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RESEARCH ARTICLE

EXPRESSION OF p53 AND ki-67 in ORAL SQUAMOUS CELL CARCINOMA WITH THE BACKGROUND OF ORAL SUBMUCOUS FIBROSIS

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ABSTRACT

Oral submucous fibrosis (OSMF) is a potentially malignant disorder with 7.6% of transformation rate into oral squamous cell carcinoma (OSCC) of which majority display low grade of tumor differentiation. The aim of the study is to assess the genetic markers p53 and ki-67 in OSMF, OSCC and OSCC with the background of OSMF. 10 cases of each group were stained with p53 and Ki-67 by immunohistochemistry. Statistically significant results were found amongst the 3 groups with moderate to severe expression ($p<0.05$) of both the markers. To conclude, these biomarkers can be useful in assessing the malignant transformation in oral precancerous conditions and may serve as intermediate points for cancer prevention programmes.

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INTRODUCTION

Oral cancer is the most common cancer in Indian males and is the third most common cancer in Indian females (1). Tobacco, alcohol, areca nut, and human papillomavirus are the common etiologic factors. Each of these agents follows a unique model of carcinogenesis that leads to a certain distinct presentation and behavior (2-4). Oral submucous fibrosis (OSMF) is a potentially malignant disorder of the oral cavity. A high incidence of oral submucous fibrosis (OSMF) is linked to areca nut (group 1 human carcinogen) chewing in the Indian subcontinent. Most of the people affected by OSMF are betel quid chewers. It is characterized by epithelial atrophy and progressive accumulation of collagen fibers in lamina propria and submucosa of the oral mucosa. 7.6% of OSMF cases undergo oral squamous cell carcinoma (OSCC) transformation of which majority display low grade of tumor differentiation (5,6). In the present paper, a hypothesis has been proposed to correlate atrophy, turnover rate and surface keratization in OSMF with degree of tumor differentiation in OSCC. A novel hypothesis for epithelial atrophy in OSMF has also been emphasized. High proliferative activity and basal cell hyperplasia in conjunction with rapid exfoliation of superficial cells and epithelial atrophy suggest that epithelial turnover rate is very high in OSMF. Presence of surface keratinized layer in this situation suggests faster maturation or differentiation of epithelium in OSMF (7). Thus, the epithelial cells are genetically programmed for high turnover rate and faster differentiation or maturation to form keratin. During malignant transformation of OSMF, the transformed epithelial cells may retain the genetic memory of faster differentiation and maturation resulting in better grade of tumor differentiation (8-10). Epithelial carcinogenesis is a multistep process. Specific genetic events lead to malignant transformation of oral epithelium (15,16). Oral squamous cell carcinoma (OSCC) may be preceded by potentially malignant lesions such as oral submucous fibrosis. Molecular biological markers have been suggested to be of value in the diagnosis and prognostic evaluation of precancerous lesions. p53 is the name of the tumor suppressor gene located on short arm of chromosome 17, as well as the protein encoded by this gene (17,18). The immunohistochemical analysis of p53 proteinis an uncomplicated method that has been broadly used;many studies have shown that p53 protein is implicatedin oral carcinogenesis and its alteration occurs early inthe progression of neoplastic

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transformation, frequently preceding identifiable histological alterations. Also, in oral premalignant lesions, expression of p53-positivecells in the suprabasal layers of the epithelium has been seen as an indication of impending malignancy (19,20). To our knowledge no studies have examined the expression of p53 protein in OSMF developing in background of OSCC. Therefore, the aim of the present study was to investigate the expression of p53 protein in plain OSCC, plain OSMF and OSMF developing in background of OSCC, using immunohistochemistry, and comparing the datas for better understanding of the lesions

MATERIALS AND METHODS

Tissue processing and TMA preparation

Study cases including, OSMF, Oral Squamous Cell Carcinoma, OSMF in background of Oral Squamous Cell Carcinoma and normal mucosa as control were selected from the archives of the Department of Oral and Maxillofacial Pathology, Dr. D.Y. Patil Dental College, Pimpri, Pune. Tissues were fixed in buffered formalin, processed using standard procedures and embedded in paraffin. Tissue blocks were stored at room temperature in the pathology archive up to 10 years before being used for TMA construction. 10 paraffin blocks of each group were selected for the study. Histological slides for each specimen were prepared for IHC staining by cutting 3 µm tissue sections on a standard microtome. Immunohistochemical technique was performed using the avidin-biotin-peroxidase protocol, according to Abrahao *et al*. (2011). Briefly, antigen retrieval wasperformed with Target antigen retrieval solution pH 9(Dako A/S, CA, USA) in a water bath, followed byincubation with 6% hydrogen peroxide to quench endogenous peroxidase. The sections were then incubated in blocking solution (3% bovine serumalbumin) for 1 hour at room temperature, followed by primary antibody incubation, previously diluted in blocking solution. Anti-p53 (clone DO-7, 1:200 dilution– DAKO A/S, CA, USA) antibody was incubated for 30minutes at room temperature. Sections were exposed to the LSABTM system (DAKO A/S, CA, USA),developed in diaminobenzidine (Dako A/S, CA, USA)and counterstained in Mayer's hematoxylin. For the antibody, positive and negative controls were used.

RESULTS

P53 expression

8 cases showed negative (Figure 1) and 2 cases showed very mild expression seen in normal mucosal tissues. Amongst 10 OSMF cases, 5 cases showed mild (+) expression (Figure 2), 1 with moderate (++) expression (Figure 3) and rest 4 cases showed no expression. The staining was limited to basal and suprabasal layers. All OSCC cases showed P53 expression. The intensity ranged from moderate (++) to intense (+++). 2 cases were stained moderate and 8 stained intense (Figure 4). All OSCC cases with the background of OSMF showed positivity for p53.The intensity ranged from moderate to intense.7 cases were stained moderate (Figure 5) and the other 3 stained intense (Figure 6). Statistically significant results were found between the normal with OSMF, OSCC and OSCC with OSMF in mild, moderate and severe expression wih $p<0.05$. Statistically significant results were found between OSMF with OSCC in mild expression ($p=0.012$) and between OSMF with OSCC and OSMF in all mild ($p=0.008$), moderate ($p=0.008$) and severe ($p=0.067$) expression with $p<0.05$. Significant results were found between OSCC with OSCC and OSMF in moderate ($p=0.028$) and severe ($p=0.028$) expression with $p<0.05$.

Ki-67 expression

In Normal oral mucosa, staining was mild to moderate and was limited to the basal layer of cells. 6 cases showed mild and 4 cases showed moderate staining (Figure 7). Oral submucous fibrosis cases showed only basal layer expression with 7 cases negative to 3 cases mild staining (Figure 8). All 10 OSCC cases showed intense staining (Figure 9). The staining intensity was moderate in 3 cases and intense in 7 cases of OSCC in the background of OSMF. Statistically significant results were found between the normal with OSMF, OSCC and OSCC with OSMF in mild, moderate and severe expression with $p<0.05$. Statistically highly significant ($p=0.000$) results were found between OSMF with OSCC and OSCC with OSMF with $p<0.05$. Similarly highly significant ($p=0.000$) results were found between OSCC with OSCC with OSMF $p<0.05$.

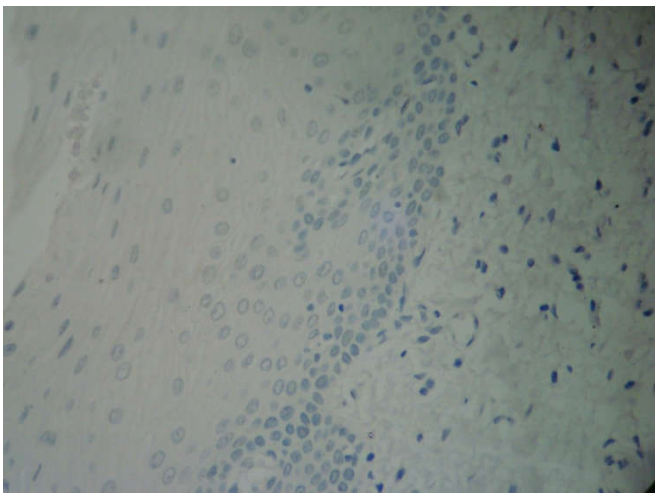

Figure 1. Negative expression of p53 in normal mucosa

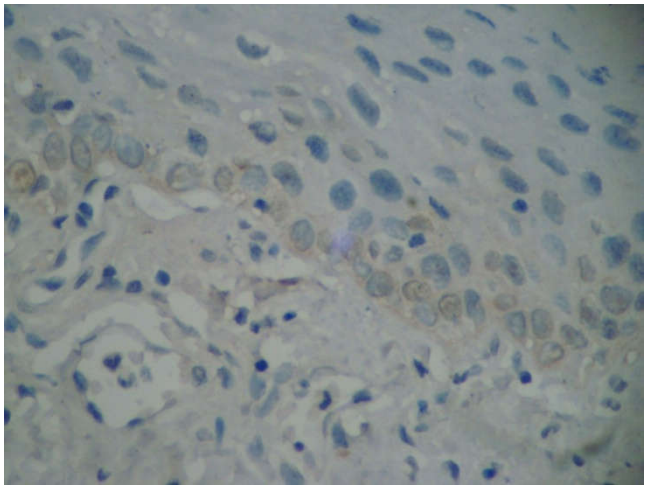

Figure 2. Mild expression of p53 in OSMF

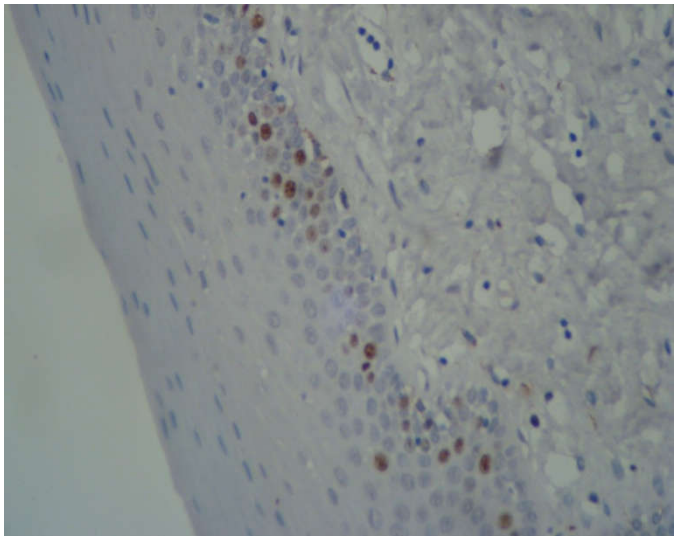

Figure 3. Moderate expression of p53 in OSMF

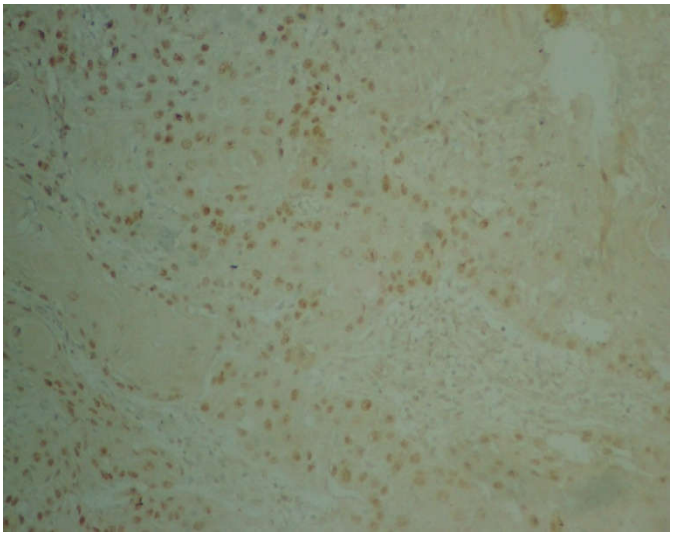

Figure 4. Intense expression of p53 in OSCC

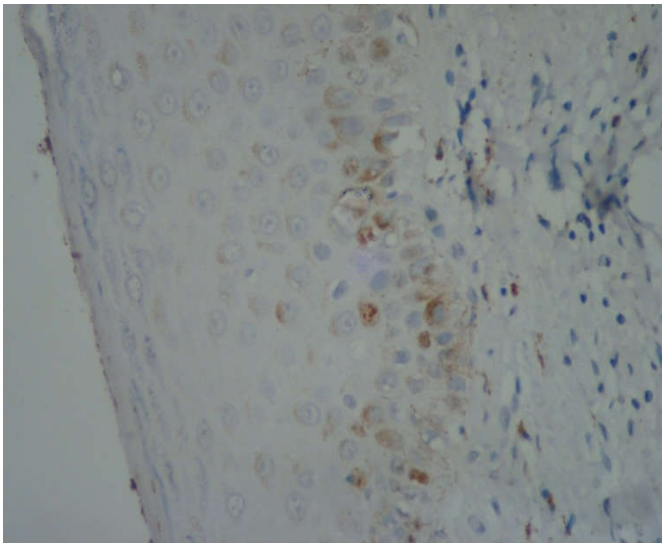

Figure 5. Moderate expression of p53 in OSCC cases with the background of OSMF

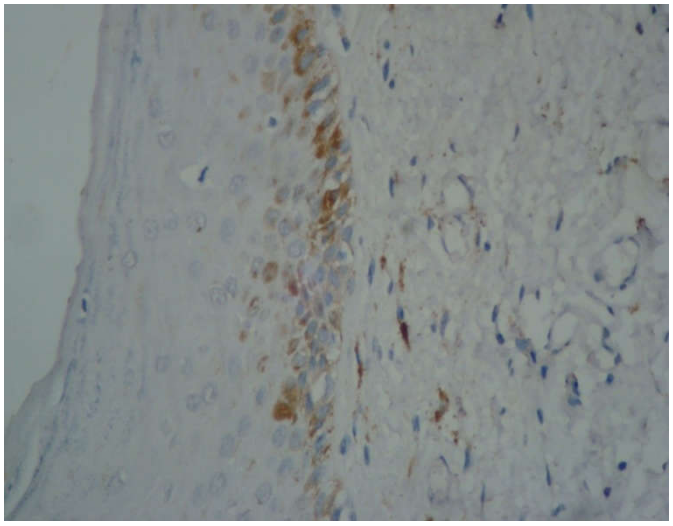

Figure 6. Intense expression of p53 in OSCC cases with the background of OSMF

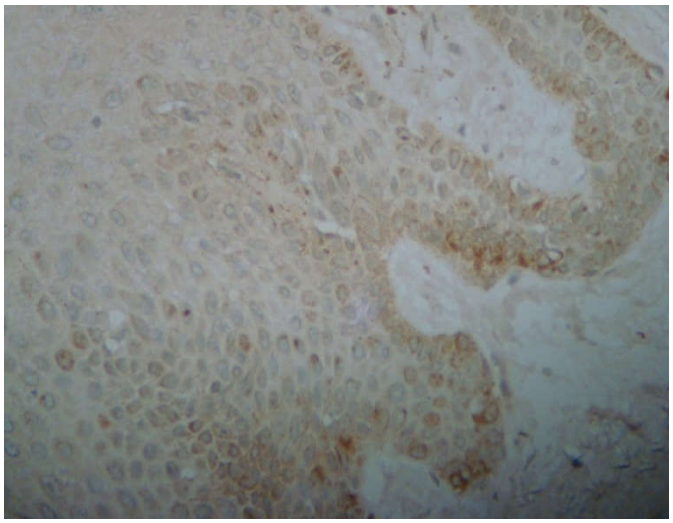

Figure 7. Moderate staining of Ki-67 in normal mucosa

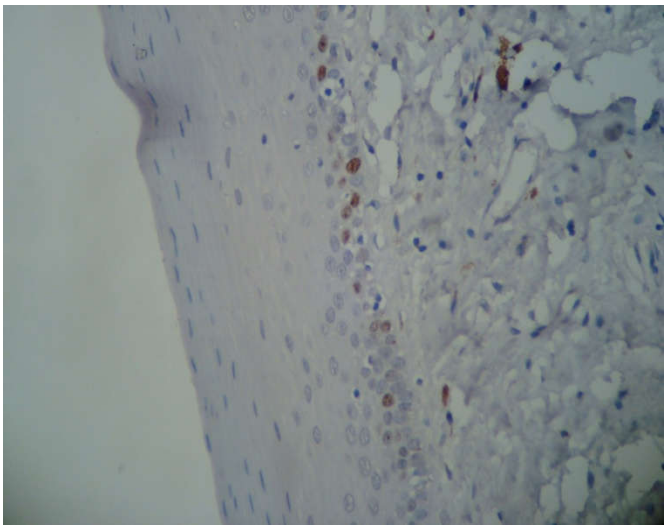

Figure 8. Mild staining of Ki-67 in OSMF

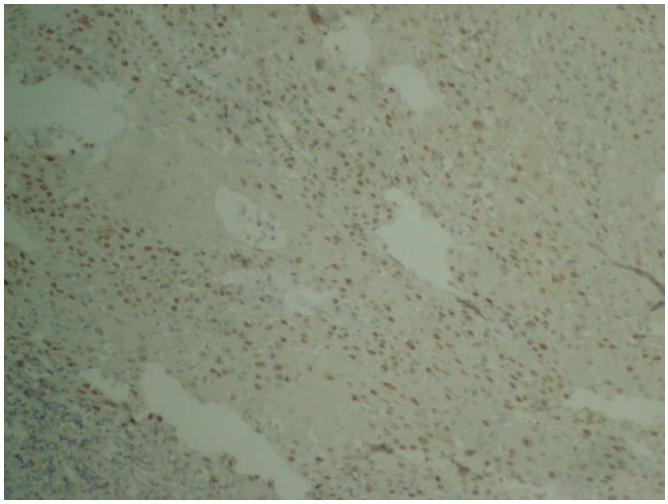

Figure 9. Intense expression of Ki-67 in OSCC

DISCUSSION

Mutations in the p53 gene are the most common genetic changes observed in OSCCs. These mutations lead to uncontrolled cell proliferation, resulting in further genetic abnormalities and finally in malignancy (10). Therefore, the nature of the p53 gene and the proliferative status of a cell are closely linked and the loss of this linkage is one of the main causes of tumor formation and is considered to be an early event in this process. To analyze the proliferative status of a cell or tissue Ki-67 marker is reliable and widely used. It recognizes a proliferation-related nuclear antigen present at all phases of cell cycle except G_0.(9) In the present study all the normal control tissues (oral mucosa) unrelated to any deleterious chewing habit exhibited positive p53 expression at the basal layer in consistent with other studies. (Win *et al*., 2005; Piattelli *et al*., 2002; Takeda *et al*., 2006) (3,11,12) The possible explanation is that genotoxic stress, caused by a physical, chemical or microbiological agent that commonly acts in the oral cavity, may lead to p53 accumulation in these epithelial cells for physiological response. (Win *et al*., 2005) (3) The other possibility for this positive expression may be defect in the degradation pathway or binding of p53 protein to other proteins such as certain DNA virus-encoded proteins. (Nylander *et al*., 2000)(5) All the samples of normal mucosa showed positive staining with ki-67 in basal layer of epithelium similar to p53; this could be due to the physiological proliferative activity in the basal cell layer. The present study revealed that the p53 expression in premalignant lesions and conditions was significantly associated with the personal habits. Increased p53-positive cells were detected in patients with both betel quid chewing and smoking habits which is in contrary to the study of Yan *et al*. (1996)(13) which revealed that Taiwanese patients without a betel quid chewing habit had a higher rate of p53 over expression than heavy chewers.

In OSMF cases, p53 expression although limited to basal layer, was very high when compared to normal control group which is consistent with the study of Win *et al*. (2005)(3) This indicates the possibility of mutations in p53 gene in the development and progression of OSMF. The most common culprit, areca nut products have a high copper content, which suggest a copper-mediated etiopathogenic mechanism for genetic aberration found in OSMF by means of binding with p53 gene. (Win *et al*., 2005; Hazarey *et al*., 2007) (3,14) The results of the present study in OSMF and OSMF with OSCC groups showed positive correlation between p53 mutations and proliferative activity (ki-67 expression) of the basal layer only in two samples each from both groups but inverse relation was observed in two samples of OSMF group. Hence, it was difficult to completely analyze the relation between p53 and ki-67 expression in OSMF and OSCC groups and further studies may be required using large samples. The present study observed that mutant p53 gene is a common event in the development of OSCC which is consistent with the report of Langdon *et al*. (21) The previous studies on p53 expression in oral premalignant lesions and OSCC showed a range of positivity of 35-90% and this finding is also observed in the present study (40-95%). Hence, the present study suggests that the ki-67 expression in OSCC is significant and useful in predicting histologic grade of differentiation and prognosis of the lesion.

Conclusion

In conclusion, the significant correlation between progression of oral epithelium from normal to neoplasia and increased expression of these antigens suggest that they may be useful biomarkers of malignant transformation in oral precancerous lesions and conditions and may serve as intermediate points for cancer prevention programmes. However, further immunohistochemical studies on large samples identifying p53, ki-67 and other associated proteins in premalignant lesions along with the mutational analysis are necessary to predict more specifically the development and malignant transformation of these oral lesions.

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