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Original Research Article

The Study of Expression of Ki67 as Proliferative Index in Premalignant and Malignant Squamous Cell Lesions of Oral Cavity at Tertiary Health Care Center in Chhattisgarh

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Abstract:

Introduction: Oral cavity is significantly more prone to an enormous number of environmental insults because of its exposure to the external environment and making it common site for many tumors and tumor like conditions. The expression of Ki67 correlates well with the disease progression from dysplasia to carcinoma of oral cavity. It is therefore marker of malignant transformation and carcinogenesis in oral premalignant lesions and in future it may serve as prognostic tool in early detection of malignancy. The aim of this study is to identify an association between Ki67 protein expression and histological grades of Oral Epithelial Dysplasia - Oral Squamous Cell Carcinomas, and the role of Ki67 protein in the prognostic index of different histological grades of Oral Epithelial Dysplasia and Oral Squamous Cell Carcinomas.

Material and Methods: The present Observational and Prospective study was to examine the Expression of Ki67 as Proliferative Index in Malignant and Premalignant Squamous Cell Lesions of Oral Cavity. This study conducted for 2 years (June 2022 –May 2024) and all biopsies reported as premalignant or malignant squamous cell lesions of oral cavity from patients in all age groups were included in the study. The relationship between various parameters such as Age, Gender, Addiction history, Histologic type, Histologic grade and the expression of Ki67 index were studied and Proliferation Index was calculated.

Results: In the present study total 62 biopsies of oral lesions were studied. It was observed that most common lesion among Oral Epithelial Dysplasia was Moderate Dysplasia (12.9%) and among Oral Squamous Cell Carcinoma was Well Differentiated Squamous Cell Carcinoma [WDSCC] (41.9%). Ki67 Proliferative Index (PI) was categorized into Low, Moderate and High proliferative Index (PI). It was observed that most cases were showing Mild proliferation in 29 cases (46.8%), Moderate proliferation in 20 cases (32.3%) and High proliferation was seen in only 13 cases (21%).

Conclusion: It was concluded in present study that Ki67 has an Inverse correlation between the degree of tumour differentiation and rate of cell proliferation. Ki67 antigen can be used as a marker for the histological reviewing of Oral Epithelial Dysplasia (OED) and Oral Squamous Cell Carcinoma (OSCC). Ki67 expression expands with the severity of Epithelial Dysplasia and it was found that expression of Ki67 is significantly higher in tissues with Poorly Differentiated Squamous Cell Carcinoma and Severe Oral Epithelial Dysplasia.

Keywords: Oral cavity, Squamous Cell Carcinoma, Expression of Ki67, Dysplasia.

Introduction

Oral cavity is significantly more prone to an enormous number of environmental insults because of its exposure to the external environment and making it common site for many tumors and tumor like conditions.[1] Oral cancer is the most common type of cancer in India in men and actually

accounted for 40% of all forms of cancers. In males, oral cancers represent 4% of total body cancers whereas in females in India 2% of all cancers are of oral cavity. [2] Ki67 is strongly associated with cell proliferation and is widely used in routine pathology. [3] The fraction of positive cells is often correlated with clinical course of the disease, and has been extensively examined in Oral Epithelial Dysplasia (OED) and Oral Squamous Cell Carcinoma (OSCC). [4] The expression of Ki67 correlates well with the disease progression from dysplasia to carcinoma of oral cavity. It is therefore marker of malignant transformation and carcinogenesis in oral premalignant lesions and in future it may serve as prognostic tool in early detection of malignancy.[5]The aim of this study is to identify an association between Ki67 protein expression and histological grades of Oral Epithelial Dysplasia - Oral Squamous Cell Carcinomas, and the role of Ki67 protein in the prognostic index of different histological grades of Oral Epithelial Dysplasia and Oral Squamous Cell Carcinomas.

Material and Methods

The present Observational and Prospective study entitled "To Study the Expression of Ki67 as Proliferative Index in Malignant and Premalignant Squamous Cell Lesions of Oral Cavity at Tertiary Health Center in Chhattisgarh" had been conducted in the Department of Pathology, Pt. Jawahar Lal Nehru Memorial Medical College, Raipur, Chhattisgarh and associated Dr. Bhimrao Ambedkar Memorial Hospital, Raipur, Chhattisgarh. Approval from the Institutional Ethics and Scientific Committees had been obtained. This study conducted for 2 years (June 2022 –May 2024) and all biopsies reported as premalignant or malignant squamous cell lesions of oral cavity from patients in all age groups were included in the study. All the patients on Neoadjuvant Chemotherapy or Radiotherapy and inadequate or poorly preserved samples were excluded from the study. All 62 biopsies of oral lesions were subjected for routine Histopathological Examination. The specimens were checked for Labelling and Patient's information including patient Name, Age, Hospital Registration Number, Date of sampling, Date of receiving of samples and Proper fixation of the specimen were verified. Proforma was filled including the patient's name, age, residential address and contact number, history of present and past illness, duration of illness, family history, clinical details, and investigation. Thereafter lesions were subjected to routine Histopathological Process, slides were prepared, examined under microscope and diagnosed as pre-malignant or malignant lesions and further subjected to the Immunohistochemistry (IHC) for Ki67 to know the expression. The relationship between various parameters such as Age, Gender, Addiction history, Histologic type, Histologic grade and the expression of Ki67 index were studied and Proliferation Index was calculated.

Results

In the present study total 62 biopsies of oral lesions were studied. The mean age of study population was 45.74+13.72 years. Most of them belonged to 30-40 Years (29%) and 40-50 Years (24.2%) and there were 44 (71%) Males and 18 (29%) Females in study group. Out of 18 cases of Oral Epithelial Dysplasia - 13 were Males and only 5 were Females whereas among Oral Squamous Cell Carcinoma - 28 were Males and 13 were Females. Overall Male Predominance was observed in present study. Most Common form of addiction observed in present study was Tobacco Chewing in 36 cases (58.1%). Most common site of involvement was Buccal Mucosa (35.5%) followed by Tongue (24.2%) and Gingiva (14.6%). Lips are involved in 12.9% whereas Alveolus and Palate are involved in 8.1% and 4.8% respectively.

A total of 66.1% of study group had Oral Squamous Cell Carcinoma; 29 % had Oral Epithelial Dysplasia and only 4.8% had Normal Oral Mucosa. This shows advanced presentation of cases in this area. It was observed that most common lesion among Oral Epithelial Dysplasia was Moderate Dysplasia (12.9%) and among Oral Squamous Cell Carcinoma was Well Differentiated Squamous Cell Carcinoma [WDSCC] (41.9%). Ki67 Proliferative Index (PI) was categorized into Low, Moderate and High proliferative Index (PI). It was observed that most cases were showing Mild proliferation in 29 cases (46.8%), Moderate proliferation in 20 cases (32.3%) and High proliferation was seen in only 13 cases (21%).

Table 1: Ki67 Expression in Oral Epithelial Dysplasia (OED) and Oral Squamous Cell Carcinoma (OSCC)

Category		N	Proliferation Index		
			Low	Moderate	High
Normal Oral Mucosa		3	3	0	0
Oral Epithelial Dysplasia (OED)	Mild Dysplasia	7	7	0	0
	Moderate Dysplasia	8	7	1	0
	Severe Dysplasia	3	2	1	0
Oral Squamous Cell Carcinoma (OSCC)	Well-Differentiated	26	9	11	6
	Moderately Differentiated	12	1	7	4
	Poorly Differentiated	3	0	0	3
Total		62	29	20	13

Table 1 showed Expression of Ki67. The samples from Normal Oral Mucosa (NOM) expectedly showed Low Proliferative Index. Similarly in OED most cases were of Moderate Dysplasia (7 out of 8) showing Low Proliferative Index. In Well-Differentiated Squamous Cell Carcinoma most cases (11 out of 26) were of Moderate Proliferative Index and similar findings were seen in Moderately differentiated squamous cell carcinoma (7 out of 12). However, all 3 cases of Poorly Differentiated Squamous Cell Carcinoma (PDSCC) showed high Proliferative Index.(Photomicrograph 1,2,3,4) Ki67 scores of mild and severe Oral Epithelial Dysplasia (OED) is significantly different (p=0.011) but there is no difference between mild and moderate Oral Epithelial Dysplasia (OED) (p=0.063) and moderate and severe Oral Epithelial Dysplasia (OED) (0.173).

Table 2. Mean Ki67 labelling score in all Cases

Group	Category	N=62	Mean	SD	p value
Normal Oral Mucosa (NOM)		3	10.00	0.00	<0.001**
Oral Epithelial Dysplasia (OED)	Mild Dysplasia	7	12.85	3.93	
	Moderate Dysplasia	8	20.00	8.45	
	Severe Dysplasia	3	26.66	7.63	
Oral Squamous Cell Carcinoma (OSCC)	Well Differentiated OSCC	26	43.07	22.71	
	Moderately Differentiated OSCC	12	47.91	12.14	
	Poorly Differentiated OSCC	3	76.66	7.63	

One Way ANOVA; **p<0.001 highly significant

Table 2 showed there was a highly significant difference (p<0.001) between Ki67 labelling score among the different categories of OED. The highest score was of severe OED followed by moderate and mild. Among the OSCC, the highest score was for poorly differentiated OSCC (76.66±7.63) followed by MDSCC > WDSCC.

Discussion

The present study included all biopsies reported as Normal, Premalignant and Malignant Squamous cell lesion of oral cavity in all age groups, coming to the Histopathology Laboratory of the Department of Pathology and reviewed microscopically for histomorphological features and Immunohistochemistry (IHC) for Ki67 labelling. Majority of oral cavity lesions are asymptomatic therefore it is difficult to diagnose clinically and need Histopathological evaluation for malignancy. We studied oral cavity lesions with its demographic profile, histopathological evaluation and Ki67 Proliferative Index in cases of Normal Oral Mucosa (NOM), Oral Epithelial Dysplasia (OED) and Oral Squamous Cell Carcinoma (OSCC).The observations of the present study were compared and correlated with recently published literature on certain relevant parameters. In the present study, the patients' age ranged from 11 years to 80 years. Out of 62 cases, most cases were in the age group 30 years to 40 years (29%)

Our study was in concordance with studies by Maheshwari V et al (2013) [6] and Verma S et al (2017) [7] all of whom reported maximum frequency in the 31-60 years age group. A total of 62 cases were included in the study in which 44 (71%) were males and 18 (29%) were female, displaying male predominance. Our study was in concordance with Maheshwari V et al (2013) [6] which observed 83% males and 17% Females considering 65 cases. The study yielded results similar to studies by Gupta M et al (2016) [8] and Tomar A et al (2021) [9]. Other studies by Verma S et al (2017) [7], Patro P et al (2020) [10], Kumar P et al (2020) [11], Chandrakanta et al (2021) [12] and Jha M et al (2023) [13] also shows male predominance. In present study articulation of Ki67 in normal oral epithelium was restricted mainly in the basal layers of epithelium. In case of epithelial dysplasia expression was limited to basal as well as parabasal and spinous layers of epithelium and its expression increases with the severity of Dysplasia. In mild OED the maximum Ki67 expression was located at basal and parabasal layers of epithelium and showed least expression. It was observed that there is no statistical significance between mild oral epithelial dysplasia (OED) and Normal oral epithelium (NOE), thus we conclude that it is difficult to predict prognosis of mild OED lesions as it has proliferative activity same as that of NOE. In cases of Moderate and Severe dysplasia Ki67 expression was located at basal, parabasal and some of the spinous layers of epithelium.

In cases of Well Differentiated Squamous cell carcinoma, Ki67 positive cells were located mainly in the periphery of tumor nests. This suggest that less differentiated cells located in the peripheral layer and cells in the center are highly differentiated keratinization ability, so no Ki67 expression was seen in central part of tumor. In cases of moderately differentiated Squamous cell carcinoma positive cells were located in periphery as well as most of the

center of tumor nests and the overall staining of Ki67 in MDSCC was more quantitative than WDSCC. However, Ki67 expression was Diffuse in most cases of poorly differentiated Squamous cell carcinoma. All the above findings were in accordance with the study done by Takkem A et al (2018) [14], Dash K C et al (2022) [15], Singh A et al (2023) [16] and Birajdar S S et al (2014) [17].(Table 3)

Table 3: Comparison of Mean Ki67 Labelling Score in Normal Oral Epithelium (NOE), Oral Epithelial Dysplasia (OED) and Oral Squamous Cell Carcinoma (OSCC)

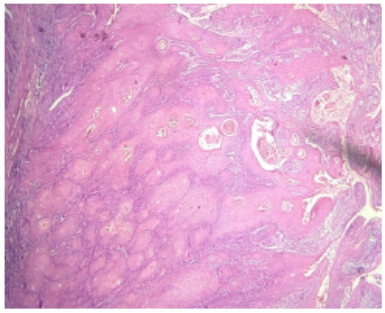
Study	Mean Ki67 labelling Score				
	NOE	OED	OSCC	P-value	Significance
Reddy V M et al (2010) [18]	8.47±1.586	25.5±3.01	29.7±3.04	>0.05	Not significant
Birajdar S S et al (2014) [17]	62.34±26.49	108.02±60.76	71.45±31.11	0.002	Significant
Takkem A et al (2018) [14]	10.6±3.41	36.83±19.56	42.87±17.99	<0.00001	Significant
Dash K C et al (2022) [15]	12.56±6.9	28.24±13.2	40.18±15.9	0.00	Significant
Present study (2024)	10±0.00	18.33±8.22	46.95±21.03	<0.001	Significant

In present study statistical comparison of Ki67 protein expression in Normal Oral Epithelium, Oral Epithelial Dysplasia and Oral Squamous Cell Carcinoma revealed a significant difference between three groups with a p-value of <0.001, which is in concordance with the study done by Birajdar S S et al (2014) [17], Takkem A et al (2018) [14] and Dash K C et al (2022) [15]. Our study was in discordance with a study by Reddy V M et al (2010) [18] which shows no significant difference between three groups with a p-value of >0.05.In present study statistical analysis between Normal Oral Epithelium and Oral epithelial dysplasia revealed Insignificant difference which is similar to study done by Reddy V M et al (2010) [18].Other similar studies done by Birajdar S S et al (2014) [17] and Takkem A et al (2018) [14] shows significant difference between Normal Oral Epithelium and Oral epithelial dysplasia which is in discordance with the present study. In present study statistical analysis between Normal oral epithelium and oral squamous cell carcinoma revealed significant difference which is similar to study done by Takkem A et al (2018) [14].

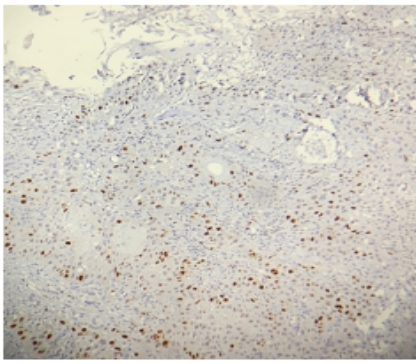
Table 4: Comparison of Mean Ki67 labelling Score in Normal oral epithelium (NOE) and Oral Epithelial Dysplasia (OED)

Study	Mean Ki67 labelling Score			
	NOE	OED	P-value	Significance
Reddy V M et al (2010) [18]	8.47±1.586	25.5±3.01	>0.05	Not significant
Birajdar S S et al (2014) [17]	62.34±26.49	108.02±60.76	<0.001	Significant
Takkem A et al (2018)[14]	10.6±3.41	36.83±19.56	0	Significant
Present study (2024)	10±0.00	18.33±8.22	0.458	Not significant

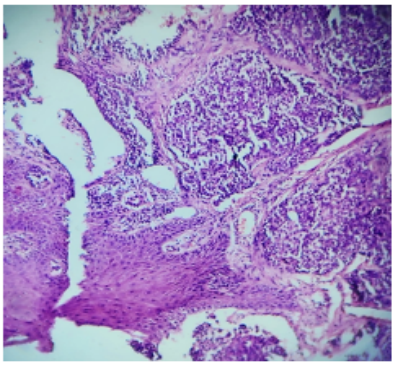
(Table 4) Other similar study done by Birajdar S S et al (2014) [17] which shows insignificant difference between Normal Oral Epithelium and Oral Squamous Cell Carcinoma which is in discordance with the present study. There was a highly significant difference (p<0.001) between Ki67 Labelling Score among the different categories of OED. The highest score was of severe dysplasia and among the OSCC, the highest score was for poorly differentiated OSCC (76.66+7.63) followed by MDSCC. The high expression of Ki67 in plays an important role in development of oral squamous cell carcinoma.



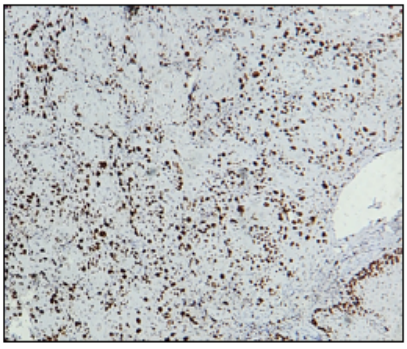
Photomicrograph 1 showed Well-Differentiated Squamous cell carcinoma(H&E, 100X)



Photomicrograph 2 showed low proliferation activity of Ki67 (IHC- Ki67, 100X)



Photomicrograph 3 showed Poorly Differentiated Squamous cell carcinoma(H&E, 100X)



Photomicrograph 4 showed high proliferation activity of Ki67 in cases of PDSCC (IHC- Ki67, 100X)

Conclusion

Ki67 proliferation marker has been recognized as an important predictive and prognostic marker in many studies. It was concluded in present study that Ki67 has an Inverse correlation between the degree of tumour differentiation and rate of cell proliferation. Ki67 antigen can be used as a marker for the histological reviewing of Oral Epithelial Dysplasia (OED) and Oral Squamous Cell Carcinoma (OSCC). Ki67 expression expands with the severity of Epithelial Dysplasia and it was found that expression of Ki67 is significantly higher in tissues with Poorly Differentiated Squamous Cell Carcinoma and Severe Oral Epithelial Dysplasia. Finally, we can say that Ki67 level may be considered a valuable biomarker in oral cavity lesions. Ki67 being an independent prognostic factor should be evaluated routinely in oral lesions.

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