ORIGINAL RESEARCH PAPER
Volume - 12 | Issue - 05 | May - 2023 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

CAN KI-67 PREDICT MALIGNANT AND PRE-MALIGNANT ORAL LESION? : AN EXPERIENCE IN A TERTIARY CARE HOSPITAL IN THE CENTRAL INDIA.

Histopathology

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ABSTRACT

Background: Oral cancer ranks the sixth commonest cancer worldwide and the third most common cancer in India. Ki67 index has been suggested to play an important role in early diagnosis and prognosis of potentially malignant disorders. This study aims to understand prognostic role of Ki 67 expression in different categories of oral mucosal neoplastic lesions in Central India, where tobacco chewing is endemic. This could help in early categorization and effective management of patients. **Aims:** To study the Ki67 expression in terms of Index of Positivity and Intensity score in oral mucosal neoplastic lesions. **Setting and Design:** Department of Pathology, Tertiary Care Hospital Observational & Prospective study **Methods & Material:** The expression of Ki-67was studied in paraffin- embedded tissue sections of histopathology proven pre- malignant and malignant lesions of oral cavity in ninety four cases. **Results:** Association of Ki67 Expression Intensity score with the type of lesion was found to be significant in premalignant and malignant lesions predominantly (p= <0.001).Similarly, Ki67 Index of positivity score was highly statistically significant (p= <0.001) in association with the type of lesion. **Conclusions:** the expression of Ki-67 correlates well with the disease progression from dysplasia to carcinoma of the oral cavity. It is therefore a marker of malignant transformation and carcinogenesis in oral premalignant lesions and in future it may serve as a prognostic tool in the early detection of malignancy.

KEYWORDS

Ki67 index, oral mucosal neoplastic lesions, malignant, premalignant lesion.

INTRODUCTION

Squamous carcinomas represent about 3% of human cancers and over 90% of malignant tumours with oral location.[1]The problem is even alarming in central Indian regions like Bhopal due to high prevalence of tobacco usage in this population.[2] It is strongly associated with tobacco consumption, betel quid chewing, alcohol consumption and poor oral hygien.[3,4]

Understanding the markers for predicting degree of dysplasia and progression to malignancy can help early identification and prompt treatment of patients with oral cancers.

Histopathology is taken into account as gold standard for diagnosis and management of oral lesions.

The most common immunohistochemical cell proliferation marker is Ki-67 antigen. It's a nuclear protein expressed in the G2 and M- phases of actively dividing cells and play a pivotal role in cell proliferation[5,6] Ki-67-positive cells are often correlated with the clinical course of the disease. The percentage of cells in a tissue stained for Ki-67, indicates the growth fraction.It provides significant information about the degree of aggressiveness and prognosis of the disease. Hence, this study is being conducted to find expression of Ki-67 in oral neoplastic lesions.[6]

Methods

The present study was carried out on the patients admitted or attending the Oncology department for some lesion in oral cavity . A concise history, clinical examination, including local examination of oral lesion was carried out. After receiving such specimen and biopsy in pathology department , histopathology of paraffin-embedded section of these lesions were done using hematoxylin and eosin (H&E) stain.

Small biopsies with insufficient tissue and autolysed bits were excluded. Total 94 lesions were studied. All lesions were graded according to Broder's Grading systemand classified based on WHO system for tumour classification.

Expression of Ki-67 was studied on these sections and it was correlated with the clinical staging and histological grading of the lesion. Only strong brown nuclear staining of epithelial cell was considered positive. Those histological sections with uniform and good intensity staining were assessed for scoring. The section stained for Ki-67 proliferation were evaluated using scores from 0 to 2.

An index of positivity (IP)was calculated which will represent the percentage of labeled cells in 1000 counted cells under the 40× microscope field. Following scoring were done.
Score 0: <10%,
Score 1: 10–50%,
Score 2: >50%.

Intensity of staining was scored as:

- Score 0: Weak
- Score 1: Moderate
- Score 2: Strong

Two pathologists reported the slides from same areas independent of each other to overcome inter and intra observer bias. Ki 67 expression was also observed in the epithelial layers- basal, parabasal and suprabasal layers. Expression of protein Ki-67 was examined by IHC in oral epithelial dysplasia, oral squamous cell carcinoma and normal oral epithelium as a control.

Data collection and analysis was done in Excel sheet version 2016 and SPSS version 25.

RESULTS

The mean age of study participants was 48.03 ± 2.05 years. Male (77.7%) dominance was observed as compared to females (22.3%) among the participants.

45.7% of participants presented the lesion on buccal mucosa, followed by tongue with 31.9% involvement and combination of gingivo-buccal sulcus with buccal cavity accounting 9.6% site involvement. Majority (35.1%) of study participants presented with exophytic ulceroproliferative fungating growth. This was followed by clinical presentation of endophytic ulcerated growth (25.5%), white patch (19.1%), swelling (9.6%), verrucous growth (6.4%) and reddish patch (4.3%).

Among 94 study participants, it was found that majority i.e., 29.8% were diagnosed on H &E-stained section as Moderately Differentiated Squamous cell carcinoma (SCC), 12.8 % Well differentiated SCC,9.6% Poorly differentiated SCC and 6.4% Verrucous Carcinoma Premalignant lesions - 6.4% OIN I (Oral Intraepithelial Neoplasia I)showing mild degree of dysplasia, 5.3% OIN III showing severe degree of dysplasia, 4.3% OIN II showing moderate degree of dysplasia and 25.6 %- Benign oral mucosal lesions.

On the basis of nature oflesion, 67% were malignant lesions followed by 17% as benign and 16% as pre- malignant lesions.

Association between Ki67 expression Intensity score and type of oral neoplastic lesion has been displayed in table 1.

Table 1 : Association Between Ki67 Expression And Type Of Oral Neoplastic Lesion (n=94)

Sl. No.	Category	Benign		Pre malignant		Malignant		Total	
		n=16	%	n=15	%	n=63	%	n=94	%
A	Ki67 Expression Intensity Score								
1	0	15	15.9	1	1.1	0	0	7	4.4
2	1	1	1.1	6	6.4	21	22.3	39	41.5
3	2	0	0	8	8.5	42	44.7	48	51.1
	Chi Square, p value	85.369, <0.001							
B	Ki67 Index Positivity Score								
1	0	14	14.8	1	1.1	0	0	6	6.4
2	1	2	2.2	9	9.6	14	14.9	31	33
3	2	0	0	5	5.3	49	52.1	57	60.6
	Chi Square, p value	85.369, <0.001							

Only one benign case showed intensityscoreof 1 staining with Ki-67 while other showed intensity score of 0. Only one premalignant case showed intensity score of 0 and all the malignant cases showed either 2 or 3 intensity score for Ki-67. Significant association was observed between type of lesion and Ki-67 Expression Intensity

Score with p value of<0.001. Among score 2 , majority (44.7%) lesion were found to be malignant followed by 8.5% premalignant and none of the benign lesions showed score 2. Table 1 also emphasizes on association between Ki67 Index Positivity Score and type of oral neoplastic lesion. Most of the benign cases showed score 0 while other two benign cases reported as score 1. Only one premalignant case reported as score 0 and all the malignant cases showed score 1 or 2 for index of positivity of Ki67 marker. Association between Ki-67 Expression Index of Positivity Score and type of lesion was found to be highly significant with chi square value of 85.369 (p<0.001).

None of the malignant cases showed score 0 for Ki-67 index positivity score. Table 2 depicts association between Ki67 expression Intensity score and Broder's Histological grade of differentiation which was found to be significant with the chi-square value 15.88 and p value <0.001. The result was significant. This table also displays association between Ki-67 Expression Index of Positivity Score and Broder's histological grade which was also found to be significant with chi square value of 10.786 (p = 0.005). The ki67 Index of positivity increased with less degree of differentiation. It is depicted in Table 2.

Table 2 : Association Between Ki67 Expression And Broder's Histological Grade Of Differentiation (n=63)

Sl. No.	Category	Grade I		Grade II		Grade III		Total	
		n=26	%	n=28	%	n=9	%	n=63	%
A	Ki67 Expression Intensity Score								
1	0	0	0	0	0	0	0	0	0
2	1	16	25.4	4	6.3	1	1.6	21	33.3
3	2	10	15.9	24	38.1	8	12.7	42	66.7
	Chi Square, p value	15.879, <0.001							
B	Ki67 Index Positivity Score								
1	0	0	0	0	0	0	0	0	0
2	1	11	17.5	3	4.8	0	0	14	22.2
3	2	15	23.8	25	39.7	9	14.3	49	77.8
	Chi Square, p value	10.786, 0.005							

DISCUSSION

The current study was done to assess if histological diagnostic modality can be aided with the proliferation index marker Ki-67 in predicting the evolution of benign into invasive carcinomas of the oral cavity and oropharynx and in grading the tumours.

In India and other Asian countries, oral and oropharyngeal carcinomas comprise up to half of all malignancies, with this particularly high prevalence being attributed to the influence of carcinogens and region-specific epidemiological factors, especially tobacco and betel quid chewing.[7]

In our study, 93% of the patients were under the influence of potential risk factors like tobacco, alcohol and betel nut. It is also a established association between these factors and oral cancer.[8]

The mean age of cases were 48.03 ± 2.05 years. In india oral squareoun cell carcinoma mainly belongs to the age group 41-50 year (30%) and with mean age of 48.4 yrs.[9,10] It is similar to our study. This forms the basis for the fact that there is a prospect of early intervention by early diagnosis and treatment to put a check on the progression to malignant lesions in third to fifth decades of life which are productive years of life.

In our study out of 94 cases, 73 were males (77.7%) and 21 were females (22.3%). Male: Female ratio was 3.5:1. Buccal mucosa was found to be the most common (45.7%) site of involvement. Most of the lesions (35.1%) were ulceroproliferative in appearance. Similar findings were seen in previous study in which Buccal mucosa (46%) and tongue (44%) were observed to be the most common sites. However many researcher found tongue (48.7%) and then buccal mucosa (20.5%) for its occurrence.[11,12] We found moderately differentiated squamous cell carcinoma as majority among all type of cases. But some studies found poorly differentiated SCC the most common grade of oral SCC.[13]

Increased cellular proliferation is associated with more advanced lesions and the distribution of proliferating cells in tissues may tell us more about the regulatory mechanisms that become dysfunctional during carcinogenesis.[14] Researcher has found Ki- 67 is good proliferation markers in comparison to others. P53 expression has low positive predictive value in this context. Ki-67 is not having such limitations, which makes it an individualistic proliferation marker it tallies with the existence as well as the virulence of dysplasia. It also express aggressiveness of a invasive squamous cell carcinoma (SCC). Apart from this Ki-6 activity also seen in neoplastic cells at the edge of infiltrating fronds and intensity of staining is mild to intense in basal layer, which makes it most promising prognostic marker.[5,15,16,17] Hyperactivity of cells is detected by Ki-67 in oral epithelial dysplasia but it is also comparable in terms of quantitative positivity for clinical course or prognostication of the disease.[18,19] This explains the role of Ki67 expression in oral cavity lesions.

In our study association of Ki67 Expression Intensity score with the type of lesion was found to be significant predominantly in premalignant and malignant lesions (p=< 0.001). Similarly, Ki67 Index of positivity score was highly statistically significant (p= < 0.001) in association with the type of lesion.

It is laso found in previous study that the expression of Ki67 was significantly higher in well differentiated carcinomas as compared to poorly differentiated carcinomas.[20] It is going against to our study observations. The survival time of these patients was influenced by clinical presentation, stages, and surgical treatment. Expression of Ki67 had no impact on survival.[21] In our study, Ki-67 expression was maximum in grade II and grade III comprising 67.0% of malignant lesions.

The immunohistochemical expression of Ki67, in precancerous lesions and oral carcinoma showed promising results in one study. It showed Immunohistochemical expression of Ki67 was a raised expression with the increasing degree of dysplasia. The expression of Ki67 was significant in differentiating normal mucosa and mild dysplasia from moderate and severe dysplasia.[22,23]

There is also significantly higher proportions of cases (66.3%) displayed positive immune-expression among malignant cases, (Chi-square – 15.4; P = 0.004).[24] Ki-67 intensity scores followed similar trend what we observed in our study. It also shows a strong intensity of staining with significantly higher number of malignant cases (60.2%) than premalignant cases (36.8%) and benign cases (10%) respectively (Chi-square – 25.0; P = 0.0001). [24]

The increased expression of Ki67 occur with the decrease of the degree of tumoral differentiation [more towards poorly differentiated][25]. It is again similar to our observations.

Similar to our study Ki-67 labelling index was observed to be significantly correlated with the increasing histological grades of Oral

squamous cell carcinoma with p-value 0.047 in a study .[9]

As observed in our study, which is similar with other study which showed significant difference between the expression of ki-76 in moderate, severe and normal oral epithelium, with P- valve of 0.000. The difference was not statistically significant between normal epithelial oral and mild oral dysplasia, concluding that the rate of malignant transformation is higher in lesions that demonstrate moderate to severe dysplasia.[22]

It is a generally increasing trend in the mean Ki-67 index with increasing Broder's grade. There was significant difference between the three groups with respect to the mean Ki-67 index.[26] It is similar to our results and findings.

The number of Ki67 labelled cells increased in basal layers of epithelium with increasing grade of dysplasia, up to the most superficial layer in OIN III i.e., carcinoma in situ. Thus, present study in accordance with other studies concludes that tumour aggressiveness in oral lesion can be assessed promisingly by Ki-67 indicator[16,25,27] and it plays important role in making strategies that inactivate Ki-67 protein for the management of oral cancer lesion treatment.[28,29]Also it was observed that Ki 67 expression raised not only in basal layer but also in suprabasal layers in pre malignant lesions. [19]Similar to our study, Other researches also observed expression of Ki-67 in oral squamous cell carcinoma which is high as compared to premalignant lesion, suggesting that Ki- 67 may be useful indicator of malignant transformation in dysplasticlesion.[30]

CONCLUSION

The cell proliferation index marker Ki-67 when expressed, could provide significant information about type of oral cavity lesion whether it is still benign/ premalignant or already progressed to malignancy as well as predicting the transformation from dysplasia into invasive carcinomas of the oral cavity.

Hence, Ki-67 expression would be useful as a prognostic indicator in determining the aggressiveness of the tumour. It is believed that the proliferation index of a lesion indicates its future behaviour pattern. Our study highlights the significance of Ki 67 in early categorization so that effective management can be set formalignant and premalignant oral neoplastic lesions.

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