IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 21, Issue 1 Ser.17 (January. 2022), PP 54-65
www.iosrjournals.org

A Comparative Study of Ki-67 Immunomarker in Oral Potentially Malignant Disorder and Oral Squamous Cell Carcinoma

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Abstract
Aims & Objective of Study: *The purpose of this study to evaluate ki67 (Proliferative marker for epithelial tissues) potential association between grades of Oral Epithelial Dysplasia (OED) and Oral Squamous Cell Carcinoma (OSCC) by the marker Ki-67 and compare it with normal mucosa as a healthy control.* ***Material & Methods:*** *The present study was done in the department of Oral Pathology & Microbiology, RUHS College of Dental Sciences, Jaipur. This retrospective study comprised 40 samples out of these 15 clinically and Histopathologically diagnosed cases of different grade of Oral Potentially Malignant Disorders (OPMD) (Oral epithelial dysplasia mild, moderate and sever), 15 different grade of Oral squamous cell carcinoma (OSCC) (well, moderately and poorly differentiated OSCC) and 10 were control group of healthy individuals, with the age group of 20-85. Histopathological examination was carried out in all the cases following incisional biopsy from the affected area of the oral cavity for OPMD AND OSCC While for control group tissue taken from oral surgery. The selected patients were explained in detail about the study and the procedure they were subjected. A formal informed written consent was taken prior to procedure and demographic details were recorded for all patients and included age, sex and personal history about alcohol consumption, tobacco chewing or smoking and other chewing habits. Immunohistochemistry staining for Ki-67 was performed on Sections of 4-micron thickness shall be cut from paraffin embedded tissue blocks and stained with Immunohistochemical staining with Ki-67 is be carried out for each case following standardized protocol in the Department Oral Pathology & Microbiology.* ***Results:*** *Statistical analysis is done by using Statistical package for social sciences (SPSS v 21.0, IBM). The proliferating cells of normal epithelium were restricted mainly in the basal layer of the epithelium. We also found to that expression of the Ki-67 protein in the located at the basal, parabasal and spinous layers of the epithelium of OPMD(OED) and its expression increased with the severity of dysplasia. In well-differentiated OSCC the nuclear Ki-67 positivity was found in the peripheral area of tumor islands. This suggests that less differentiated cells are located in the peripheral layer and the central cells are highly differentiated with the ability of keratinization, thus, no expression of Ki-67 was observed in the central cells of the tumor island.* ***Conclusion:*** *The findings of this study suggest that expression of Ki-67 may be used as an assessment of prognosis, patient survival and also with tumor staging. The higher immunomarker for proliferation/expression status may be considered a useful indicator for initial changes occurring in neoplastic cells (progression of tumor).*

Key words: *ki67, OPMD, Epithelial Dysplasia and OSCC*

Date of Submission: 16-01-2022 Date of Acceptance: 31-01-2022

I. Introduction

Head and neck cancers pose a serious threat to the society. The problem is further amplified by the fact that number of new cases goes up every year.[1] Oral cancer is a significant disease worldwide with up to 400000

new cases every year and almost 130,000 deaths annually.[2] Incidence rates are much higher in developing regions like Southeast Asia, where they account for up to 50 % of all malignant tumors.[3] Malignancies of head and neck are notoriously known for their varied biologic behavior.

The current evidence recognizes carcinogenesis of the epithelium as a multistep, progressive, cumulative process of genetic mutations which culminate in tumor formation, and ultimately invasion and metastasis. In this model, simple epithelial hyperplasia progresses through mild Oral Epithelial Dysplasia (OED) to more severe dysplastic changes with increasing genetic aberrations.[4] Oral Potentially Malignant Disorders (OPMD) have shown an increased risk of progressing to cancer. The various rates of conversion include 14%–51% for erythroplakia, 60%–100% for proliferative verrucous leukoplakia and 7%–26% for oral submucous fibrosis (OSMF). Oral Squamous Cell Carcinoma is commonly preceded by a range of tissue and cellular alterations consistent with carcinoma, which are restricted to the surface epithelial layer and termed oral epithelial dysplasia (OED).[5] 'Dysplasia is encountered principally in the epithelia. "It comprises a loss in the uniformity of the individual cells, as well as a loss in their architectural orientation." Dysplastic cells exhibit considerable pleomorphism (variation in size and shape) and often possess deeply stained (hyperchromatic) nuclei, which are abnormally large for the size of the cell. The criteria used for diagnosing dysplasia include architectural changes (tissue changes) and cytological changes (individual cell changes/cytological atypia).[4] The WHO three-tier grading of oral dysplasia is traditionally used by pathologists, in which OED is graded as mild, moderate and severe Grading of OED is used to assess the probability of malignant transformation. World Health Organization (WHO) criteria for epithelial dysplasia (2017). Based on the architectural and cytological alterations, the epithelium is divided into "thirds," and the lesions are classified into five categories.[6]

1. Hyperplasia (Squamous hyperplasia): Lesions with an increase in cell number in the spinous layer and/or in the basal/parabasal cell layers. There is presence of regular stratification and no cellular atypia.
2. Mild dysplasia: Architectural disturbance present only in the lower third of the epithelium with cytological atypia.
3. Moderate dysplasia: The criteria postulate that architectural disturbance extending into the middle third of the epithelium, but the degree of cytological atypia may require upgrading it to "severe dysplasia"
4. Severe dysplasia: Architectural disturbance observed in greater than two thirds of the epithelium, with cytological atypia.
5. Carcinoma *in situ* (CIS): Is a noninvasive carcinoma, classified as a precursor lesion of OSCC. CIS is characterized by full thickness or almost full thickness of epithelial architectural disturbance in the viable cell layers accompanied by pronounced cytological atypia.

The binary system for grading epithelial dysplasia categorizes OED into low risk and high risk for undergoing malignant transformation Although the three-tier grading systems (mild, moderate and severe) is widely used, the binary system complements the WHO classification systems, and it has merit as it helps clinicians to make critical clinical decisions particularly in cases with moderate dysplasia. It also facilitates a standardized approach to overcome some difficulty in subjectivity in reporting of epithelial dysplasia.[7] Oral epithelial dysplasia in the clinical context. The histopathologic assessment for the presence of OED is considered the current gold standard for predicting malignant transformation of OPMDs. The presence of epithelial dysplasia is an indicator of the malignant potential of OPMDs, and the risk of these lesions to progress to carcinoma increases with the increasing grades of epithelial dysplasia. [8,9]

Oral Squamous Cell Carcinoma is a malignant tumor derived from the stratified squamous epithelium of the oral mucosa. Various histopathological grading systems of OSCC have been discussed in literature and Broder's grading has been popular for a long time. Broder's criteria to classify as well-differentiated squamous-cell carcinoma (WDSCC), moderately differentiated squamous-cell carcinoma (MDSCC) and poorly differentiated squamous-cell carcinoma (PDSCC). Conventional (OSCC) can present as several variants that make up in aggregate about 10-15% of all squamous cell carcinomas. These variants include verrucous carcinoma (VC), adenoid/ acantholytic/ pseudo glandular SCC (AdSCC), spindle cell/sarcomatoid carcinoma (SCSC), adenosquamous carcinoma (ASC), basaloid SCC (BSCC) and papillary SCC (PSCC). Each of these variants has a unique histomorphological appearance. [10]

There are multiple genetic mutations that must occur for normal tissues to progress to dysplasia or OSCC. Biopsy remains the gold standard for the Introduction 4 investigating for Oral epithelial dysplasia and oral squamous cell carcinoma.3,18 In oral epithelial tissues, accumulating mutations, chromosomal damage, and loss of cellular control functions, these changes are manifested as the transition from normal histology to dysplasia, to superficial cancer and invasive squamous cell carcinoma.[10] Oral cancer originates as an epithelial dysplasia and is characterized by the altered proliferation of dysplastic squamous cells on the surface of the epithelial layer, which subsequently degrades the subepithelial basement membrane (BM). Degradation of the BM results in local destruction and distant invasion via metastasis. Local invasion to the underlying tissue occurs via the islets and cords of epithelial cells. The ability to metastasize is directly associated with the differential grade of tumor cells, similar to that of the neoplastic tissue architecture and normal epithelium.

Despite refinement of surgical techniques in the past few decades once invasive cancer is formed, the prognosis is poor with an average 5-year survival rate of 40% of affected patients. A key factor in the lack of improvement in prognosis over the years is the fact that a significant proportion of OSCC are not diagnosed or treated until they reach an advanced stage. The prognosis for patients with OSCC that is treated early is much better, with 5-year survival rates as high as 80%.[11]

Markers of proliferation could potentially be good candidates for improving the prognostic evaluation of premalignant lesion and OSCC. until now, a panel of molecular markers has not been determined that allows for a prognostic prediction of oral squamous cell carcinoma. However, these new markers could be considered complementary to conventional prognostic evaluation. The control on cell proliferation biological process is thought to be lost in cancer and many studies have reported that abnormal cell proliferation appears to be a precursor and may be a predictor of tumorigenesis. The most common immunohistochemical markers used to study cell proliferation are proliferating cell nuclear antigen Ki-67 antigen. The Ki-67 protein, which encodes two protein isoforms with molecular weights of 345 and 395 kDa, was identified by Scholzer and Gerdes in the early 1980s.[12]

The Ki-67 protein is present during all active phases of the cell cycle (G1, S, G2, and M), but is absent in resting cells (G0). The prognostic value of Ki-67 protein has been investigated in a number of studies with its potential as a reliable marker having been shown in cancers of the breast, soft tissue, lung, prostate, cervix and central nervous system. It has been shown that blocking of Ki-67 protein either by microinjection of antibodies leads to inhibit the progression of the cell cycle. An increasing number of studies have suggested that Ki-67 protein may be an important factor in cancer grading and prognostic.[12]

The aim of this study was to identify an association between Ki-67 protein expression and histological grades of oral epithelial dysplasia - oral squamous cell carcinomas. And the role of Ki-67 protein in the prognostic of different histological grades of oral epithelial dysplasia and oral squamous cell carcinomas.

II. Material And Methods

SOURCES OF DATA The present retrospective study comprised 40 samples out of these 15 clinically and Histopathologically diagnosed cases of different grade of Oral Potentially Malignant Disorders (OPMD) (Oral epithelial dysplasia mild, moderate and sever), 15 different grade of Oral squamous cell carcinoma (OSCC) (well, moderately and poorly differentiated OSCC) and 10 were control group of healthy individuals with the age group of 20-85, from the Department of Oral Pathology and Microbiology, RUHS College Of Dental Sciences, Jaipur. Demographic details were retrieved of all patients including age, sex and personal history about alcohol consumption, tobacco chewing or smoking and other chewing habits from the archives.

INCLUSION CRITERIA

- Clinically and histologically diagnosed 15 cases of OED and 15 cases of OSCC
- Healthy individual 10 cases as control group

EXCLUSION CRITERIA

- Patients who have undergone treatment for the OPMD (OED) and OSCC.
- Medically compromised and uncooperative patients

All the patients were subjected to incisional biopsy and the soft tissue specimens were routinely fixed in 10% formalin (24-48 hours), processed (4µ thickness formalin fixed paraffin embedded tissue blocks) and stained with Hematoxylin & Eosin in the Department. OED specimens were histologically graded as mild, moderate and severe and OSCC graded as well differentiated, moderately differentiated, and poorly differentiated SCCs, according to the differentiation of cells and the resemblance of neoplastic cells to that of epithelial cells.

Materials

- Semiautomatic soft tissue microtome (Radical).
- Tissue floatation bath with thermostat (Yorco).
- Glass slide coated with Poly-L-lysine solution/ positive charge slides.
- Universal hot air oven
- Decloaking chamber(biocare)
- Humidity chamber
- Xylene, Alcohol, Deionized water, Tissue bond (working solution - 7 ml tissue bond in 350 ml acetone)
- Hydrogen Peroxide (working solution- 1ml H_2O_2 in 300 ml methanol)
- Primary antibody (Ki-67)
- Universal IHC kit
- Secondary antibody (MACH 1 mouse probe and universal HRP polymer)
- Trish buffer 40x (working solution-60 ml TBS +2400 ml deionized water)
- Citrate buffer 20x (working solution- 25 ml diva decloaking solution in 5000 ml deionized water).

- DAB (3,3 Diaminobenzidine) chromogen with DAB substrate. (working solution- 1 drop DAB chromogen in 1 ml DAB substrate) Material and Method 33
- Harris's hematoxylin
- Trinocular light microscope with digital camera (Motic)

Immunohistochemical staining Ki-67 Marker procedure

Sections of 4µ thickness from formalin fixed paraffin embedded tissue blocks were cut and taken on glass slides (poly L –lysine coated/ positive charge) and placed for intubation in universal hot air oven at 60℃ for 60 min. after slides were Cleared in 3 changes of xylene 5 min each and Rehydrated with 3 changes of descending grades (100%, 80%, 50%) of alcohol for 3-5 min each and kept in running water for 5 min. Slides were kept in deionized water 2 changes 30 seconds each and in 0.3% peroxidase solution for 7-10 min. washed with TBS for 2-3 min and Heat induced epitope retrieval - in closed decloaking chamber with citrate buffer. The temperature was set at 950 C for 60 min. and cooled down for 20 min at room temperature. Slides were arranged on racks of humidity chamber, washed with TBS two times and marked by pap pen. Primary antibody Ki-67(BioCare) was applied for 90 min and subsequently washed by tris buffered (TBS) at pH 7.4. Intubated and secondary antibody MACH 1 conjugated with HRP mouse probe was applied for 30min, washed with TBS. DAB chromogen (1 drop DAB chromogen in 1 ml DAB substrate) was applied for 1-2 min and counterstain with Harris's hematoxylin for 1-2 min. Mounted with DPX.

Evaluation of Staining

The intensity of Immunohistochemical staining will be graded based on subjective evaluation of color exhibited (brown color) by antigen, antibody and chromogen complex as: negative (-, no color), mild (+, light brown color), moderate Material and Method 35 (++, dark brown color) or intense (+++, very dark brown color). The distribution of stained cells is counted per high power 3 field (400x) magnification with help of one observer, per microscopic area. Only nuclear staining of epithelial cells was observed, and the nuclei with clear brown color, regardless of staining intensity, were regarded as positive. The percentage of positive cells is recorded. The area with maximum number of positive cells was considered in each section. Known positive Immunostaining slides were used as positive controls. The parameters used to analyze the expression of ki-67 antigen are:

- Pattern or distribution of expression in the per microscopic area.
- Intensity of staining in each slide;
- The percentage of positive cells or labeling index (LI).

Fig: 1 Deloaking chamber (biocare)

Fig:2 Universal IHC Kit with Ki-67 Primary Antibody

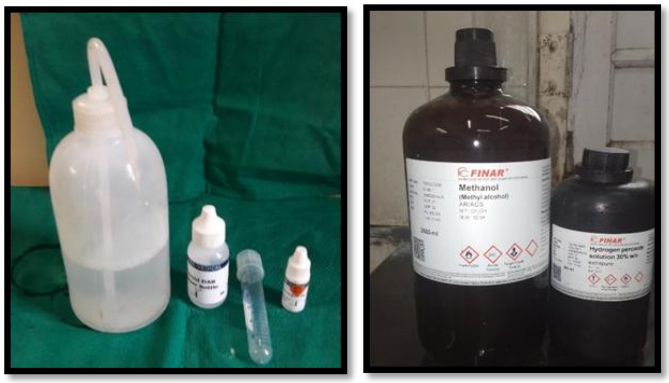


Fig: 3 IHC- Reagents

Fig: 4 Immunostaining Humidity Chamber

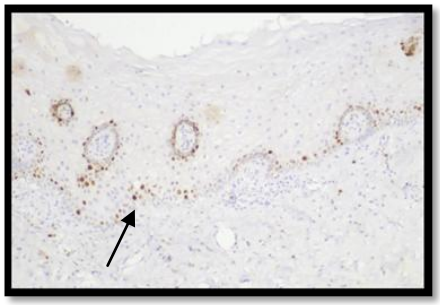


Fig: 5 Positive immunohistochemical Staining of Ki-67 of Normal Oral Mucosa at 10x

Fig: 6 Positive immunohistochemical Staining of Ki-67 of Mild Oral Epithelial Dysplasia

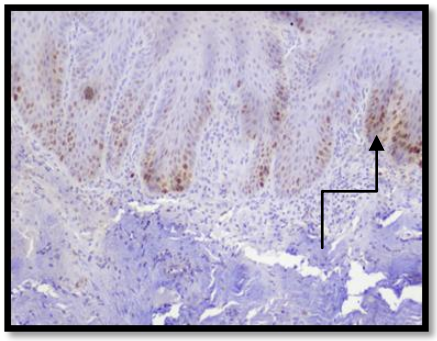


Fig: 7 Positive Immunohistochemical Staining of Ki-67 of Moderate Oral Epithelial Dysplasia at10

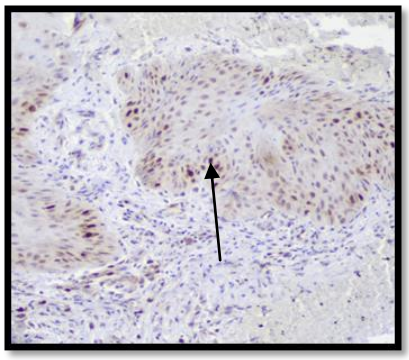


Fig: 8 Positive Immunohistochemical Staining of Ki-67 of Severe Oral Epithelial Dysplasia at10x

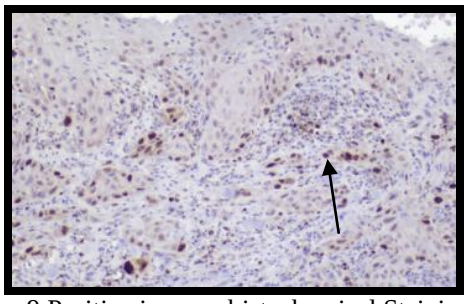


Fig: 9 Positive immunohistochemical Staining of Ki-67 of Well Differentiated OSCC at 10x

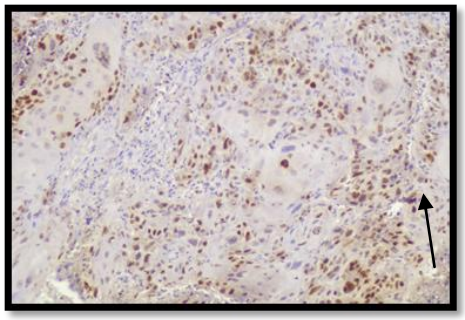


Fig: 10 Positive immunohistochemical Staining of Ki-67 of moderately Differentiated OSCC at10x

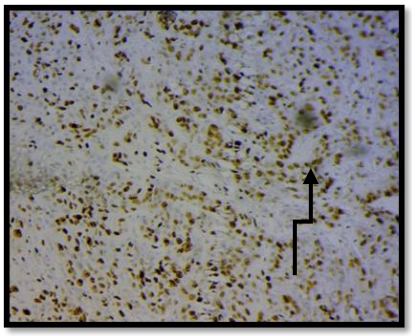


Fig: 11 Positive immunohistochemical staining of Ki-67 of Poorly Differentiated OSCC at 10x

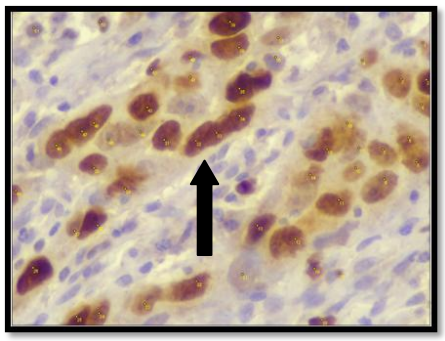


Fig: 12 Positive Immunohistochemical Staining of Ki-67 Cells Expression Count at (Per high power field) 40x

Statistical Analysis

Data obtained was compiled on a MS Office Excel Sheet (v 2010, Microsoft Redmond Campus, Redmond, Washington, United States) and statistical analysis using Statistical package for social sciences (SPSS v 21.0, IBM). Descriptive statistics like frequencies and percentage for categorical data, Mean & SD for numerical data has been depicted. Inter group comparison (>2 groups) was done using one way ANOVA followed by pair wise comparison using post hoc test. Comparison of frequencies of categories of variables with groups was done using chi square test. For all the statistical tests, p0.05) for all tables Groups are as follow:

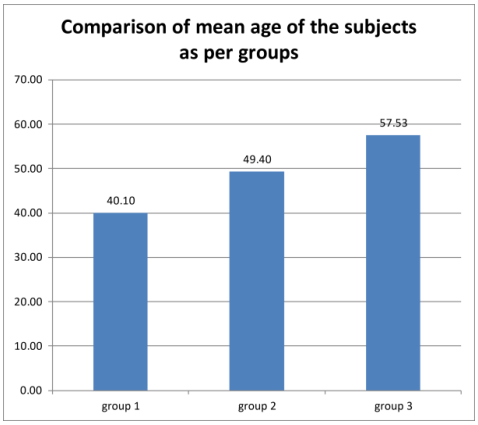
- Normal Mucosa healthy control
- OPMD (OED)
- OSCC

The percentage of positive cells or the LI for each slide in each group was calculated by dividing the number of positive cells by the total number of cells counted in the slide. Mean LI and standard deviation was calculated for all groups using descriptive analysis. As the scores were not continuous variables, statistical analysis was done using non-parametric methods. The one-way ANOVA test was done to find out if there is any significant difference of mean LI between the groups for each antibody. LIs of ki-67 for all groups were compared to find out relationship if any by using Spearman rank correlation test. χ 2 test was used to find out if there is any association between pattern or distribution, LI and intensity of staining for each antibody between the groups. P-value is considered significant where it is less than 0.05.

III. Result

Table: 1 showing mean age of the subjects

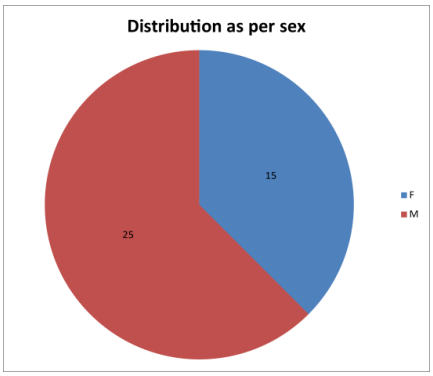
	N	Minimum	Maximum	Mean	Std. Deviation
age	40	20	85	50.13	17.144
Valid N (listwise)	40				



Graph:1 Comparison of mean age of the subjects as per groups

Table:2 Distribution as per sex

	Frequency	Percent
F	15	37.5
M	25	62.5
Total	40	100.0



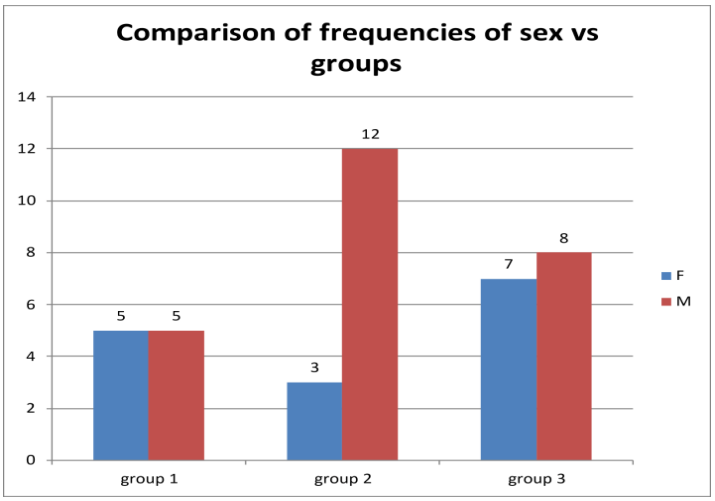
Graph:2 Distribution as per sex

Table-1 & graph-1 shows the mean age & SD of taken individuals for the study and maximum & minimum age of the patients. Mean value of the age & SD found 50.13±17.14 and minimum age 20 years and maximum age 85 years of the patients. Table-2 & graph-2 shows the distribution of the patients according to sex, number of male & female respectively 25 & 15 and according to age group: group-1:20-40 years, group-2:40-60 years &group-3 >60 years respectively number of patients. percentage wise group-1:14, group-2:14 & group-3:12, while the number of patients involved sex vs in each group in table:3 & graph 3 as followed group-1 number of female & male patients 5 (50 %) & 5(50%) Group-2 OPMD female & male number of patients 3(20%) & 12(80%) Group-3 OSCC female & male number of patients 7(46.67%) & 8(53.33

Table:3 Comparison of frequencies of sex vs groups

SEX	groups			Total	Chi square value	p value of chi square test
	1	2	3			
F	5	3	7	15		
M	5	12	8	25	3.164	0.206#
Total	10	15	15	40		

There was a statistically non-significant difference seen for the frequencies between the groups (p>0.05)

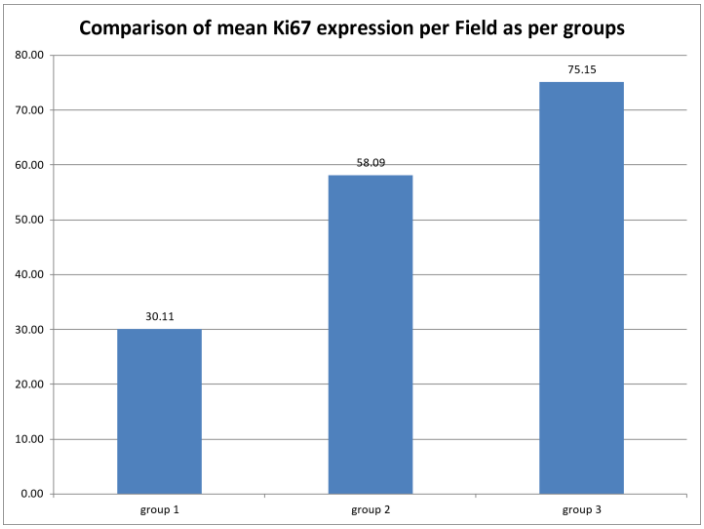


Graph: 3 Comparison of frequencies of sex vs groups

Table: 4 Comparison mean of Ki67 expression per Field as per groups

	N	Mean	Std. Deviation	Std. Error	Minimum	Maximum	F value	p value of one way ANOVA
1	10	30.110000	1.9353151	.6120004	27.0000	32.7000		
2	15	58.093333	13.4625123	3.4760057	36.0000	74.3000	38.064	.000**
3	15	75.153333	15.4679792	3.9938151	62.4000	101.3000		
Total	40	57.495000	21.5446341	3.4065057	27.0000	101.3000		

There was a statistically significant / highly significant difference seen for the values between the groups ($p<0.01, 0.05$) with higher values in group3 and least in group 1.



Graph:4 Comparison of mean ki67 expression per field as per groups

Table: 5 Pair wise comparison using Tukey's Post Hoc Tests

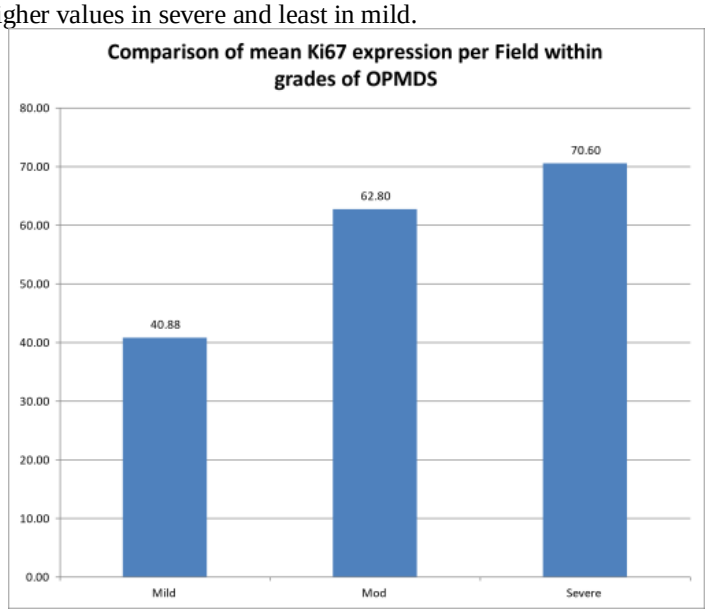
Groups I	Group J	Mean Difference (I-J)	Std. Error	p value
1	2	-27.9833333*	5.1642725	.000**
1	3	-45.0433333*	5.1642725	.000**
2	3	-17.0600000*	4.6190657	.002**

There was a statistically significant / highly significant difference seen for the values between the groups (p<0.01, 0.05) between the pairs like Group 1 vs 2, group 1 vs 3 and group 2 vs 3. Table 4 &5 and Graph -4 shows the distribution of ki67 in per field as per group -1, gtoup-2 & group-3. Here the table-9 shows the mean value and SD of ki67 distribution as per in group-1:30.11±1.93 and minimum and maximum distribution of ki67 as per field in group-1: 27.00 & 32.70. In group-2 OPMD 58.09±13.15 and minimum & maximum ki67 as per field distribution 36.00 & 74.300. Group-3 OSCC 75.15±15.46 and minimum & maximum Ki67 distribution per field in OSCC 62.40 & 101.30.

Table: 6 Comparison of mean Ki67 expression per Field within grades of OPMDS

	N	Mean	Std. Deviation	Std. Error	Minimum	Maximum	F value	p value of one way ANOVA
Mild	5	40.880000	3.8107742	1.7042300	36.0000	44.7000		
Mod	5	62.800000	4.4710178	1.9994999	60.3000	70.7000	87.395	.000**
Severe	5	70.600000	2.4979992	1.1171392	67.3000	74.3000		
Total	15	58.093333	13.4625123	3.4760057	36.0000	74.3000		

There was a statistically significant / highly significant difference seen for the values between the groups (p<0.01, 0.05) with higher values in severe and least in mild.



Graph: 5 Comparison of mean ki67 expression per field within grades of OPMDS

Table:7 Pair wise comparison using Tukey's Post Hoc Tests

(I) HP	(J) HP	Mean Difference (I-J)	Std. Error	p value
Mild	Mod	-21.9200000*	2.3310084	.000**
Mild	Severe	-29.7200000*	2.3310084	.000**
Mod	Severe	-7.8000000*	2.3310084	.015*

There was a statistically significant / highly significant difference seen for the values between the groups (p<0.01, 0.05) between the pairs like Group 1 vs 2, group 1 vs 3 and group 2 vs 3. Table: 6 & 7 and Graph: 5 shows mean value ± SD and comparison of mean value of Ki 67 distribution in the OPMD grades mild, moderate and severe. Mean value and SD as followed in each group (1) mild: 40.88±±3.81 (2) moderate: 62.800±4.47 (3) sever: 70.00±2.49 with the respected minimum and maximum value of each group: 36.00 & 44.700, 60.30 & 70.70 and 67.30 & 74.30. In table:7 shows pair wise comparison using turkey's post hoc tests shows the significant differences between mild vs moderate, mild vs severe and moderate vs severe group of OPMD.

Table:8 Comparison of mean Ki67 expression per Field within grades of OSCC

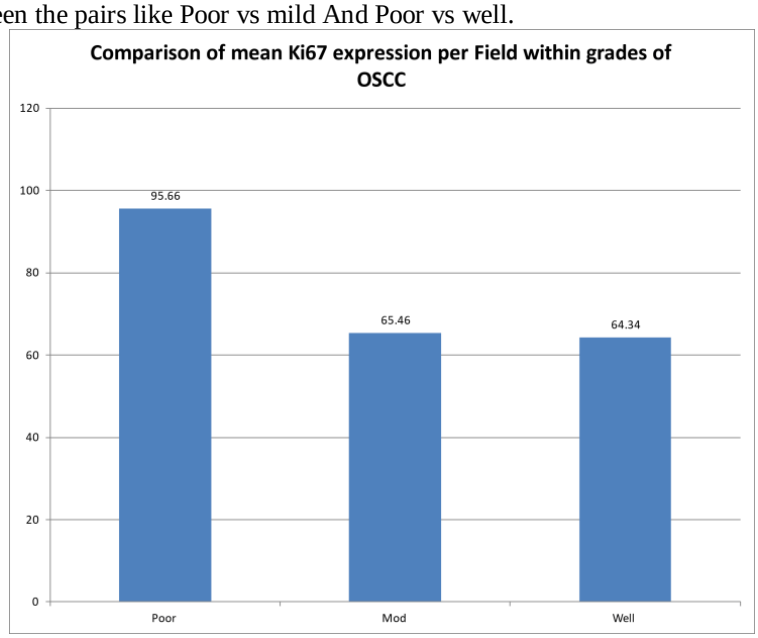
	N	Mean	Std. Deviation	Std. Error	Minimum	Maximum	F value	p value of one way ANOVA
Poor	5	95.660000	6.6387499	2.9689392	86.0000	101.3000		
Mod	5	65.460000	1.3240091	.5921149	63.7000	67.3000	98.373	.000**
Well	5	64.340000	1.5208550	.6801470	62.4000	65.7000		
Total	15	75.153333	15.467979	3.9938151	62.4000	101.3000		

There was a statistically significant / highly significant difference seen for the values between the groups ($p<0.01, 0.05$) with higher values in poor and least in well

Table: 9 Pair wise comparison using Tukey's Post Hoc Tests

(I) HP	(J) HP	Mean Difference (I-J)	Std. Error	Sig.
Poor	Mod	30.2000000*	2.5334825	.000**
Poor	Well	31.3200000*	2.5334825	.000**
Mod	Well	1.1200000	2.5334825	.899#

There was a statistically significant / highly significant difference seen for the values between the groups ($p<0.01, 0.05$) between the pairs like Poor vs mild And Poor vs well.



Graph: 6 Comparison of mean ki67 expression per field within grades of OSCC

Table: 8 & 9 and graph: 6 shows mean value ± SD and comparison of mean value of Ki 67 distribution in the OSCC grades well, moderate and poorly. mean value and SD as followed in each group (1) well: 64.34±1.52 (2) moderate: 65.460±1.32 (3) poorly: 95.66±6.69 with the respected minimum and maximum value of each group: 62.40 &65.700, 63.70 & 67.30 and 86.30 & 101.30. In table: 9 shows pair wise comparison using turkey's post hoc tests shows the significant differences between well vs moderate, well vs poorly and moderate vs poorly group of OSCC.

IV. Discussion

Oral mucosa is made up of stratified squamous epithelium; the stratification is the result of cell proliferation and sequential differentiation. The transition of the normal oral epithelium to dysplasia and to malignancy is featured by increased cell Proliferation and differentiation are controlled by autocrine and paracrine factors generated by the keratinocytes; the cytokines and growth factors originating in the underlying connective tissue and the circulating systemic factors.[13,14] The control on cell proliferation is thought to be impeded in cancer, and many studies have reported that abnormal cell proliferation appears to be a precursor as well as a predictor of tumorigenesis.[4,15]

Cell proliferation markers play an important role in the biological behavior of neoplasms and discovery of various proliferation markers has enabled the detection of the hyperactive state of the epithelium and has been

suggested to be of prognostic significance. Immunostaining with antibodies to Ki-67 antigen is well established as a quick and efficient method for evaluating growth fractions of various tumor types because of its distinctive reaction patterns that exclusively involves the proliferating cells.[16]

The expression of Ki-67 is higher in to severity of disease or increasing in dysplastic changes so it can be used as a prognostic marker.[17] In this study, the expression of the cell cycle-associated protein Ki-67 was examined by immunohisto- chemistry in oral epithelial dysplasia (OPMD)Fig: 6,7 & 8, oral squamous cell carcinomas (OSCC) Fig: 9,10,11 &12, normal oral epithelium (NOE) as a control Fig: 5. this study also reviews the significance of ‹Ki-67 protein as markers in diagnosis and prognostic assessment of severity of OPMD and histological grades of OSCC.

The basal cell layer is the proliferative compartment of normal oral epithelium, but increasing levels of dysplasia may be recognized by the presence of proliferating cells in both suprabasal and superficial cell strata. Ki-67 as a marker for cell proliferation is used in histopathological evaluation of patients with both premalignant and malignant diseases. Expression pattern and intensity of Ki-67 have been correlated to grade of dysplasia in Oral Leukoplakia and cancer transformation (Kushner *et al.* 1997). [4]

The mean value and standard of deviation of Ki-67 protein was found in normal oral epithelium 30.11±1.93, while in orally potential malignant disorders 58.09± 13.463 and according to grading histologically with mild, moderate and severe respected mean value and standard of deviation as followed 40.88±3.81, 62.80±4.47 and 70.60±2.49. In OSCC 75.15±15.716 also graded histologically in well, moderate and poorly differentiated carcinoma with as followed mean value and standard of deviation are 64.34±1.52,65.46±1.32 and 95.66±6.63. so, it is clearly showing that expression of ki-67 is increasing from normal mucosa-epithelia dysplasia-oral squamous cell carcinoma as well as in grades of OED & OSCC. these similar findings were found in study (Donia SD, et al. 2011). [7,13,18]

In this study also measured various criteria except Ki 67, like age group, sex, most common site of the lesion, Comparison of frequencies of gross morphology and social habit of the patients. So according to result patients mean age group ± standard of deviation is 50.13±17.144 with minimum age of patient 20 years while maximum age of patients 85 years. Distribution of patients as per sex number of patients male and female respectively 25 &15 while according to percentagewise 62.5% male and 37.5% female There were no significant differences between groups by age, sex, and site. These similar finding are found in the study (Kumar V, et al. 2015).[19,20] In this study the most common site affected by the lesion is buccal mucosa of the oral cavity and compared the frequencies of gross morphology of lesion in affected individuals. So most common morphological lesions were found ulcerative proliferative in nature in both epithelial dysplasia and oral squamous cell carcinoma. According to result most common social habit of tobacco in the form of chewing and smocking.

The high expression of the Ki-67 protein in oral squamous cell carcinoma tissues may play an important role in the development of oral squamous cell carcinoma in our study similar result are found which is done by previous study done (Takkem A *et.*, al 2018).[11] Ki-67 protein increase with decreasing tissue differentiation of OPMD and OSCC. The difference between OPMD and OSCC was not so significant between the moderate and severe epithelial dysplasia and oral cancer. There by signifying the fact that dysplastic epithelium holds a high potential for malignant transformation.

V. Conclusion

Expression of Ki-67 protein in all proliferating cells because of that have prognostic value in early diagnosis severity of epithelial dysplasia and cancers, a potential therapeutic target in cancer, and strategies that inactivate Ki-67 protein are a promising anti-proliferative approach, with potential applicability in cancer treatment. In conclusion, the cell proliferation in Oral epithelial dysplasia and oral squamous cell carcinomas can be determined by its growth rate using anti-Ki-67 monoclonal antibody. The nuclear protein Ki-67 is an established early prognostic and predictive marker for the assessment of biopsies from patients with oral epithelial dysplasia and oral squamous cell carcinoma.

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