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Original Research Article

Immunohistochemical Expression of P53 and Ki-67 Antigen in Premalignant and Malignant Oral Lesions

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Abstract

Background: p53 protein is a product of TP53 tumor Suppressor gene. TP53 mutations leading to loss of function are the commonest type of genetic damage found in human cancers and oral squamous cell carcinomas, often preceding recognizable histological alterations. Ki-67 has been shown to be excellent for the estimation of the growth fraction in both normal and malignant human tissue and this antibody is now used as the usual standard for the assessment of cell proliferation. *Aim:* To study the expression of p53 and Ki-67 in premalignant and malignant lesions of oral cavity. *Material and Methods:* The immunohistochemical expression of Ki-67 and p53 was studied on 110 cases of premalignant and malignant lesions of oral cavity during a period from July 2017 to June 2018. *Results:* The immunohistochemical expression of Ki-67 and p53 showed similar trends, and increased with the degree of dysplasia. On analyzing statistically, a high significant association was found between p53 expression and higher grades of tumour differentiation. However expression of Ki67 did not show significant association with various grades of differentiation of oral squamous cell carcinoma. *Conclusion:* p53 and Ki-67 are useful biomarkers of malignant transformation in oral precancerous lesion and may serve as intermediate points for cancer prevention programmes.

Keywords: Ki-67; p53; premalignant; malignant; oral lesions

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Introduction

Carcinoma of the oral cavity is one of the most common cancer occurring worldwide and comprise for 90% of head and neck lesions [1]. Annually, 3,00,000 new cases arise globally,out of which 62% occur in developing countries with the Indian sub-continent accounting for one-third of the world burden [2]. Oral squamous cell carcinoma being most prevalent type of cancer represents about 91% of the diagnosed cases of malignant tumors of the mouth [3]. Many oral squamous cell carcinoma develop from premalignant conditions of the oral cavity, including leukoplakia, erythroplakia,

palatal lesions of reverse cigar smoking, oral lichen planus, oral submucous fibrosis, discoid lupus erythematosus and hereditary disorders such as dyskeratosis congenital and epidermolysis bullosa [4,5]. Studies have shown that about 80% of oral cancers were preceded by oral precancerous lesions [6]. Histological examination of tissue remains the gold standard for diagnosis and identification of malignant oral lesions. Immunohistochemical staining is widely used in diagnosis of abnormal cells such as those found in cancerous tumours [7]. Mutation in the p53 gene, one of the most common genetic alterations in human cancer, are implicated in tumorigenesis and tumour progression. A higher rate of p53 detection was observed among large tumours and in those with a prominent depth of invasion, lymphatic and vascular invasion and lymph node involvement. Prognosis was significantly worse for patients with p53 positive staining tumours. Ki-67 is a nuclear protein and is a excellent marker to determine the growth fraction of a given cell population. High Ki-67 is a sign of poor prognosis associated with a good chance of clinical response to chemotherapy. The aim of present study was to study immunoexpression of p53 and Ki-67 in benign, premalignant and malignant lesions of oral cavity.

Materials and Methods

The present study was conducted on 110 formalin fixed liquid paraffin sections from various oral lesions including benign, premalignant and malignant lesions in the department of pathology during a period of one year from July 2017 to June 2018. Informed consent was taken from all the patients. For histopathological examination, Haematoxylin and Eosin stained formalin fixed paraffin embedded tissue sections were examined, then the immunohistochemical expression of p53 and Ki-67 were analysed in all the section diagnosed histopathologically. For immunohistochemical analysis, sections were incubated using the avidin-biotin-peroxidase complex with the following antibodies: p53 (clone DO7, dilution 1:150; Neomarkers, Union City, CA, USA), Ki-67 (clone BGX-Ki67, dilution 1:50; Biogenex, The Hague, Neitherlands). For each antibody, positive and negative controls were used. Two independent observers (ED, FA) reviewed the immunohistochemically-stained sections. Statistical analysis was performed using the Chi-square test. The difference in values was considered significant when $p < 0.05$.

The intensity of immunohistochemical staining was 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 (++, dark brown color) or intense (+++, very dark brown color). The distribution of staining was graded as confined only to basal layer, both basal and supra basal layers, and all layers of the epithelium. Only nuclear staining of epithelial cells was observed, and the nuclei with clear brown color, regardless of staining intensity, were regarded as positive. The pattern of expression was also analysed semi quantitatively by counting the number of positive cells per 100 basal or parabasal cells and was recorded as percentage. The percentage of positive cells was scored as 0-5%; 6-25%; 26-60%; 61-99%. The percentage of positive cells for each slide in each group was calculated by dividing the number of positive cells by the total number of cells counted in the slides.

Results

Out of total 110 cases studied, 11cases (10%) were normal, 33 cases (30%) were premalignant and 66 cases (60%) were malignant. The age of the patients ranged from 21-84 years with mean age of 42.6 years with male to female ratio 3:1. Most of the cases belonged to rural area (70%) with a ratio of rural: urban 3:1. The most common site of involvement in premalignant lesions was buccal mucosa (54.55%) followed by tongue (21.21%). Among malignant lesions, the most common site of involvement was also buccal mucosa (56.06%) followed by tongue (36.37%). Most of the premalignant as well as malignant cases were associated with tobacco in one or in another form.

Out of 33 premalignant cases, the commonest lesion was leukoplakia (72.73%) followed by submucous fibrosis (27.27%). Dysplasia was observed in 20 cases (60.60%). These cases of squamous epithelial dysplasia were graded into mild, moderate and severe dysplasia as per WHO criteria. Commonest grade was mild dysplasia (30.30%) followed by moderate dysplasia (24.24%).

Out of total 66 malignant lesions, squamous cell carcinoma was the predominant histologic type, constituting 97% of all cases. Rest two cases were of verrucous carcinoma and adenoid cystic carcinoma each (1.5%). Out of 64 cases of squamous cell carcinoma, maximum cases (34 cases, 53.13%) were well differentiated squamous cell carcinoma.

On analyzing the expression of p53 in normal mucosa, most of the cases (81.81%) were negative. The expression was limited exclusively to the lower third of epithelium most of the cases. Among 33 premalignant cases, p53 expression was seen in 27 cases (75.75%). Expression of p53 was increased with the degree of dysplasia. Majority of non-dysplastic cases (61.53%) showed low immunostaining (6-25%), 3 cases showed moderate positivity while only 2 cases showed 0-5% positivity while 45% of dysplastic cases expressed low immunopositivity and moderate expression was seen in 6 cases (Fig. 1, Table 1).

The difference of p53 expression between normal and premalignant lesions was found to be highly significant statistically. ($p<0.001$) Among premalignant dysplastic cases, p53 expression showed significant correlation with degree of dysplasia ($p=0.030$). Out of 64 squamous cell carcinoma, the expression of p53 was found to be positive in 47 cases (73.43%). Immunoposivity was observed in all layers of epithelium as well as in infiltrated tumor cell nests. On analyzing the percentage positivity of p53 in various grades of differentiation of squamous cell carcinoma, p53 was more expressed in moderately to poorly differentiated than well differentiated squamous cell carcinoma. (Figs. 2,3) Immunohistochemical expression of p53 was increased as the degree of severity of lesion increased. A highly significant correlation was found between p53 expression and higher grades of tumour differentiation. ($p<0.001$) Single case of adenoid cystic carcinoma showed high positivity (61-90%) with p53 while one case of verrucous carcinoma showed low expression (6-25%) and was limited to basal and suprabasal layers (Table 3).

Analyzing the pattern of p53 expression in normal oral mucosa, staining was nuclear and was confined to the basal layer of cells. Among premalignant cases, majority of cases (84.85%) showed basal layer of expression of p53 except in 5 cases (15.15%) which showed basal as well as suprabasal expression. All 64 cases of squamous cell carcinoma showed staining in all layers of epithelium as well as infiltrated tumor cell nests. Adenoid cystic carcinoma also showed p53 positively in all layers and tumor cell nests but p53 expression was limited to basal and suprabasal layer in veruccous carcinoma.

On analyzing expression of Ki-67 in normal oral mucosa, intense Ki67 expression was detected in all cases and was restricted to basal layer. Among premalignant lesions, Ki-67 was expressed in 87.88% of cases. Increased Ki-67 expression was seen in dysplastic lesions when compared to non-dysplastic lesions and was confined to basal and suprabasal layers. Ki-67 expression was increased with progressive grade of dysplasia. ($p=0.022$) (Table 3).

Table 1: Expression of p53 in various grades of oral dysplastic lesion

% positivity	0-5%	06-25%	26-60%	61-99%	Total	Chi-square Test
Mild dysplasia (n=10)	04 (40%)	06 (60%)	0	00	10	Chi-square=10.7 p- value p=0.030 Significant
Moderate dysplasia (n=8)	01 (12.5%)	03 (37.5%)	04 (50%)	00	08	
Severe dysplasia (n=2)	00	00	02 (100%)	00	02	
Total	05	09	06	00	20	

Table 2: Correlation of p53 in various grades of oral squamous cell carcinoma

% positivity	0-5%	06-25%	26-60%	61-99%	Total	Chi-square Test
Well differentiated (n=34)	12	2	18	2	34	Chi-square= 25.7
Moderately differentiated (n=20)	5	1	4	10	20	p - value p < 0.001 Highly significant
Poorly differentiated (n=10)	0	0	2	8	10	
Total	17	3	24	20	64	

Table 3: Correlation of Ki-67 with various grades of oral dysplasia

% positivity	0-5%	06-25%	26-60%	61-99%	Total %	Chi-square test
Mild dysplasia	00	08 (80%)	02 (20%)	00	10	Chi-square=7.6 p - value p = 0.022 Significant
Moderate dysplasia	00	02 (25%)	06 (75%)	00	08	
Severe dysplasia	00	00	02 (100%)	00	02	
Total	00	10	10	00	20	

Table 4: Correlation of expression of Ki-67 in various histological grades of oral squamous cell carcinoma

Staining	0-5%	06-25%	26-60%	61-99%	Total	Chi square Test
Well differentiated (n=34)	6 (17.65%)	4 (11.76%)	8 (23.52%)	16 (47.05%)	34	Chi-square=5 p - value p = 0.544 Not significant
Moderately differentiated (n=20)	3 (15%)	1 (5%)	5 (25%)	11 (55%)	20	
Poorly differentiated (n=10)	0	0	2 (20%)	8 (80%)	10	
Total	9 (14.06%)	5 (7.80%)	15 (23.44%)	35 (54.69%)	64	

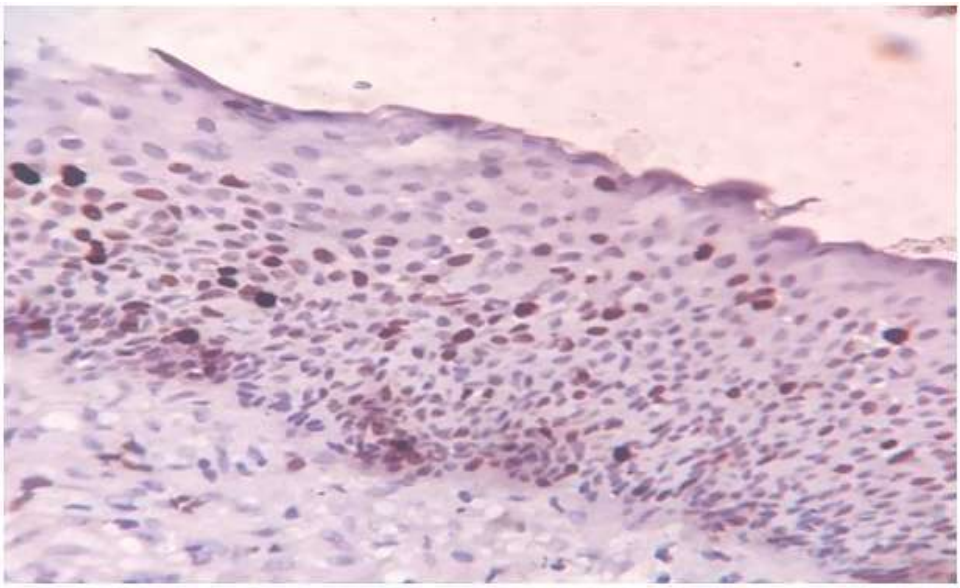

Fig. 1: Microphotograph showing immunohistochemical expression of Ki-67 in oral intraepithelial dysplasia grade-3 (x400)

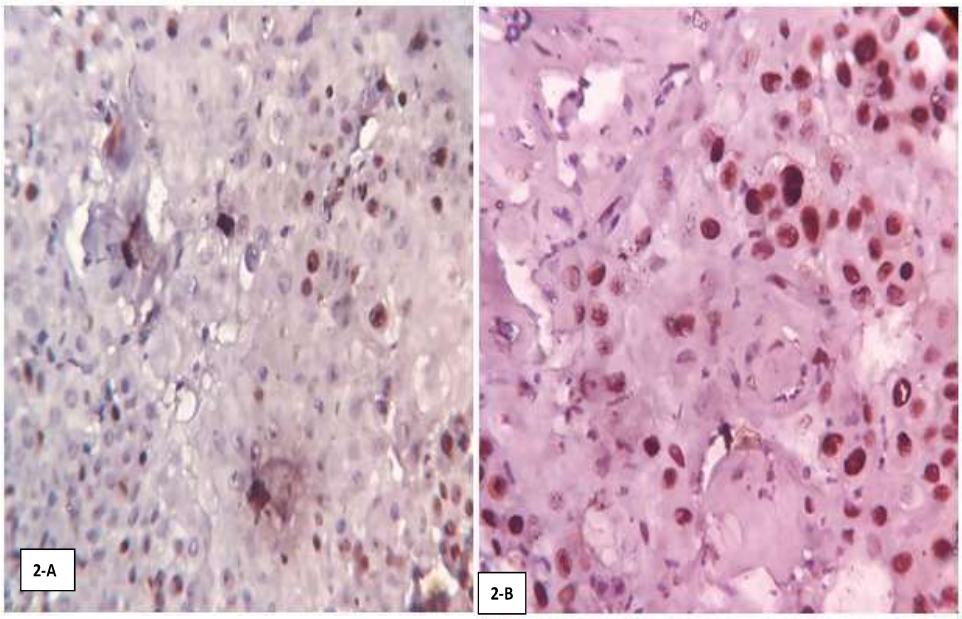

Fig. 2: Immunohistochemical expression of p53 (2-A) and Ki-67 (2-B) in well differentiated squamous cell carcinoma of oral cavity (x400)

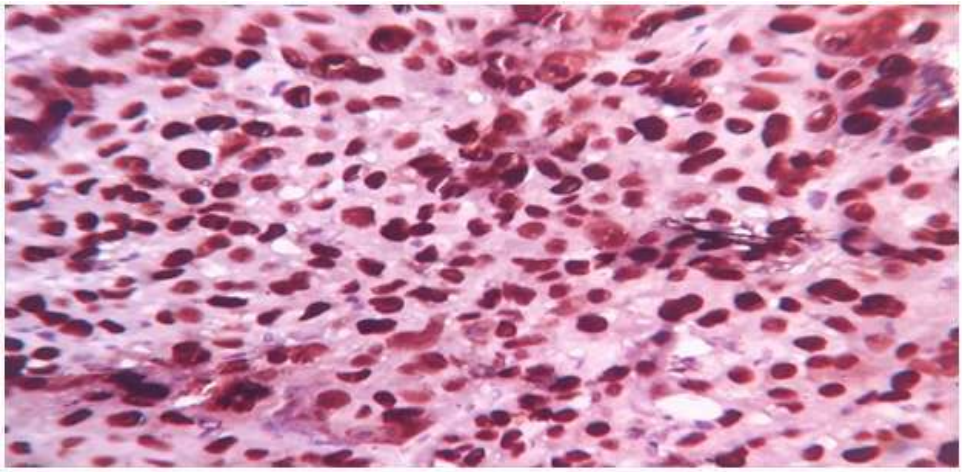

Fig. 3: Immunohistochemical expression of p53 in moderately differentiated squamous cell carcinoma of oral cavity (x400)

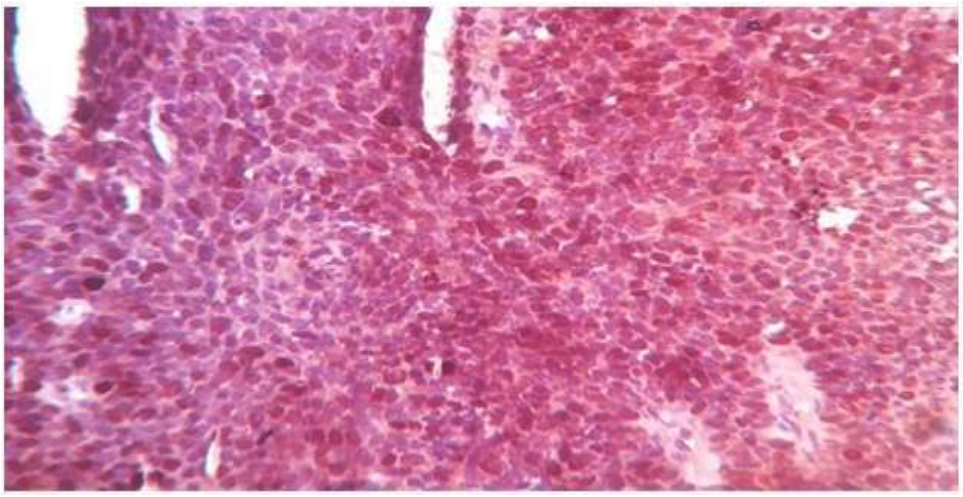

Fig. 4: Immunohistochemical expression of Ki-67 in poorly differentiated squamous cell carcinoma of oral cavity (x400)

In malignant lesions, the expression of Ki67 was seen in 85.90%. High proliferation ws observed in 54.69% cases while moderate proliferation was seen in 23.44% cases. The percentage of Ki67 positive cells in normal mucosa was 0-5% which increased to 95% in malignant mucosa (Table 4).

On correlating the association of expression of Ki-67 with various histological grade of oral squamous cell carcinoma, Ki-67 expression was highest in poorly differentiated squamous cell carcinoma (80%) than well differentiated squamous cell carcinoma (47.05%) and moderately differentiated squamous cell carcinoma (55%). The difference of expression of Ki67 between oral premalignant and malignant lesion was found to be highly significant. ($p<0.001$) However expression of Ki67 did not show significant association with various grades of differentiation of oral squmous cell carcinoma ($p=0.544$) (Fig. 4).

Discussion

Out of total 110 cases, 33 cases (30%) were premalignant lesions, 66 cases (60%) were malignant and 11 cases (10%) were normal. Our results are comparable with the study of Jagtap SV *et al.* [9] in which out of total 173 cases, 21.96% were premalignant and 78.04% were malignant lesions.

In present study buccal mucosa was the most common site constituting 54.55% followed by the tongue (21.21%) in premalignant as well as malignant lesion, which is in accordance with various authors [9–13].

In normal mucosa, p53 expression was negative and it was expressed in less than 5% of cells and was limited to lower third of the epithelium. Among 33 premalignant cases p53 expression was seen in 27 cases (75.75%). Out of 33 premalignant cases, 13 cases (39.39%) were non dysplastic and 22 cases (60.60%) were dysplastic. Majority (61.53%) of non dysplastic as well as dysplatic (45%) cases showed low expression (6-25%). This finding was in well accordance with the study of Humayun S *et al.* [14] and Bhattacharya I *et al.* [15].

On comparing the expression of p53 with various grade of dysplasia, a progressive increase was observed from mild to moderate to severe dysplasia. This finding was in agreement with Angiero *et al.* [16] and Takeda *et al.* [17].

On analyzing the expression of p53 in malignant cases, p53 positive expression was detected in 73.43% cases of oral squamous cell carcinoma which is in accordance with Bhattacharya I *et al.* [15], Dragomir LP *et al.* [18]. In comparision to leucoplakia, p53 expression was higher in oral squamous cell carcinoma. This finding was in agreement with Bhattacharya I *et al.* [15] and Humayun S *et al.* [14]. In Humayun S *et al.* [14] study, the percentage of p53 positive cells in normal mucosa was 15-25% which was increased to 95% in malignant mucosa. In the present study majority of premalignant leson showed low staining (6-25%) in contrast to oral squamous cell carcinoma where most had 26-100% immunopositive cells. Huang WY *et al.* [20] observed significant predilection for basal and suprabasal staining pattern with progression of lesion towards malignancy compared to strictly basal layer staining in normal mucosa.Cruiz IB *et al.* [21] reported 86% of premalignant lesions showing p53 expression above basal layer developed into squamous cell carcinoma. They stated that p53 expression above basal cell layer is an early event in oral carcinogenesis.

In present study p53 expression with histological grade, was significantly more expressed in moderate to poorly differentiated cases than in well differentiated cases. ($p< 0.001$). Our findings are in well correlated with Humayun S *et al.* [14], Bhattacharya I *et al.*, [15] Kannan *et al*. [19] who reported that poorly differentiated tumors had prominent alteration in p53 expression than well differentiated one. Immunostaining was strongly positive in areas of carcinoma infiltration.

The immunohistochemical expression of p53 and Ki-67 showed similar trends, and increased with degree of dysplasia. The expression of Ki-67 was significant in differentiating normal mucosa and mild dysplasia on one hand and from moderate to severe dysplasia and carcinoma on the other hand. Our findings are in well accordance with various authors [14,15,18,22]. Hyperproliferation is thought to be an early marker of disorderly growth. It is generally accepted that increased proliferation is associated with more advanced lesion and the distribution of proliferating cells in many tissue may reveal more about the regulatory mechanism that become dysfunctional during multistep process of carcinogenesis (Liu SC *et al.*).

In present study Ki-67 expression with the histological grade was more in moderate to poorly differentiated cases than in well differentiated cases which was accordance Bhattacharya I *et al*. [19], Dragmoir LP *et al*. [18], Birajder SS *et al*. [22] Poorly differentiated squamous cell carcinoma Ki67 expression was diffuse and more intense s the cells were less differentiated than well differentiated squamous cell carcinoma as well as moderately differentiated squamous cell carcinoma. More number of cells were in proliferative phase and hence showed an increase Ki67 expression than well differentiated squamous cell carcinoma and moderately differentiated squamous cell carcinoma.

Conclusion

The significant correlation between progression of oral epithelial from normal to neoplastic and increased expression of Ki67 and p53 antigens suggest that they may be useful biomarkers of malignant transformation in oral premalignant lesions. Higher p53 expression and suprabasal expression of p53 can be a predictive marker for poor prognosis in premalignant cases, the expression of p53 and Ki67 is associated linearly with the decrease of degree of tumour differentiation and with degree of dysplasia. Thus, the findings emphasizes that Ki67 and p53 together will be of great value as an adjuvant in assessing malignant potential of premalignant lesion so that early diagnosis and intervention is possible, thereby treatment outcome and patient survival can be improved. However, further immunohistochemical studies on large samples using ki67, p53 and other associated proteins are necessary to predict more specifically the development and malignant transformation of oral lesions.

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