A study of Ki 67, p53 and their combination as immunomarkers in squamous cell carcinoma of oral cavity

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1. Introduction

Oral cancer is the most common cancer and constitutes a major health problem in the world; being a major cause of death. Although representing 2-4% of the malignancies in the west, this carcinoma accounts for almost 40% of all cancers in the Indian subcontinent.1

In India, oral cavity cancers are the most common cancers in the males and third most common in females.2 Oral cavity includes lip, floor of the mouth, oral tongue(anterior 2/3), buccal mucosa, gingiva, retromolar trigone, hard palate, base of tongue, tonsillar area and soft palate. Squamous cell carcinoma represents 90% to 95% of the oral cavity malignant neoplasm. Most cases occur...
in men over the age of 50, although the relative incidence among women and younger patients seem to be increasing. Some cases have been documented in children, particularly in the tongue.3

The prognosis for patients with oral squamous cell carcinoma that is treated early is much better, with 5 year survival rates as high as 80%.

A significant proportion of oral squamous cell carcinoma develop from premalignant lesions such as leukoplakia and oral submucosal fibrosis. Histological examination of tissue remains the gold standard for diagnosis and identification of malignant oral lesions.1

Tobacco and alcohol remain the most important etiologic factors. Consumption of tobacco and betel quince can cause genetic and molecular alterations in clinically distinct oral premalignant lesions and conditions4

Infection by human papilloma virus (H.P.V.) and herpes simplex virus (H.S.V) are considered of high risk cancer development.5,6

Squamous cell carcinoma can be well differentiated (histological grade I) or moderately (histological grade II) or poorly differentiated (histological grade III). The staging of this neoplasia is done according to the TNM system of the international union against cancer with assessment of tumor size and lymph node involvement. The prognostic evaluation of this neoplasia is given by histological and immune histochemical finding, including staging, histological grade, tumor size, lymph node involvement, immune histochemical expression of p53 and Ki 67.7,8

Molecular level changes in the lesion occur before clinical and histopathological changes. Molecular biological markers have been suggested to be of value in the diagnosis and prognostic evaluation of the lesions. Markers of proliferation, epithelial differentiation and genomic markers could potentially be good candidates for improving the prognostic evaluation of precursors of oral cancer.

In recent years, the number of molecular based assays has increased but histopathology remains the gold standard for most diagnostic and therapeutic decisions. Tumor suppressor genes, oncogenes, cell proliferation markers, angiogenic markers and cell adhesions molecules have been studied as potential tools to predict the prognosis of patients with oral squamous cell carcinoma.9

The staging systems are mostly clinical staging, based on the best possible estimate of the extent of disease before first treatment. Imaging techniques like computed tomography, magnetic resonance imaging (MRI), positron emission tomography (PET), and ultrasonography may be utilized and, in advanced tumor stages, have added to the accuracy of primary tumour(T) and nodal (N)staging. Andiagnostic information that contributes to the overall accuracy of the pre-treatment assessment should be considered in clinical staging and treatment planning. When surgical treatment is carried out, cancer of the head and neck can be staged using all information available from clinical assessment, as well as from pathologic study of resected specimen.10

In squamous cell carcinoma of oral cavity, p53 and Ki-67 are related to large tumors metastatic to lymph nodes and poor prognosis.11

p 53 is known as the "guardian of genome" is a tumor suppressor gene. P53 assists in DNA repair by arresting cell cycle in G1 phase and inducing the DNA repair genes. A cell with damaged DNA that cannot be repaired is directed by P53 to undergo apoptosis. With homozygous loss of P53, DNA damage goes unrepaired; mutations become fixed in dividing cells, and this leads to malignant transformation of the cell.12 Ki-67 is associated with cell proliferation. The role of Ki-67 as a prognostic marker in various neoplasms has been discussed widely in several studies, albeit with conflicting and debatable results.13

2. Materials and Methods

The prospective study was done in department of Pathology, N.I.M.S. medical college during the period of 2015 to 2019. The study was carried out with 108 cases of oral cavity squamous cell carcinoma with neck lymph node resection. Processing and staining of the specimen was done. The haematoxylin and eosin along with immune histochemically stained sections were examined. The histological grading of the tumors were done as well, moderate or poorly differentiated according to world health organization (3). As to lymph node involvement; the criteria used was the presence of metastasis seen on microscopy. The cases were broken down into two groups: 1-Cases with negative metastatic lymph nodes and 2: cases with positive metastatic lymph nodes. As to the histological grade of the neoplasia, the cases were broken down into two groups: 1 Low grade (including grade I/well differentiated) and (2) high grade (including grades II and III / moderately and with little differentiation). As to pathological staging (pT) of the neoplasia, the cases were broken down into two groups: (1) lesions with local involvement (including pT1, p T2, pT3) and (2) lesions involving neighbouring structures (pT4).

All the immunostained sections of different grades are to be scanned randomly at 100 x magnification for the most densely labelled areas. Then nuclear counting is performed at 400x. A total of 1000 nuclei are counted in the most densely labelled microscopic fields. Counting is carried out in a systemic manner using a grid by keeping in mind not to repeat tumor nuclei while counting. Vascular endothelial cells, lymphocytic cells and necrotic areas were excluded from counts. The MIB –1 and p53 labelling indices were expressed as the percentage of MIB -1 and P53 immunolabelled tumor nuclei per 1000 tumor nuclei counted. The following quantification was used.11

Negative expression: 0 to 09% of the neoplastic cells having antibody expression and the Positive expression:10 to 100% of neoplastic cells having antibody expression.
3. Results

The maximum number of cases was found to be in the age group of 40-60 years (61.8%), mostly being men (85.1%). Female comprised of only 14.9% of the patients.

Maximum number (92.6%) of the patient’s chewed tobacco, followed by smoking (77.78%) and alcohol drinking (55.56%). The most common site of cancer was buccal mucosa (53.7%).

All cases were subjected to immunohistochemistry for Ki 67 and p53. Positive expression of Ki 67 detected was in 92 cases and p53 was detected in 72 cases. Both Ki-67 and p53 was positive in 58 cases.

As to pathological staging, 92(85.2%) patients had tumors which did not invade adjacent structures (pT1, 2, 3) and 16 had tumors invade adjacent structures (pT4), corresponding to 14.8%. Out of non-invading 92 cases 60(65.2%) were positive for p53 and out of 16 invading cases 12 (75%) were positive for p53. While in histological grading 48(44.45%) were low grade and 60 (55.6%), were high grade. Out of 48 low grade cases 26(54.1%) cases and out of 60 high grade cases 46(76.6%) were positive for p53. Both pathological staging and histopathological grading did not show any significant statically p value.

Lymph node status was seen in all cases. Out of 108 cases, lymph node were positive in 48(44.4%) cases and 60(55.6%) cases were negative. Out of 48 lymph node positive cases, 40(83.3%) cases were positive for p53 and 8(16.7%) were negative for p53. Out of 60 lymph node negative cases, 32(53.3%) cases were p53 positive and remaining 28(46.7%) were p53 negative. The relationship of p53 expression was statistically significant when compared with lymph node status.

Ki-67 marker was tested in all 108 cases. Out of total 108 cases 92 (85.2%) were found to be positive for Ki-67 and only 16(14.8%) were found negative. Out of 48 low grade cases 14(29.2%) cases and out of 60 high grade cases 44(73.3%) were positive for p53 and Ki 67 which was statistically significant. The relationship of p53 and Ki-67 expression was statistically significant when compared with lymph node status.

4. Discussion

Oral cavity and tongue carcinomas have been broadly studied and discussed. Oral squamous carcinomas, though its incidence and biological behaviour, continue to represent a health problem worldwide. Recent studies have clearly
Fig. 4: Distribution of variable in 108 cases by Ki67

Fig. 5: Pathological Staging and Ki-67 Status

Fig. 6: Both Ki-67 and p53 expression in relation to Histological grading

Fig. 7: Both Ki-67 and p53 expression in relation to Lymph-Node Status

Fig. 8: Both Ki-67 and p53 expression in relation to Pathological Staging

Fig. 9: Moderately differentiated squamous cell carcinoma of tongue (H&E, 100x)
shown the importance of immunohistochemistry in the evaluation of predictive values and prognosis of oral cavity squamous cell carcinomas. Although the lesions are easily accessible for clinical examination, their prognosis can be difficult to assess in the context to location variability, risk factors, histopathological and molecular aspects involved in their appearance and progression.

The study aims at assessing the p53 and Ki-67 immunohistochemical markers and to correlate the expression of these markers with other prognostic factors such as age, sex, lymph node status, histological staging and histological grading. In our study, the maximum number of cases was found to be in the age group of 40-60 years (61.8%), mostly being men (85.1%). Female comprised of only 14.9% of the patients. Thus the male to female ratio in our study is 5.7:1. Most number of cases were in the fourth
Male are affected more often than females because of heavier indulgence in both tobacco and alcohol habits in most countries. In India the highest rates of intraoral cancer may be found in women who chew tobacco heavily. High proportion of cases among males may be due to high prevalence of tobacco consumption habits in them, coupled with smoking whereas in our society females less commonly indulge in tobacco smoking.

In our study group, maximum number (92.6%) of the patients chewed to bacco, followed by smoking (77.78%) and alcohol drinking (55.56%). The most common site of cancer was buccal mucosa (53.7%) followed by tongue (29.63%) comparable to other studies. However it is mentioned in world health organization classification of tumors that the most sites vary geographically reflecting different risk factors.

In India, where tobacco chewing along with betel nuts consumption and reverse smoking (placing the lit end in the mouth) are practised, there is striking incidence of oral cancer.

The wild form of P53 plays an important role in arresting the cells with damaged DNA that pass from G1 phase to S phase of cellular cycle and in inducing their apoptosis, the alteration of the expression being documented in various localisations of malignant cells. The positivity percentage for p53 in squamous oral carcinomas varies in different studies from 0-100%, correlating to the severity of the injury. Some authors have shown its correlation with degree of differentiation (2), while others have refuted this association.

In our study p53 marker was done in all 54 cases. As to pathological staging 92(85.2%) patients had tumors which did not invade adjacent structures (pT1,2,3) and 16 had tumors invading adjacent structures (pT4), corresponding to 14.8%. Out of non invading 92 cases 60 (65.2%) were positive for p53. While in histological grading 48(44.4%) were low grade (well differentiated), and 60(55.6%), were high grade (moderately and poorly differentiated). Out of 48 low grade cases 26(54.1%) cases and out of 60 high grade cases 46(76.6%) were positive for p53. However both pathological and histological grading were not statistically significant when co related with p53 positivity. In our study, the marker showed expression in all layer of dysplastic epithelium adjacent to oral squamous cell carcinoma in 40% of cases. Also, the suprabasal expression of p53 is considered by some authors as being a risk factor for subsequent occurrence of epithelial dysplasia and oral squamous cell carcinoma as in other studies.

A significantly increased positivity with increasing tumor grade was noticed (24). This indicates a higher p53 expression indicates poorer prognosis. The relationship between p53 mutations and tumor grade has been evaluated in many studies.
Lymph node statuses were also seen in all cases. Out of total 108 cases, lymph node were positive in 48 (44.4%) cases, and 60 (55.6%) cases were negative for lymph node, and out of 48 positive cases, 40 (83.3%) cases are seen to be positive for p53. The relationship of p53 expression was statistically significant when compared with lymph node status in other study.11,25–27

Ki-67 marker is tested in all 108 cases. Out of total 108 cases 92 (85.2%) were found to be positive for Ki-67 and only 16 (14.8%) were found negative. As to the pathological staging 92 (85.2%) patients had tumors which did not involve adjacent structures (pT1,2,3) and 08 had tumors invading adjacent structures (pT4), corresponding to 14.8%. Out of non-invasive 92 cases 78 cases (84.8%) were positive for Ki-67 and out of 16 invading cases (87.5%) were positive for Ki-67. While in histological grading 48 (44.4%) were low grade (well differentiated), and 60 (55.6%), were high grade (moderately and poorly differentiated). Out of 48 (44.4%) low grade cases 34 (70.8%) cases and out of 60 (55.6%) high grade cases 58 (96.7%) were positive for Ki-67. Pathological staging did not show any statistical significance while histological grading show statically significant p value.

The Ki-67 immunoreaction in well differentiated oral squamous cell carcinoma revealed a medium PI-Ki-67 of 22%, in moderately differentiated oral squamous cell carcinoma was 32%, and a medium value of 53% in case of poorly differentiated in concordance with other study.27,86 In our study out of total 108 cases, lymph node were positive in 48 (44.4%) cases, and 60 (55.65) cases were negative, and out of 48 positive cases 42 (87.5%) cases were seen to be positive for Ki-67. The relationship of Ki-67 expression did not show statistically significant p value when compared with lymph node status.

Statically analysis of Ki-67 also carried out with other variables in our study and it did not prove to be significant when compared to age, sex, gender and tumor volume. The literature shows that the co expression of p53 and Ki-67 when compared to age, sex, gender and tumor volume showed statically significant when compared with lymph node status.

-67 expression did not show statically significant p value when compared with lymph node status.

-67 expression did not show statically significant p value when compared with histological differentiation. The co expression of p53 and Ki-67 was significantly associated with those cases with positive lymph node status and higher histological grading.

5. Conclusion
Oral squamous cell carcinoma is significantly associated with elderly age group and with males. Commonest site being buccal mucosa and is most commonly associated with tobacco chewing. p53 was significantly associated with cases with metastasis to neck lymph node an Ki-67 was statically significant when compared with histological differentiation. The co expression of p53 and Ki-67 was significantly associated with those cases with positive lymph node status and higher histological grading.

6. Source of Funding
None.

7. Conflict of Interest
None.

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Cite this article: Gupta S, Khatri SL, Deepak, Khatri J. A study of Ki 67, p53 and their combination as immunomarkers in squamous cell carcinoma of oral cavity. *IP J Diagn Pathol Oncol* 2020;5(2):179-186.