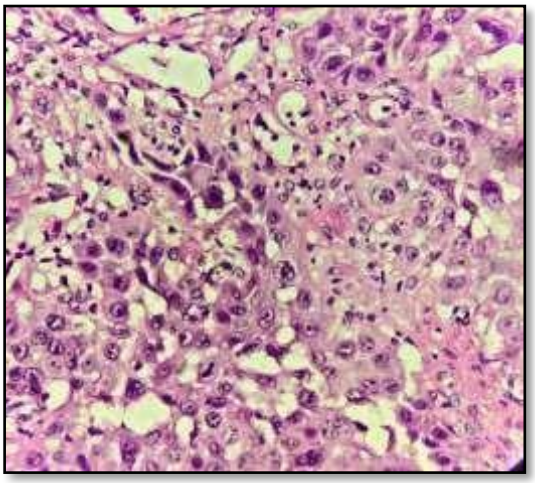


Figure 38: Photomicrograph showing individual cell morphology in MD OSCC with an atypical mitotic figure in the center. (H&E 400x)

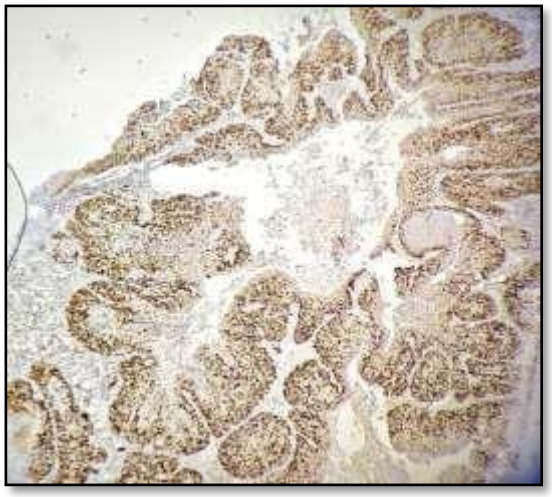


Figure 39: Photomicrograph showing Ki-67 expression in MD OSCC (IHC 100x)

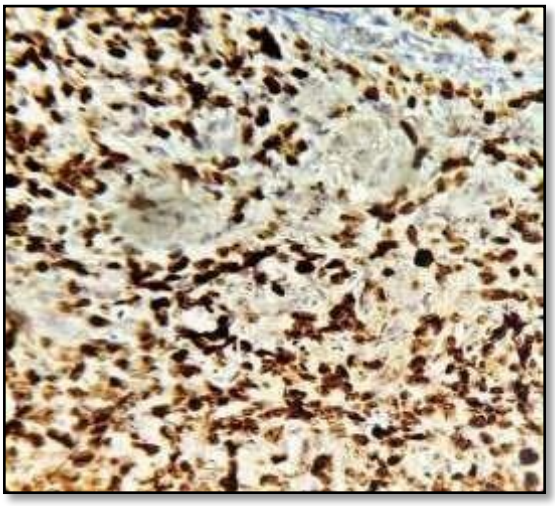


Figure 40: Photomicrograph showing 70% Ki-67 LI in MD OSCC (IHC 400x)

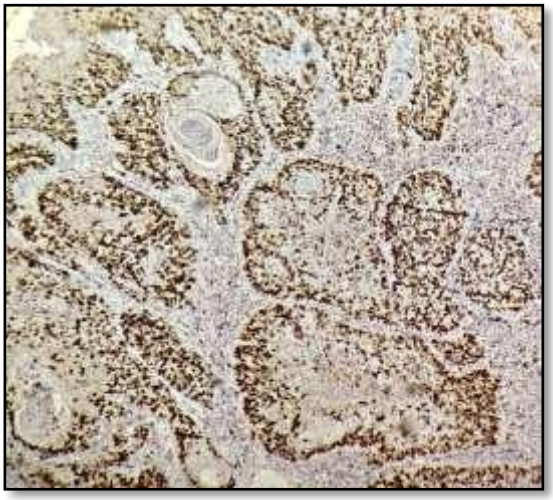


Figure 41: Photomicrograph showing Ki-67 expression in MD OSCC (IHC 100x)

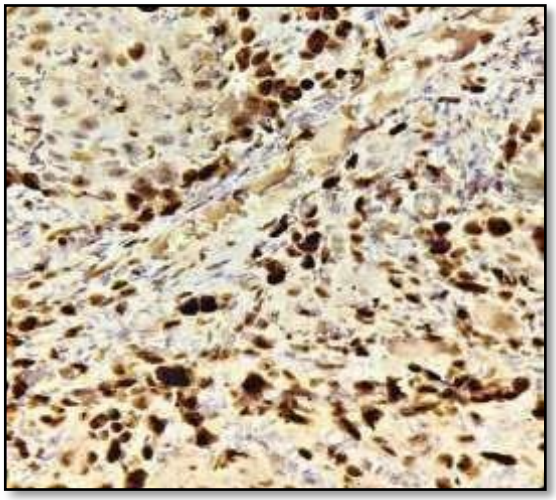


Figure 42: Photomicrograph showing 60% Ki-67 LI in MD OSCC (IHC 400x)

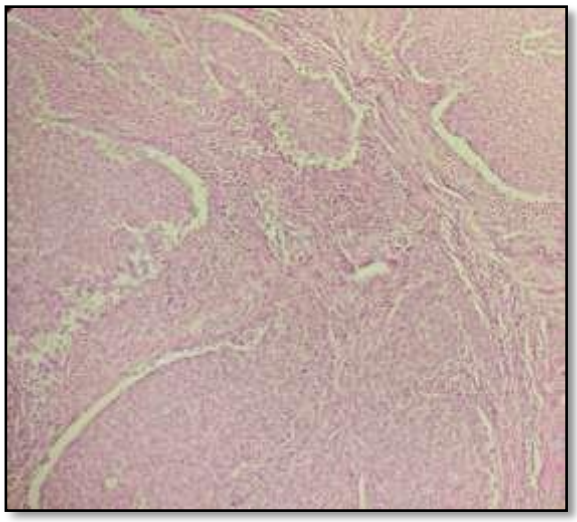


Figure 43: Photomicrograph showing PD OSCC (H&E 100x)

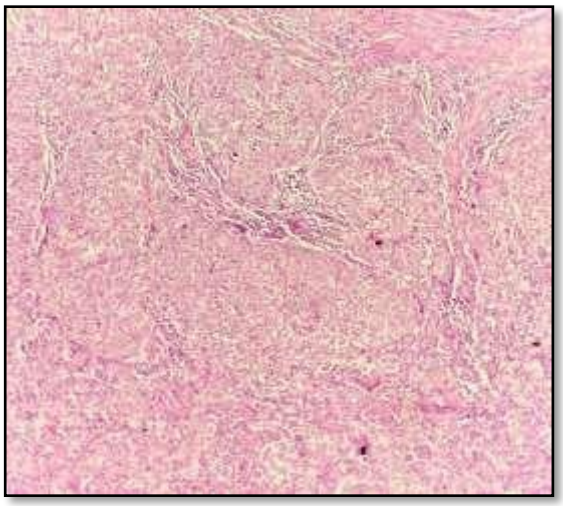


Figure 44: Photomicrograph showing PD OSCC (H&E 100x)

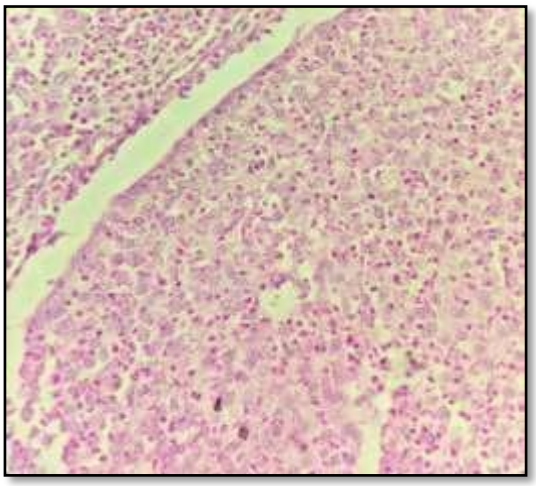


Figure 45: Photomicrograph showing individual cell morphology in PD OSCC. (H&E 200x)

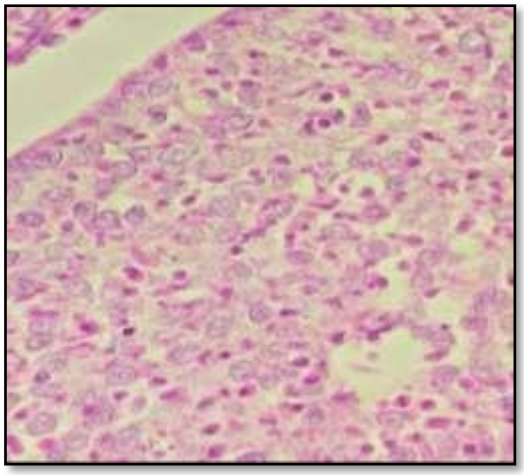


Figure 46: Photomicrograph showing individual cell morphology in PD OSCC.(H&E 400x)

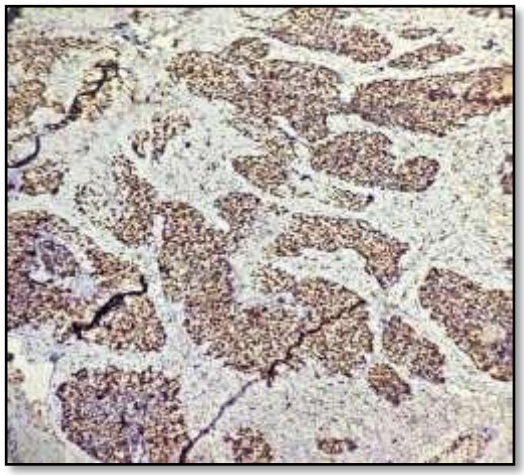


Figure 47: Photomicrograph showing Ki-67 expression in PD OSCC
(IHC 100x)

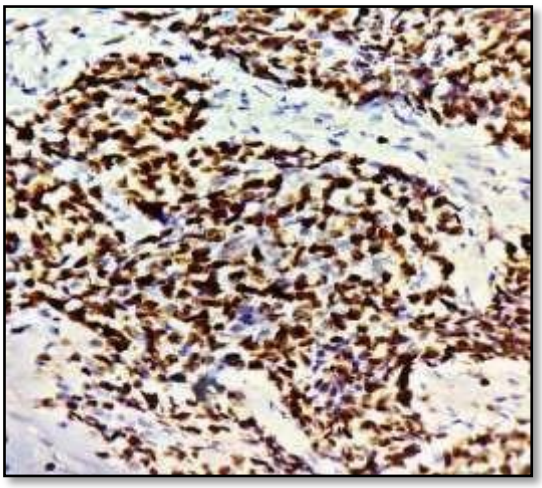


Figure 48: Photomicrograph showing Ki-67 expression in PD OSCC
(IHC 200x)

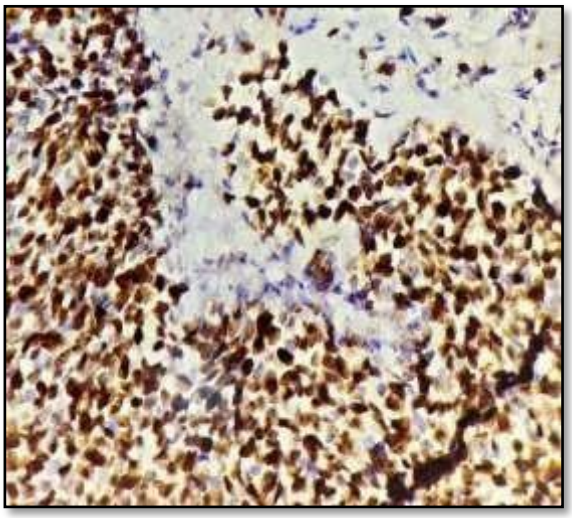


Figure 49: Photomicrograph showing 80% Ki-67 LI in PD OSCC
(IHC 400x)

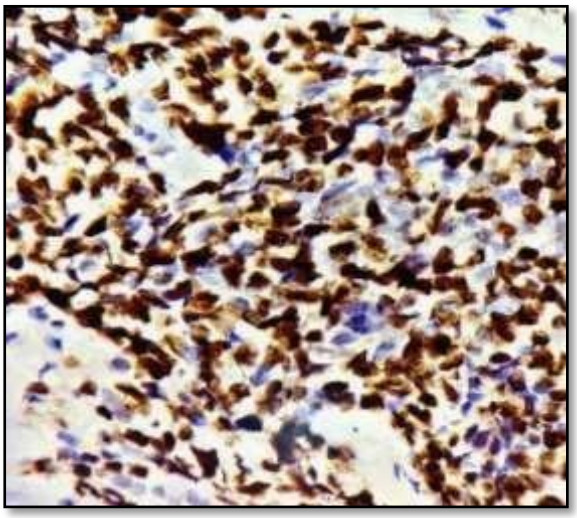


Figure 50: Photomicrograph showing 80% Ki-67 LI in PD OSCC
(IHC 400x)

DISCUSSION

The present study included 64 histopathologically diagnosed cases of epithelial dysplasia and squamous cell carcinoma of the oral cavity. Out of these 64 cases, 16 (25%) were diagnosed as Epithelial dysplasia, and 45 (75%) were diagnosed as Squamous cell carcinoma.

Out of 45 cases of OSCC, maximum number of cases were found in the age group of 41-60 years, amounting to 53%. In a study by Chen S *et al.* [43], the average patient age was 54.6±12.2 years. The most common age of presentation for OSCC in various studies was between 41 to 60 years of age. [33, 44,45,46]. Age incidence for OSCC in the present study is also correlating with the study done by other authors' findings. In a study done by various authors, it was observed that most of the cases were males, ranging from 62.1% to 81.5%. [12,33,34,45,46] Similar observations were noted in the present study, where 48 cases were males amounting to 75%, and 16 cases were females amounting to 25%. The male: female ratio in the present study was 3:1. Our findings correlate with the study by Gonzales *et al.* [45], where the male: female ratio was 3:1.

In a study done by Chandrakanta *et al.* [34], the tongue was the most common site (50%), followed by buccal mucosa (18.42%). In the study done by Krishna *et al.* [46], the commonest site of malignancy in the oral cavity was buccal mucosa (35.5%), followed by alveolus (28%), tongue (17%) and gingivo-buccal cavity (10%). In a study done by Farhood Z *et al.,* [12] most common OCSCC subsites were the floor of mouth and tongue, amounting to 34% each. Similar findings were observed in the present study, where the most common site for OSCC was buccal mucosa amounting to 41%, followed by the tongue, amounting to 31%, and Gingivobuccal sulcus, amounting to 11%. The percentage of the other affected sites like the cheek, lip,

retromolar trigone, hard palate, and posterior cricoid was less than 10 %.

In the study done by Sharma G *et al.* [33], when correlation was done between age and Ki67 expression, high Ki67 expression was noted between the age group of 45-84 years. Patients under 45 years of age showed low Ki67 expression, and more than 45 years showed high Ki67 expression, amounting to 50%. They have also done the correlation between Ki67 expression and gender using the Chi-square test, where it was observed that Ki67 expression was higher in males than females. However, the difference was statistically not significant, with a p-value being 0.344. Lim *et al.* [47], in their study, also reported that there was no correlation between Ki67 expression and factors like age and sex in OSCC. Similar findings were observed in our study, with slightly higher Ki67 expression in males than in females. However, the difference was statistically not significant.

Diffuse basal, with or without parabasal expression of Ki67, was considered as a sign of uncontrolled proliferation and an important hallmark of malignancy. [50] In a study done by Gonzales *et al.* [45], it was reported that increased proliferation of cells in the parabasal layers of pre-malignant oral epithelium is more likely to be linked with loss of heterozygosity (LOH) in 9p, 3p, and 17p.40 This behaves as a marker of precancerous fields and increases the risk of developing multiple tumors. A collection of highly proliferating parabasal cells may serve as a target for further late oncogenic events that could create more subclones of cells from the field with a higher capacity to invade. Hence, an increase in the proliferation rate of cell clones transforming into malignancy appears to be important for a precancerous field to generate a tumor. [45,51,52]

In the present study, in all cases of moderate dysplasia, Ki67 expression was seen in the basal and parabasal layers. In 66.7% of cases of severe dysplasia, Ki67

expression was noted in the basal, parabasal, and spinous layers of the epithelium, suggesting increased proliferation of the cells in parabasal layers of the epithelium. However, the number of moderate and severe dysplasia cases studied is few in the present study. Takkem *et al.* [1], Dwivedi N *et al.* [32], and Takeda T *et al.* [53], in their study, mentioned that Ki67 expression was located at basal and parabasal layers in all the cases of mild OED. In moderate and severe OED, Ki67 expression was located at basal, parabasal, and spinous layers in all the cases. They also mentioned that Ki67 positivity was increased with the severity of dysplasia. Our findings are also correlating with the results mentioned by these authors. In a study done by Pagalla AK *et al.* [54], expression of the Ki-67 protein was categorized as low grade and high grade. They observed that "in low grade dysplasia, the maximum expression of Ki-67 was located at the basal and parabasal layers of the epithelium, and in high grade dysplasia, the nuclear Ki-67 positivity was found in the basal, parabasal, and some of the spinous layers of the epithelium." These findings suggest that in the future, the Ki-67 protein may serve as a prognostic tool in detecting malignant transformation in oropharyngeal lesions. [54]

TABLE 17: COMPARISION OF GRADES OF OSCC WITH OTHER AUTHORS' STUDIES

Grade of OSCC	Chandrakanta *et al.* [34]	Pires *et al.* [55]	Sharma G *et al.* [33]	Present study
WD OSCC	26.3%	30.7%	32%	29%
MD OSCC	55.3%	45.5%	62%	69%
PD OSCC	18.4%	23.8%	6%	2%

In various studies of OSCC, it was observed that the percentage of MD OSCC was the highest, and that of PD OSCC was the lowest. Similar findings were noted in the present study.

In a study done by Chandrakanta *et al.* [34] and Gonzales *et al.* [45], the evaluation of Ki67 expression was done as per the labelling index (LI). The Ki67 LI was calculated as the percentage of the cells in a tissue staining for this marker. [34,45]

TABLE 18: COMPARISION OF KI67 LI WITH OTHER AUTHORS' STUDIES

Grade of OSCC	Chandrakanta *et al.* [34]	Gonzales *et al.* [45]	Present study
WD OSCC	28.52 ± 21.25%	46.1 ± 26.1%,	54 ± 10.01%
MD OSCC	42.85 ± 18.2%	57.8 ± 28.6%	59 ± 15%
PD OSCC	68.57 ± 17.6%	69.6 ± 21.8	80 %

Ki67 LI was highest in PD OSCC compared to WD OSCC and MD OSCC. [34,45] Similar findings were noted in the present study. Chandrakanta *et al.* [34], in their study, concluded that Ki67 acts as an excellent marker of cellular proliferation.

In the present study in 3 cases of OSCC, additional findings were seen after

the Ki67 IHC study. One case was diagnosed as Suspicious for malignancy on the histopathology slide, whereas after the Ki67 immunohistochemistry study, it was diagnosed as Severe dysplasia with SCC showing minimal invasion. It showed high Ki67 expression in the basal and the suprabasal layers of the epithelium and mild foci of invasion with Ki67-positive tumor cells in the sub-epithelium. Two cases were diagnosed as MD OSCC on the histopathology slide, and after the Ki67 IHC study, a focus of severe dysplasia with minimal invasion was noted. In the focus of severe dysplasia, Ki67 positivity was noted in basal, parabasal, and spinous layers of the epithelium with a focus of minimal invasion in the subepithelial tissue suggesting that severe dysplastic lesion might have undergone malignant transformation.

SUMMARY

A descriptive cross-sectional study was done on 64 specimens of clinically suspected cases of epithelial dysplasia and malignancy of the oral cavity received in the Histopathology section of the Department of Pathology, from 1st December to 31st July 2022. For each case, two sections of 4 μm were prepared. One section was stained by routine Haematoxylin and eosin (H&E) stain, and another section was used for IHC staining of Ki-67.

Out of 64 cases, 45 cases were histopathologically diagnosed as Oral squamous cell carcinoma, and 19 cases were diagnosed as Oral epithelial dysplasia.

Maximum number of cases were found in the age group of 41-60 years, amounting to 53%.

The majority of the cases were males amounting to 75%, with male: female ratio of 3:1.

In OED, 79% were diagnosed as mild dysplasia. Maximum number of cases of OED was observed in the age group 41-50 years. Mild dysplasia was noted in the younger age group of whereas severe dysplasia was noted in the elderly age group. In OED, out of 15 cases of mild dysplasia, 12 cases showed Ki67 positivity in the basal layer amounting to 80%. In moderate dysplasia, Ki67 positivity was noted in the basal and parabasal layers. Two cases of severe dysplasia showed Ki67 positivity in basal, parabasal, and spinous layers of epithelium.

When correlation was done between histopathological grading of OED and Ki67 expression, a statistically significant difference was noted with a p-value of 0.03 (<0.005). When correlation was done between age and gender with Ki67 expression in OED, Ki67 expression was slightly higher in females and in elderly patients, however the difference was statistically not significant.

In OSCC, maximum number of cases were MD OSCC amounting to 69%. The most common site for OSCC was buccal mucosa followed by the tongue.

In OSCC, correlation between histopathological grading of OSCC and Ki67 expression, showed higher Ki67 expression in PD OSCC as compared to WD OSCC and the difference was statistically significant with a p-value of 0.001.

CONCLUSION

In the present study, Ki67 expression was found in all the layers in severe dysplasia. It may be possibly due to the higher malignant transformation rate in a lesion that demonstrates severe dysplasia. Ki-67 expression was also significantly higher in poorly differentiated OSCC as compared to well differentiated OSCC, indicating that Ki67 expression increases with decreasing tissue differentiation.

Thus, it can be used as a prognostic and predictive marker for determining the severity of OED and histological grading of OSCC. Also, Ki67 may be used as a prognostic tool in detecting malignant transformation in epithelial dysplasia.

Ki67 can be used as predictor of proliferative marker to assess epithelial dysplasia and OSCC. Thus, it can further aid in diagnosing oral pre-malignant and malignant lesions at an early stage, which can further help in the early treatment and better survival of the patient.

Limitation of the present study:

Sample size of moderate OED and PD OSCC are very less.

Recommendations:

Since Ki-67 is a proliferative marker, it can be useful as a prognostic marker and further help in evaluating the treatment of the patients with OED and OSCC. In OED, it helps in predicting the malignant transformation of dysplastic cases.

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ANNEXURE-I

ETHICAL CLEARANCE

IEC/100-09/2021
Date-22/01/2021

B.L.D.E. (DEEMED TO BE UNIVERSITY)

(Declared vide notification No. F.9-37/2007-U.3 (A) Dated: 29-2-2008 of the MHRD, Government of India under Section 3 of the UGC Act, 1956)

The Constituent College

SHRI. B. M. PATIL MEDICAL COLLEGE, HOSPITAL AND RESEARCH CENTRE

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The Institutional ethical committee of this college met on 11-01-2021 at 11-00 am to scrutinize the synopsis of Postgraduate students of this college from Ethical Clearance point of view. After scrutiny the following original/corrected and revised version synopsis of the Thesis has been accorded Ethical Clearance

Title: Evaluation of Ki-67 expression in epithelial dysplasia and squamous cell carcinoma of oral cavity

Name of PG student: Dr Payal Doctor, Department of Pathology

Name of Guide/Co-investigator: Dr S U Arakeri , Professor & HOD of Pathology

DR.S.V.PATIL
CHAIRMAN, IEC.

Institutional Ethical Committee
B L D E (Deemed to be University)
Shri B.M. Patil Medical College,
VIJAYAPUR-586103 (Karnataka)

Following documents were placed before Ethical Committee for Scrutinization:

1. Copy of Synopsis / Research project
2. Copy of informed consent form
3. Any other relevant documents.

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ANNEXURE- II

B.L.D.E.U.'s SHRI B.M. PATIL MEDICAL COLLEGE HOSPITAL AND RESEARCH CENTER, VIJAYAPUR-586103

INFORMED CONSENT FOR PARTICIPATION IN DISSERTATION/RESEARCH

I, the undersigned,_____, S/O D/O W/O_____, aged_____years, ordinarily resident of_____do hereby state/declare that Dr _____of_____Hospital has examined me thoroughly on _____at_____(place) and it has been explained to me in my own language that I am suffering from_____disease (condition) and this disease/condition mimic following diseases . Further Doctor informed me that he/she is conducting dissertation/research titled_____under the guidance of Dr_____requesting my participation in the study. Apart from routine treatment procedure, the pre-operative, operative, post-operative and follow-up observations will be utilized for the study as reference data.

Doctor has also informed me that during conduct of this procedure adverse result may be encountered. Among the above complications most of them are treatable but are not anticipated hence there is chance of aggravation of my condition and in rare circumstances it may prove fatal in spite of anticipated diagnosis and best treatment made available. Further Doctor has informed me that my participation in this study will help in evaluation of the results of the study which is useful reference to treatment of other similar cases in near future, and also, I may be benefited in getting relieved of suffering or cure of the disease I am suffering.

The Doctor has also informed me that information given by me, observations

made/ photographs/ video graphs taken upon me by the investigator will be kept secret and not assessed by the person other than me or my legal hirer except for academic purposes.

The Doctor did inform me that though my participation is purely voluntary, based on information given by me, I can ask any clarification during the course of treatment / study related to diagnosis, procedure of treatment, result of treatment or prognosis. At the same time, I have been informed that I can withdraw from my participation in this study at any time if I want or the investigator can terminate me from the study at any time from the study but not the procedure of treatment and follow-up unless I request to be discharged.

After understanding the nature of dissertation or research, diagnosis made, mode of treatment, I the undersigned Shri/Smt _____ under my full conscious state of mind agree to participate in the said research/dissertation.

Signature of patient: Signature of doctor:

Witness: 1.

2.

Date:

Place

ANNEXURE- III

PROFORMA FOR STUDY

NAME : OP/IP No. :

AGE :

SEX :

OCCUPATION :

RESIDENCE :

Presenting Complaints

Past history :

Personal history :

Family history :

Treatment history :

General physical examination:

Pallor present/absent

Icterus present/absent

Clubbing present/absent

Lymphadenopathy present/absent

Built poor/average/well

VITALS: PR: RR:

BP: TEMPERATURE: WEIGHT:

SYSTEMIC EXAMINATION:

LOCAL EXAMINATION:

Site of lesion:

Size of lesion:

Lymph node status:

CLINICAL DIAGNOSIS:

INVESTIGATIONS:

Histopathological examination of tissue sections:

Epithelial Dysplasia of oral cavity (Mild/Moderate/Severe):

Squamous Cell Carcinoma of oral cavity (Poorly/Moderately/Well differentiated):

Immunohistochemistry: - Ki-67 IHC Marker:

Ki-67 expression score evaluation will be done as follows:

Score 1- (1- 25% of tumour cells stained by Ki-67):

Score 2- (26% to 50% of tumour cells stained by Ki-67):

Score 3- (51% to 75% of tumour cells stained by Ki-67):

Score 4- (76-100% of tumour cells stained by Ki-67):

TABLE 1: Association Ki-67 expression score in epithelial dysplasia of oral cavity.

Grading of Epithelial Dysplasia	Basal layer positivity	Basal and parabasal layer positivity	Basal, parabasal and suprabasal layer positivity
Mild epithelial dysplasia			
Moderate epithelial dysplasia			
Severe epithelial dysplasia			

TABLE 2: Association of Ki-67 expression score of oral squamous cell carcinoma of oral cavity

Grading of oral squamous cell carcinoma (OSCC)	Ki-67 Expression Score 1 (1-25%)	Ki-67 expression Score 2 (26% to 50%)	Ki-67 expression Score 3 (51% to 75%)	Ki-67 expression Score 4 (76% to 100%)
Well differentiated OSCC				
Moderately differentiated OSCC				
Poorly differentiated OSCC				

KEY TO MASTER CHART

S. No	-	Serial Number
I.P Number	-	Patient's unique ID number
HPR No.	-	Histopathology Number
M	-	Male
F	-	Female
HPR Diagnosis	-	Histopathology Diagnosis
Well Diff SCC	-	Well differentiated Squamous cell carcinoma
Mod Diff SCC	-	Moderately differentiated Squamous cell carcinoma
Poor Diff SCC	-	Poorly differentiated Squamous cell carcinoma

MASTER CHART

S.NO	NAME	AGE	GENDER	IP NO.	HPR NO.	TYPE OF SPECIMEN	HPR DIAGNOSIS	KI67 POSITIVITY RANGE	KI67 POSITIVITY AVERAGE	KI67 POSITIVTY SCORE
1	Mahananda	48	F	117582	2414/22	Buccal mucosa	Well diff SCC	55-65%	60%	3
2	Rachawwa	75	M	177076	3835/21	Buccal mucosa	Mod diff SCC	55-65%	60%	3
3	Shankar	40	M	58314	1548/22	Buccal mucosa	Well diff SCC	53-63%	58%	3
4	Pundalik	52	M	142339	2666/22	GB sulcus	Mod diff SCC	57-67%	62%	3
5	Girish	28	M	91444	1677/22	Buccal mucosa	Mod diff SCC	55-65%	60%	3
6	Riyaj	44	M	229889	4579/21	Buccal mucosa	Mod diff SCC	60-70%	65%	3
7	Hanamanth	50	M	195315	3640/22	Tongue	Mod diff SCC	55-65%	60%	3
8	Mahananda	48	F	117582	2216/22	Buccal mucosa	Mod diff SCC	56-66%	61%	3
9	Vardhaman	40	M	118101	2214/22	Buccal mucosa	Mod diff SCC	66-76%	71%	3
10	Guranna J	60	M	223945	5048/21	Buccal mucosa	Well diff SCC	47-57%	52%	3
11	Shankar B	75	M	244507	4762/21	Retromolar trigone	Mod diff SCC	50-55%	53%	3
12	Guranna J	60	M	223945	4806/21/A	Cheek	Mod diff SCC	53-62%	60%	3

13	Indrawwa	70	F	73115	2634/22	Buccal mucosa	Mod diff SCC	55-65%	60%	3
14	Janappa	68	M	176458	3845/21	Buccal mucosa	Mod diff SCC	20-30%	25%	1
15	Guruprada	65	M	107311	2535/22	Tongue	Well diff SCC	47-57%	52%	2
16	Indrawwa	72	F	160419	3059/22	Buccal mucosa	Well diff SCC	45-55%	50%	2
17	Gallappa	68	M	287926	5431/21	GB sulcus	Mod diff SCC	45-55%	50%	2
18	Mahesh	35	M	264889	5078/21	Tongue	Well diff SCC	25-35%	30%	2
19	Siddanagouda	60	M	270786	5160/21	Tongue	Mod diff SCC	20-30%	25%	1
20	Siddappa	66	M	303200	5671/21	Tongue	Mod diff SCC	55-65%	60%	3
21	Hanamanth	55	M	31479	510/22	Tongue	Mod diff SCC	75-85%	80%	4
22	Shrishail	68	M	171156	3211/22	Buccal mucosa	Mod diff SCC	15-25%	20%	1
23	Girish	28	M	91444	2025/22	Buccal mucosa	Mod diff SCC	75-85%	80%	4
24	Gadigerappa B	36	M	298488	5586/21	GB sulcus	Mod diff SCC	75-85%	80%	4
25	Inawwa	65	F	310451	5816/21	Buccal mucosa	Mod diff SCC	75-85%	80%	4
26	Ramesh	51	M	116945	2173/22	GB sulcus	Mod diff SCC	63-73%	68%	3
27	Shankar K	45	M	58314	1001/22	Buccal mucosa	Mod diff SCC	57-67%	62%	3
28	Chandrakanth	72	M	51994	4527/22	Tongue	Mod diff SCC	63-73%	68%	3
29	Sangabasappa	72	M	51931	154/21	Post cricoid	Mod diff SCC	47-57%	52%	3
30	Mallikarjun	50	M	117689	984/21	Buccal mucosa	Mod diff SCC	55-65%	60%	3
31	Ashok	57	M	85671	634/21	Tongue	Mod diff SCC	56-66%	61%	3
32	Ameena	65	F	70856	640/21	Hard palate	Mod diff SCC	57-67%	62%	3
33	Mallappa	75	M	32768	1076/21	Tongue	Well diff SCC	67-77%	72%	4

34	Bagavantgouda	60	M	131958	1189/21	Tongue	Mod diff SCC	57-67%	62%	3
35	Ameensa N.	55	M	142812	1286/21	Tongue	Mod diff SCC	45-55%	50%	2
36	Mallanagouda	67	M	146482	1348/21	Tongue	Mod diff SCC	45-55%	50%	2
37	Seetawwa	45	F	147473	1401/21	Buccal mucosa	Well diff SCC	55-65%	60%	3
38	Sharanappa	55	M	136918	1551/21	Buccal mucosa	Well diff SCC	44-54%	49%	2
39	V M Biradar	51	M	88362	2528/21	GB sulcus	Well diff SCC	40-50%	45%	2
40	Nagappa	42	M	89485	2533/21	Tongue	Mod diff SCC	45-55%	50%	2
41	Shanta irakal	45	F	89421	2536/21	Buccal mucosa	Well diff SCC	55-65%	60%	3
42	M.dodda	55	M	95449	2616/21	Lip	Mod diff SCC	60-70%	65%	3
43	Shantappa	45	M	76543	3212/20	Tongue	Well diff SCC	45-55%	50%	2
44	Kasturi	43	F	8723	3383/20	Buccal mucosa	Well diff SCC	55-65%	60%	3
45	Mairabanu	65	F	12233	2182/20	Buccal mucosa	Poor diff SCC	60-70%	65%	3
46	Ganpathi	49	M	10469	one/21	Tongue	Mild dysplasia	basal layer		
47	Suresh	44	M	38722	seven/21	Tongue	Mild dysplasia	basal layer		
48	Honagouda	45	M	13395	546/21	Buccal mucosa	Mild dysplasia	basal parabasal layer		
49	Sharanappa	42	M	3736	779/20	Lip	Mild dysplasia	basal parabasal layer		
50	Rajkumar	36	M	77999	1270/20	Buccal mucosa	Mild dysplasia	basal layer		
51	Girimallappa	75	F	137236	2222/20	Tongue	Mild dysplasia	basal parabasal layer		
52	Bhimabai	60	F	71652	2465/20	Buccal mucosa	Mild dysplasia	basal layer		
53	Kamlesh	50	M	163152	2973/20	Tongue	Mild dysplasia	basal layer		
54	Rakhee	26	F	23969	3894/20	Cheek growth	Mild dysplasia	basal layer		

55	Sharanappa	55	M	136918	1219/21	Lip	Severe Dysplasia	basal parabasal suprabasal layer		
56	Shivaji	18	M	4592	4026/20	Cheek	Mild dysplasia	basal layer		
57	Laxmibai	56	F	44563	1801/20	Tongue	Mild dysplasia	basal layer		
58	Nithin	26	M	23969	3894/20	Cheek	Mild dysplasia	basal layer		
59	Ravikumar	36	M	77987	1272/20	Buccal mucosa	Mod dysplasia	basal parabasal layer		
60	Amarappa	50	M	163142	2173/20	Tongue	Mild dysplasia	basal layer		
61	Shankaremma	76	F	58017	987/22	Tongue	Severe Dysplasia	basal parabasal layer		
62	Annapuran	70	F	82993	1458/22	Tongue	Mild dysplasia	basal layer		
63	Sangamesh	50	M	99894	2199/21	Tongue	Severe Dysplasia	basal parabasal suprabasal layer		
64	Channapa	60	M	73115	2263/22	Buccal mucosa	Mild dysplasia	basal layer		

Evaluation ofki67 in oed and oscc

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