Original Article

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A comparative study of p53 expression in hyperplastic , dysplastic epithelium and oral squamous cell carcinoma

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Abstract

Aim: To study oral hyperplastic epithelium, dysplastic epithelium and squamous cell carcinoma to determine (1) the prevalence of p53 protein immunoreactivity, (2) number of p53 positive cells, and (3) the area of localization of p53 protein immunoreactivity. **Methods:** Two contiguous sections from 30 tissue specimens (10 each from oral hyperplastic epithelium, dysplastic epithelium and squamous cell carcinoma) were subjected to hematoxylin and eosin (H/E) staining for histopathological diagnosis and immunohistochemical (IHC) staining for demonstration of p53. p53 positivity was looked for in each IHC stained slide and the number of positive cells amongst 1,000 epithelial cells were recorded. The localization of these p53 positive cells within the strata (i.e. basal/suprabasal, spinous and superficial layers) of epithelium between 3 groups, and also within each group according to histological grades was recorded. **Results:** Higher p53 positive cell counts were demonstrated in oral squamous cell carcinoma compared to hyperplastic and dysplastic tissues. The expression of p53 in epithelial hyperkeratosis was mainly localized to basal epithelial cells whereas in epithelial dysplasia, it was predominantly localized to spinous epithelial cells. **Conclusions:** Qualitatively p53 is not a specific marker for malignancy of oral epithelium. However the quantitative analysis of p53 positive cells and their localization in oral epithelium is of importance as a marker for oral squamous cell carcinoma.

Keywords: dysplasia, p53, oral squamous cell carcinoma, oral leukoplakia.

Introduction

Oral squamous cell carcinoma constitutes the sixth most common cancer worldwide and the third most common cancer in the developing countries[1]. Oral squamous cell carcinoma is believed to develop through sequential stages of premalignant/pre-invasive lesions: hyperplasia, mild, moderate, severe dysplasia, carcinoma in situ, and finally invasive squamous cell carcinoma[2].

Leukoplakia and erythroplakia are recognized oral precancerous lesions and may exhibit the histopathological features of epithelial dysplasia ranging from mild to severe. The proportion of epithelial dysplastic lesions that progress to squamous cell carcinoma varies between 6.6 and 36%, and the period over which this occurs may be $\geq$20 years. Higher transformation rates are quoted when lesions exhibit epithelial dysplasia[3].

Genetic changes leading to alterations in structure, function or expression

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level of proteins involved in cell cycle regulation are known to be one of the key events in the malignant transformation of the tissue[4]. Mutations of the tumor suppressor gene, particularly p53, are the most commonly identified events in various human cancers[5].

The grading and staging of squamous cell carcinoma is indicative of the prognosis and the clinical course of the disease. The quantitative study of p53 suggested their aberrant expression in oral cancer. p53 over expression was also detected in 15-19% of oral pre-malignant lesions, including lesions with mild dysplasia in head and neck cancer patients[6].

The present study was conducted to correlate the p53 expression with histological diagnosis of oral hyperplastic, dysplastic lesions and oral squamous cell carcinoma. To the best of our knowledge, there are only few published studies on similar lines reported from India found on Pubmed search[7].

Material and methods

The sample selected for this study consisted of 30 tissue specimens from 30 different patients, 10 each of oral hyperplastic epithelium, dysplastic epithelium and squamous cell carcinoma. The sections were sourced from the archives of the Department of Oral and Maxillofacial Pathology, DJ College of Dental Sciences and Research, Modinagar, UP, India. Breast carcinoma tissue section was used as positive control whereas the negative control was the normal tissue from the palatal mucosa.

Each tissue specimen was fixed in 10% neutral buffered formalin and processed for histopathology. In each case, two contiguous 4-µm-thick sections were cut. Of these, one section was stained with H/E staining and the second was subjected to immunostaining for p53 (Fig. 1-7) using the protocol proposed by Abbas NF[8].

p53 positivity was looked for in each IHC slide. One thousand epithelial cells were counted and the number of p53 positive cells was recorded.

The localization of these p53 positive cells within the strata of epithelium between hyperplastic and dysplastic groups and also within each grade of dysplastic group was noted with respect to basal/suprabasal, spinous and superficial layers.

Results

Immunohistochemical staining for p53 was found exclusively in the nuclei of epithelial cells. Breast carcinoma tissue section was used as positive control whereas the

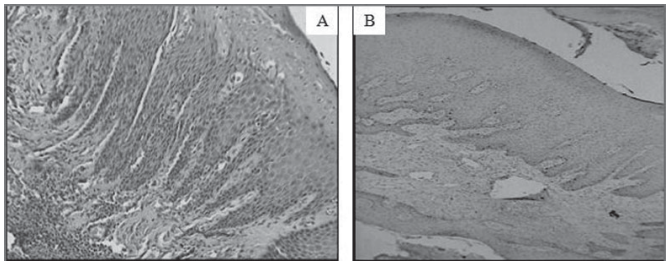

Fig. 1 H/E (A,10X) and IHC staining with p53 expression (B,4X) showing hyperplasia.

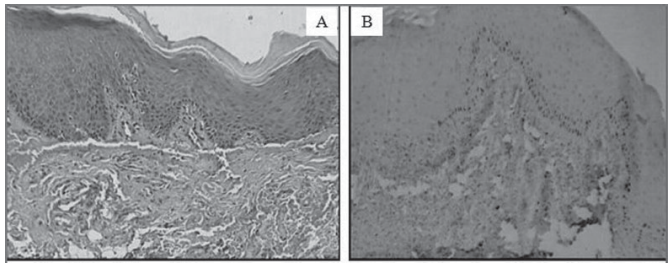

Fig. 2 H/E (A) and IHC staining with p53 expression (B) showing mild dysplasia (10x).

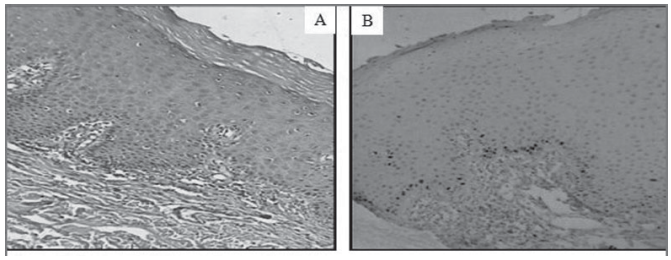

Fig. 3 H/E (A) and IHC staining with p53 expression (B) showing moderate dysplasia (10x).

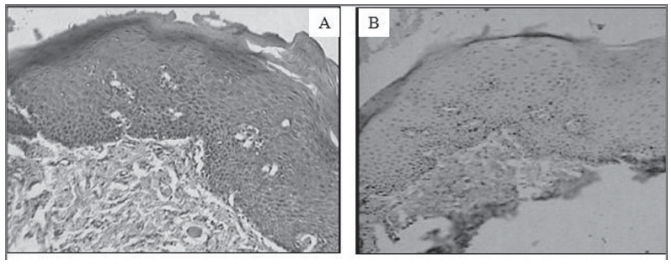

Fig. 4 H/E (A, 10X) and IHC staining with p53 expression (B, 4X) showing severe dysplasia.

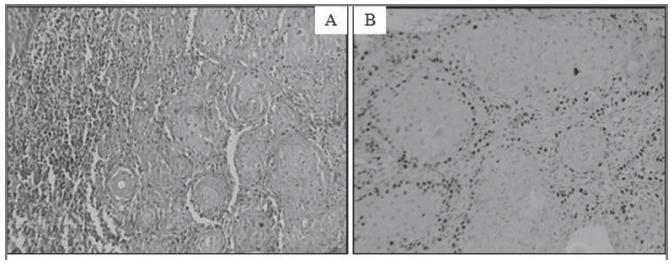

Fig. 5 H/E (A) and IHC staining with p53 expression (B) showing well differentiated squamous cell carcinoma (10x).

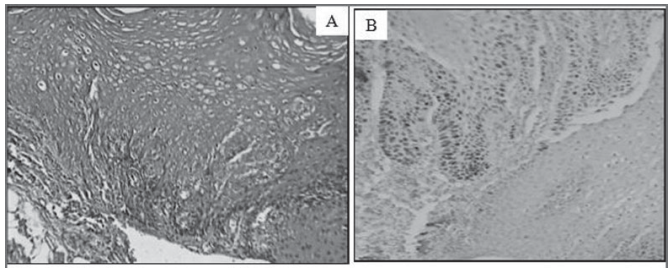

Fig. 6 H/E (A) and IHC staining with p53 expression (B) showing moderately differentiated squamous cell carcinoma (10x).

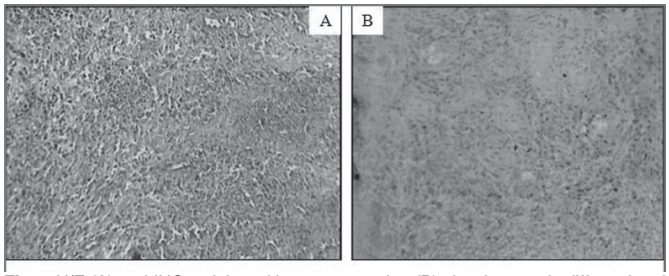

Fig. 7 H/E (A) and IHC staining with p53 expression (B) showing poorly differentiated squamous cell carcinoma (10x).

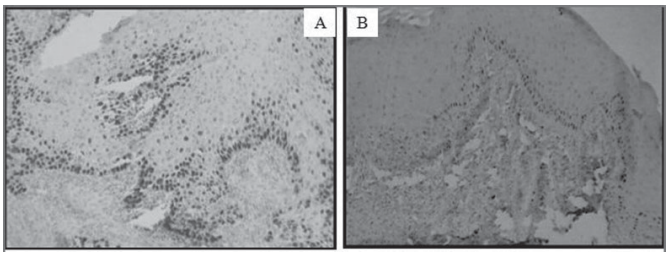

Fig. 8 IHC staining with p53 (10x) showing well differentiated squamous cell carcinoma (A) with increased number of p53 positive cells as compared to dysplastic epithelium (B).

negative control was the normal tissue from the palatal mucosa. Negative control did not display brown staining in epithelial or any other cells. i.e., it was negative for p53. Sections of breast cancer tissue showed consistent nuclear staining of neoplastic cells.

p53 positivity was observed in each histological group. p53 positive cells were counted in each group for each patient. In different class intervals, the number and percentage of patients in each group were counted (Table 1). The localization of p53 positive cells in the hyperplastic and dysplastic group and also within each grade of dysplastic group were recorded (Table 2).

Table 1. Number and percentage of patients in each group in different class intervals of p53 positive cells.

Type of tissue	p53 positive cells per 1,000 epithelial cells			
	0-150	151-300	301-450	451-600
Hyperplastic	8 (80%)	1 (10%)	1 (10%)	0
Dysplastic	3 (30%)	5 (50%)	2 (20%)	0
Oral squamous cell carcinoma	4 (40%)	1 (10%)	0	5 (50%)

Table 2. Distribution of cases according to predominant localization of p53 cells in the different study groups.

Type of tissue		Layers of epithelium		
		Basal	Basal & Spinous	Superficial
Hyperplastic		7	3	0
Dysplastic	Mild	2	0	0
	Moderate	3	3	0
	Severe	0	2	0

p53 immunostaining was positive in all the tissues of hyperplastic oral epithelium, dysplastic oral epithelium and oral squamous cell carcinoma. Hence, there was no significant qualitative difference in p53 positivity among three groups (Fig. 1-7).

Higher p53 positive cell counts were demonstrated in oral squamous cell carcinoma with respect to hyperplastic tissue and dysplastic tissue (Fig. 8). The number of p53 positive cells increased from hyperplastic and dysplastic lesions to oral squamous cell carcinoma. However, there were no significant differences in the expression of these proteins, within a group according to grading.

In this study, p53 varied in location between the three groups, and also within different grades of dysplasia. The expression of p53 in epithelial hyperplasia was mostly limited to basal and parabasal epithelial cells, whereas in the epithelial dysplasia group it also extended to the spinous

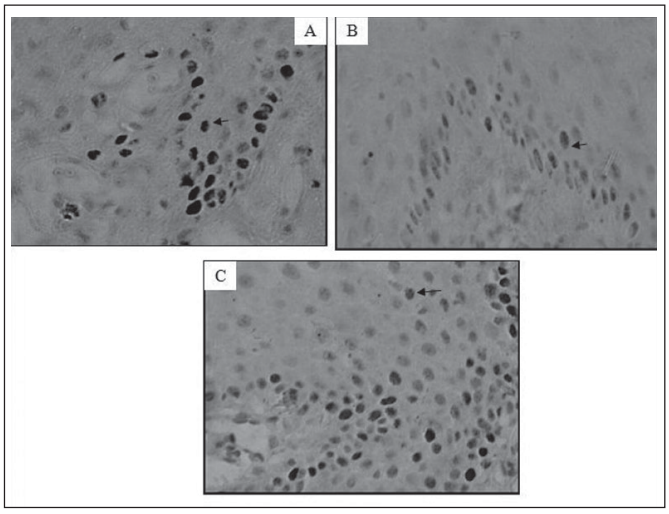

Fig. 9 IHC staining showing basal and suprabasal position of p53 positive cells (marked by arrows) in hyperplasia (A) and mild dysplasia (B), and spinous position in severe dysplasia (C) in different layers of oral epithelium.

strata in some cases (Fig. 9A and C).

In the dysplasia group, as we move from mild to moderate and to severe dysplasia, it was observed that the p53 positive cells was predominantly localized to basal strata in mild dysplasia, localized to either basal alone or together in basal and spinous strata in moderate dysplasia and predominantly localized to spinous strata in severe dysplasia (Fig. 9B and C).

Discussion

The present study was carried out in oral hyperplastic and dysplastic epithelium and squamous cell carcinoma to determine p53 protein immunoreactivity (both presence and quantification) and to determine the area of its localization in tissue strata in different grades of dysplasia. This was achieved by performing H/E and p53 (IHC) staining on two contiguous sections from each tissue specimen.

In the present study, all cases in all three groups showed p53 positivity. The reasons for this might be due to small sample size or the criteria applied for qualitative analysis for determining p53 positivity[9]. Hence, there was no significant qualitative difference in p53 positivity among the three groups. This is similar to the findings of Abbas et al.[8] p53 regulates cell cycle proliferation, and so all tissues may be expected to exhibit some amount of cell proliferation. Hence,

some p53 positive cells are expected in all cases including normal tissue[8,10-11].

In this study, the number of p53 positive cells per 1,000 epithelial cells was found to be in the range of 0-150 in the hyperplastic group, 150-300 in the dysplastic group and more than 450 in the squamous cell carcinoma group. Thus higher p53 positive cell counts were demonstrated in oral squamous cell carcinoma compared to hyperplastic tissue and dysplastic tissue suggesting that the p53 expression peaked close to the time of transition from the precancer state to cancer. Several studies have shown that the proportion of cases with positive p53 expression increases from hyperplasia to dysplasia to oral squamous cell carcinoma[8-10,12].

The number of immunopositive cells in mild, moderate and severe grades of dysplastic tissue and in different grades of oral squamous cell carcinoma was counted.

There were no significant differences in the expression of these proteins, within a group according to grading. The results of the present study were in accordance with those of Abbas et al.[8] and Regezi et al.[13], who could not find a clear correlation between grades of dysplasia and the percentage of p53 positive cells in oral premalignant lesions.

In contrast, Wood et al.[14] reported a significant correlation between p53 expression and grades of dysplasia. In that study, a significantly higher number of p53 positive cells were found in the lesions showing moderate or severe dysplasia than in the lesions showing mild dysplasia.

The distribution patterns or localization of p53 in different strata of epithelium were also assessed in the present study. The expression of p53 in epithelial hyperplasia was localized mainly in basal and parabasal epithelial cells whereas in epithelial dysplasia, p53 positive cells occupied lower two-third (basal, suprabasal and spinous cell layers) of whole thickness of epithelium.

The results of this study are in accordance with those of Piattelli et al.[12] who correlated the progression of preneoplastic lesions to neoplasms with the extension of expression of p53 from suprabasal to spinous layers.

Within different grades of dysplasia, as we move from mild dysplasia to moderate dysplasia and then to severe dysplasia, it was observed that the location of p53 positive cells extended from suprabasal cell layer to the spinous layer, indicating the association of suprabasal staining with the increasing severity of the grades of dysplasia.

The results of the present study are similar to those of Nylander et al.[15], who reported that the suprabasal expression of p53 could indicate the proliferative activity of the suprabasal cells, rather than just the basal cells as expected in normal epithelium. These authors also suggested that there are more chances of malignant transformation in lesions with suprabasal staining.

Based on the obtained results, it may be concluded that presence of p53 is not a specific marker for malignancy of oral epithelium. However, the quantitative analysis of p53 positive cells and their localization in oral epithelium may be a significant biomarker for diagnosis of oral epithelial dysplasia and squamous cell carcinoma.

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