RESEARCH ARTICLE

EXPRESSION OF p53 AND Ki-67 IN ORAL DYSPLASIA AND SQUAMOUS CELL CARCINOMA: AN IMMUNOHISTOCHEMICAL STUDY

Sharmistha M Patel, Kinjal A Patel, Prashant R Patel, Bhavna Gamit, Rasik N Hathila, Swati Gupta
Department of Pathology, Government Medical College, Surat, Gujarat, India

Correspondence to: Kinjal A Patel (kinjalpatel2086@gmail.com)

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ABSTRACT
Background: Oral cancers are a major health problem in developing country. They usually progress from premalignant conditions of oral cavity. Early diagnosis of these premalignant lesions by histopathology with help of immunohistochemistry can prevent morbidity and many other complications.
Aims & Objectives: To investigate the expression of p53 protein and Ki67 antigen in normal, oral premalignant and oral malignant lesions and to find correlation of the expression of this antigen in various premalignant and malignant oral lesions.
Materials and Methods: This study comprises of 39 cases of oral lesions (premalignant and malignant). After processing of representative tissue block, H&E and IHC stain with Ki-67 and p53 immunomarkers were carried out.
Results: Total 39 cases of premalignant and malignant oral lesions were reported in our study. Strong association was found in expression of both p53 and Ki-67 immunomarkers in premalignant and malignant oral lesions in compared to normal mucosa in our study.
Conclusion: Increased expression of p53 and Ki-67 immunostain was significantly correlated with progression of oral epithelium from normal to neoplasia and increased expression of these antigens suggest that they may be useful indicator of malignant transformation in dysplastic lesions.
Key Words: Oral Squamous Cell Carcinoma; Oral Dysplasia; Immunohistochemistry (IHC); p53; Ki-67

Introduction

Oral cancer constitutes a major health problem in developing countries and represents leading cause of death. Squamous carcinomas represent about 3% of human cancers and over 90% of malignant tumors at oral location, being diagnosed worldwide each year in over 350,000 new cases.[1]

The etiology of oral squamous cell carcinoma (SCC) is multifactorial and the most important risk factors include personal habits of tobacco use and alcohol consumption. Tobacco is considered the most important risk factor in the development of oral dysplasia and oral SCC. Various genetic and molecular alterations are observed in oral premalignant and malignant lesions due to consumption of tobacco. Carcinogens in tobacco affect the normal oral epithelium by increasing the number of aneuploid nuclei. Most of the oral SCC develops from precursor lesions, such as leukoplakia and oral submucous fibrosis.[2,3]

Leukoplakia, erythroplakia and submucous fibrosis are recognized oral precancerous lesions, and may exhibit the histopathological features of epithelial dysplasia, ranging from mild to severe.[4] Among these lesions, leukoplakia is the most common one, with malignant transformation rate ranging from 0.6% to 18%. This may be directly related to the severity of dysplasia, as it ranges from 5% for leukoplakia with mild dysplasia to 43% for leukoplakia with severe dysplasia.[5] Transformation rate of leukoplakia to oral cancer is based on the microscopic assessment of dysplasia and the degree to which it occurs.

The tumor suppressor gene p53 acts as a "monitor" in guarding the integrity of the genome and plays a central role in controlling the progression of the cell cycle from the G1- phase to the S-phase. Normal or wild– type p53 gene is a negative regulator of cell proliferation, whereas mutations in the gene are main culprit for malignant transformation. Gene mutation and overexpression of p53 play a vital role in tumour development. Mutated or inactivated p53 are incapable to induce apoptosis in cells. So the damaged cells continue to proliferate forming a tumour.[6] Ki-67 is a nuclear protein expressed in the G2- and M-phases of actively dividing cells. This antigen is a proliferation marker that correlates with the presence and severity of epithelial dysplasia. It provides significant information about the degree of aggressiveness and prognosis of oral Squamous cell carcinoma (OSCC).[7]

The purpose of this study was to investigate the expression of p53protein and Ki67 antigen in normal,

oral premalignant and oral malignant lesions, and to find the correlation of expression of this antigen in various premalignant and malignant oral lesions.

Materials and Methods

This study comprises of 39 cases of oral lesions (premalignant and malignant) who attended New Civil Hospital, Government Medical College, Surat, from the year August 2011 to October 2013.

Clinical history was taken from all the cases with more stress on probable risk factors of tumors. Patients were questioned about habits of smoking, alcohol consumption and tobacco chewing. Patients were also questioned about their socioeconomic status, dietary habits and were examined for oral hygiene.

The samples were collected from 39 cases reported to the Department of ENT, New Civil Hospital, Government Medical College, Surat, which consisted of 30 patients with OSCC, 3 each with mild, moderate and severe dysplasia (total 9) and 5 with normal oral mucosa as controls.

After receipt of biopsy specimens, it was fixed as early as possible by 10% neutral buffered formalin & processed preferably within 24 hrs of taking the biopsy. Representative tissue was submitted for processing and hematoxylin and eosin (H&E) stain for routine histological diagnosis. Immunohistochemistry (IHC) for P53 and Ki-67 were performed on representative blocks of paraffin embedded tissue. 4 µm thick sections were submitted for IHC Staining. Sections were taken on slides previously coated with poly L lysine. Antigen retrieval was done by Heat Induced Epitope Retrieval (HIER) method using Citrate buffer at 2.5 PH.

The intensity of immunohistochemical staining was graded based on subjective evaluation of colour exhibited (brown colour) by antigen, antibody and chromogen complex as:

- Negative: -, no colour
- Mild: +, light brown colour
- Moderate: ++, dark brown colour
- Intense: +++, very dark brown colour

The pattern of staining was graded as confined only to basal layer, both basal and suprabasal layers, and all layers of the epithelium.

The labelling index (LI) was counted by counting the number of positive cells per 100 basal or parabasal cells and was recorded as percentage. The nuclei with clear brown colour, regardless of staining intensity, were regarded as positive. The area with maximum number of positive cells was considered in each section. Known positive immune-staining slides were used as positive controls.

The parameters used to analyze the expression of both p53 and ki67 antigen are:

- Pattern of staining in epithelial layers
- Intensity of staining in each slide
- The percentage of positive cells or labelling index (LI).

Results

The most common age of presentation for malignancy was between 41 to 60 years of age (61.36%). The most common age group for SCC was 51-60 years (34.09%) followed by 41-50 years (27.27%). Mean age was found to be 49 ± 12.79 years in cases of SCC. Mean age in females in SCC group was found to be 43.12 ± 11.16 years. In present study, most common site of SCC was tongue followed by buccal mucosa.

Table-1: Age Distribution of patients at presentation

Age Group	Histopathology Diagnosis		Total
	Dysplasia	Squamous cell Carcinoma	
21-30	0	3	3
31-40	0	6	6
41-50	3	8	11
51-60	5	9	14
>61	1	4	5
Total	9	30	39

Table-2: Site of involvement

Site	No of cases	Percentage
Tongue	19	48.71
BM	8	20.51
BOT	6	15.38
Hard palate	3	7.69
Lip	1	2.56
Floor of Mouth	1	2.56
Alveolus	1	2.56
Total	39	100.00

Table-3: p53 LI in normal, dysplastic and malignant cases

Diagnosis	Expression of p53				Total
	<5%	6-25%	26-60%	61-99%	
Normal	5	0	0	0	5
Dysplasia	1	7	1	0	9
SCC	2	2	3	23	30

Table-4: Ki-67 LI in normal, dysplastic and malignant cases

Diagnosis	Expression of Ki-67				Total
	<5%	6-25%	26-60%	61-99%	
Normal	0	5	0	0	5
Dysplasia	0	6	3	0	9
SCC	0	5	15	10	30

Table-5: Association of P53 in various lesions

P53	Normal	Dysplasia	SCC	P Value
Positive	0	8	28	0.000029
Negative	5	1	2	
Total	5	9	30	

In present study, 28 out of 30 (93.33%) cases of SCC showed positive history of tobacco chewing. 55.55% of dysplastic lesions were associated with tobacco chewing. P value for tobacco chewing habit was found to be 0.0002, which was highly significant (P<0.05). So, there is strong association between malignancy and tobacco chewing.

The summary of immuno-staining data and staining intensity of p53 and KI-67 according to LI in Normal, dysplastic and malignant cases is given in Table No 3 and 4 respectively.

Chi-square test was used (Table 5) to compare frequency of Protein P53 expression in Normal, Dysplastic and Malignant cases. Strong association was observed in expression of p53 in dysplastic and malignant epithelium. P Value was highly significant (P= 0.000029). As Ki-67 is a normal proliferative marker, it shows strong positivity in all malignant as well as in dysplastic epithelium.

Discussion

p53, a tumour suppressor protein, acts as a "molecular break" to critically regulate the cell cycle. This DNA-binding protein has also been involved in DNA repair and synthesis, cell proliferation, cell differentiation, programmed cell death, and in the maintenance of genomic stability.[8] Mutations in the p53 gene is the most common genetic change observed in human carcinomas. These mutations lead to uncontrolled cell proliferation, resulting in further genetic abnormalities and finally in malignancy.[9]

In a normal cell, the p53 protein is kept at a low concentration by rapid degradation, on immunostainning <5% basal epithelial cells shows p53 positivity. In our study all control cases were negative for p53 staining.

The distribution of p53 confined to basal layer only in normal epithelium but in dysplasia it may be found in suprabasal layer depending on severity of molecular damage. In our study 3 cases showed basal layer localization of p53 while 6 cases of moderate and severe dysplasia showed both basal as well as suprabasal localization of p53. Similar results were observed by Kerdpon et al and Nylander et al in their studies.[10,11] These findings are suggestive that the expression of p53 above the basal layer could be an early event in oral carcinogenesis and an indicator of developing carcinoma. As p53 protein has been reported to be expressed at high levels in premalignant and malignant lesions. In our study 8 (88.89%) dysplastic lesions and 28 (93.33%) SCC cases showed p53 overexpression. Similar findings were observed by Panjwani et al in their study that showed 75% positive cases. Higher p53 positive cell counts were demonstrated in oral squamous cell carcinoma compared to hyperplastic tissue and dysplastic tissue by Sucheta Bansal.[12]

The mean p53 LI in dysplastic lesions, in the present study, was found to be 13.33%, and in carcinoma it was 71.83%, while normal mucosa was negative for p53. This suggests p53 immunolocalization increases as normal mucosa becomes dysplastic and undergoes malignant transformation. This finding is similar with study of S. Humayun et al and supported by other reports such as the study of S Kannan et al.[13,14] These findings suggest a strong correlation between p53 expression and degree of dysplasia, thus confirming that p53 may be involved in proliferative events as well as in neoplastic transformation.

In the present study, 93.33% of SCC cases were positive for p53, which is comparable with the studies of L.P. Dragomir et al, Raju et al (100%) and S. Humayun et al (100%).[1,2,13]

Ki67 is one of the mitotic indicators in proliferative activity of tumors. Expression of Ki-67 in mean of proliferative activity of tumor cells is one of the indicators for tumor invasion potential and invasive activity of cancers related to degree of malignant neoplastic cells.[15] In our study, all the cases including normal controls showed Ki-67 expression. Raju et al observed Ki-67 expression in 93.10% of premalignant lesion and 90% of oral cancer.[2] L.P. Dragomir et al in their study observed that all cases of Oral SCC expressed Ki-67. Motta RDR showed 92.75% cases with Ki-67 expression.[2,15]

In the present study, all the dysplastic lesions expressed Ki-67 in >5% of cells. S Kannan et al in their study observed in 6 out of 7 dysplastic lesions, Ki-67 expression was in >5% of cells. They also observed Ki-67 expression in >25% of cells in 58% of dysplastic lesions.[14] In the present study, Ki-67 expression in >25% of cells was found in 33.33% of dysplastic lesions.

In the present study, mean LI in SCC group was found to be 56.00%. 83.33% of carcinoma cases had Ki-67 LI>25%. S. Kannan et al observed that in 67% of carcinoma cases, Ki-67 LI was >25%.[14] Thus, in oral mucosal lesions, the expression of Ki-67 has been reported to increase according to the proliferative activity and degree of epithelial dysplasia, suggesting that it is a marker of the presence and severity of epithelial dysplasia.[2]

In the present study, 2 cases of carcinoma showed Ki-67 expression only in basal layer, 4 cases showed both basal and suprabasal staining and remaining 24 cases showed Ki-67 expression in all layers. S. Humayun et al observed Ki-67 expression in basal and suprabasal layer in one case while in other two cases in all layers in their study.[13]

In oral SCC co-expression and correlation between p53 and Ki-67 have been demonstrated and suggest that alteration in p53 lead to increased cell proliferation and its co-expression in dysplastic lesions are indicator of carcinoma development.[2] In our study, p53 and Ki-67 were co-expressed in 94.87% of the cases, which is comparable with the study of Raju et al, who found it to be 80%.[2] A positive correlation was observed between LI of p53 and Ki-67, which indicates that there is increase or decrease in p53 LI with increase or decrease of Ki-67 LI in oral premalignant lesions and OSCC.

Conclusion

Expression of both p53 and Ki-67 were found to be increased in Dysplasia and SCC as compared to normal mucosa in our study. These findings are significantly correlated with progression of oral epithelium from normal to neoplasia and increased expression of these antigens suggests that they may be useful indicators of malignant transformation in dysplastic lesions. However, to validate these findings, larger studies are necessary in oral lesions in India.

Abbreviations

H&E: Haematoxylin and Eosin; IHC: Immunohisto-chemistry; SCC: Squamous cell carcinoma; OSCC: Oral Squamous cell carcinoma; HIER: Heat Induced Epitope Retrieval; LI: Labeling Index

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