

IMMUNOHISTOCHEMICAL EXPRESSION OF PROTEIN p53 IN ORAL EPITHELIAL DYSPLASIA AND ORAL SQUAMOUS CELL CARCINOMA

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ABSTRACT

The study was carried out to determine the frequency of p53 protein expression in oral dysplastic lesions and oral squamous cell carcinoma and to compare them in both the lesions. Sixty cases (30 each) of oral epithelial dysplasia (OED) and oral squamous cell carcinoma (OSCC) were retrieved from the record files along with their paraffin blocks at the Armed Forces Institute of Pathology, Rawalpindi including twenty fresh/frozen sections. The immunohistochemical marker p53 was applied using Streptavidin-Biotin technique. Staining and intensity of the marker was recorded and frequencies were compared in both the lesions. Protein p53 expression was seen in 60% of oral epithelial dysplastic lesions and 67% of oral squamous cell carcinomas. The results signify the importance of p53 for the early diagnosis and early treatment of dysplastic (precancerous) lesions.

Key words: Oral epithelial dysplasia (OED), oral squamous cell carcinoma (OSCC), p53 protein expression

INTRODUCTION

Oral Epithelial Dysplasia (OED) is a histopathological term. Majority of the patients present with OED in their 6th decade with tongue, buccal mucosa and floor of the mouth being the most commonly affected sites.¹ Dysplasia is categorized into mild, moderate and severe degrees. Severe dysplasia is a precancerous lesion showing a high risk of malignant transformation.² Leukoplakia and erythroplakia are two precancerous (dysplastic) lesions which white and red respectively on clinical examination and show dysplastic epithelial changes on histopathological examination.³ These lesions show a higher risk of transforming into malig-

nancy and the risk increases with increasing histological grade of dysplasia.⁴

Squamous Cell Carcinoma usually originates from a precancerous progenitor followed by proliferation of the clonal foci.⁴ Tobacco is considered the most important risk factor in the development of oral dysplasia and oral SCC. Carcinogens in tobacco affect the normal oral epithelium by increasing the number of aneuploid nuclei.⁵

The tumour suppressor gene p53 acts as a “monitor” in guarding the integrity of the genome.⁶ After DNA damage by carcinogenic agents, wild-type p53 gets activated as a transcription factor to arrest the cell

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cycle for repair of the damaged DNA.⁷ Normal or wild-type p53 gene is a negative regulator of cell proliferation⁸, whereas mutations in the gene are critical for malignant transformation.⁹ It encodes a 393-aminoacid nuclear phosphoprotein¹⁰, which exists normally as wild-type p53 with a short half-life of 6-30 minutes. Mutated p53 has a long half-life and so it can be detected by immunohistochemistry.¹¹

Gene mutation and overexpression of p53 plays a vital role in tumour development.¹² Mutated or inactivated p53 are incapable to induce apoptosis in cells. So the damaged cells continue to proliferate forming a tumour.¹³

In Pakistan, OSCC is among the initial 10 most common neoplasms.¹⁴ Because of a high prevalence of oral cancer in developing countries, the focus should be to detect and treat the precancerous lesions in their early stages.⁴

METHODOLOGY

A total of 60 subjects (30 each) of oral dysplasia and oral squamous cell carcinoma were selected by convenient sampling. Forty formalin-fixed and paraffin-embedded (FFPE) tissue samples were taken from the archives of the Armed Forces Institute of Pathology, Rawalpindi between January 2007 and December 2010. Twenty fresh/frozen sections were taken from the patients of the Oral Surgery Department of the Armed Forces Institute of Dentistry (AFID). Informed written consent was obtained from all such patients.

The patients included 34 males and 26 females, aged between 21-86 years. The data on age, gender and site of involvement were extracted from the clinical records. Histopathological features of the selected samples were reviewed from freshly prepared Hematoxylin & Eosin sections. Diagnosis of oral dysplasia and OSCC was made according to the criteria given by WHO, and the new diagnosis was confirmed by a consultant histopathologist.

Immunohistochemistry: Sections of 5 µm thicknesses were cut from FFPE tissue, and were dewaxed in two washes of xylene. Endogenous peroxidase activity was blocked by incubating in 0.5% hydrogen peroxide in methanol for 10 minutes. Antigen retrieval was done in

citrate by giving pressure for one minute in pressure cooker. One to two drops of Protein Blocking Serum were instilled on each section for 10 minutes in order to block extra antigens. All incubations were done in humidity chamber. Primary antibody p53, a mouse monoclonal antibody recognizing mutant p53 (NovoCastra, 15Mm Sodium Azide, NCL-p53-BP) was used. After washing gently with PBS buffer, the sections were incubated in Biotinylated Secondary Antibody, then in Streptavidin-Biotin complex and in DAB-chromogen for 10 minutes each. Lastly, the sections were washed in tap water and counter-stained with Hematoxylin for 20 seconds. Colon carcinoma was taken as positive control. For negative control, all the steps of IHC were followed except that the primary antibody p53 was omitted. The percentage was evaluated by counting 100 tumour cells in the area of best staining and were then subdivided broadly into four groups;

- , absence of staining or occasional keratinocytes staining
- +, staining of 10- 33% of keratinocytes
- ++, staining of 33-66 % of keratinocytes
- +++, staining of greater than 66% of keratinocytes.

The p53 immunoreactivity was considered positive if greater than 10% of the tumour cells nuclei were stained. The intensity was graded subjectively as mild, moderate and marked.

Data analysis

The data collected in the form of variables were analyzed using the SPSS version 17.0. Frequency of p53 protein expression was calculated along with 95% confidence intervals.

RESULTS

In the 60 patients studied, the male distribution was more as compared to females (1.5:1). Mean age of OED was 54.23years while mean age of OSCC was 55.66years. Buccal mucosa and tongue were the most frequent sites involved by OED and OSCC.

The summary of immunostaining data and staining intensity of p53 in oral dysplasia and OSCC is given in tables 1 & 2 respectively.

Photomicrographs of immunohistochemical p53 staining in oral dysplasia and oral squamous cell carcinoma are shown in figures 1 & 2.

Chi-square test was used to compare frequencies of protein p53 expression in oral dysplasia and oral squamous cell carcinoma. There was no significant difference between the frequencies (table 3).

Frequencies of p53 protein expression in OED and OSCC are shown in table 4.

TABLE 1: EXPRESSION OF p53 IN OED AND OSCC

Lesion type	No. of specimens showing each level of p53 IHC staining				Total
	—	+	++	+++	
OED	12	8	8	2	30
OSCC	10	6	10	4	30

Key:

_ Absence of staining or staining of only occasional keratinocytes, + staining of 10-33% of keratinocytes, ++ staining of 33-66% of keratinocytes, +++ staining of greater than 66% of keratinocytes.

TABLE 2: STAINING INTENSITY OF p53 IN OED AND OSCC

Intensity of p53 staining	OED frequency (n=30)	OSCC frequency (n=30)
Mild	13.3%	10%
Moderate	30%	16.6%
Marked	16.6%	40%

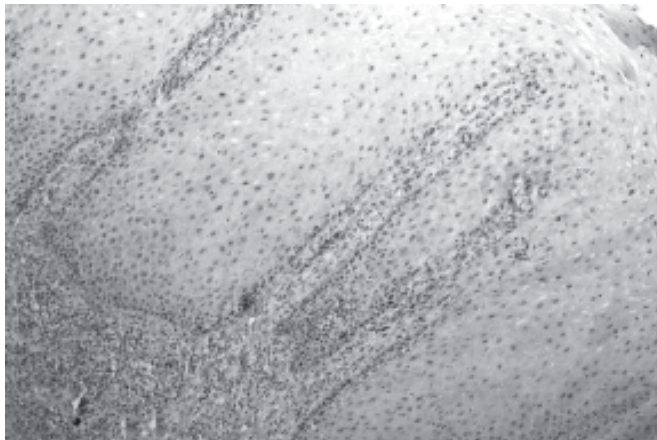


Fig 1: Photomicrograph of p53 Immunostaining in oral dysplasia x100.

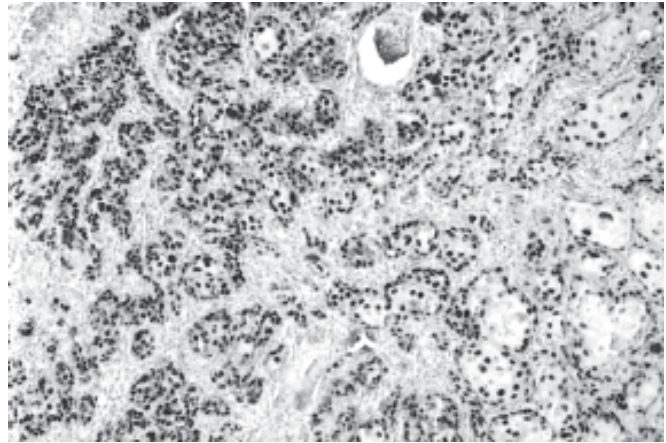


Fig 2: Photomicrograph of p53 Immunostaining in Oral Squamous cell carcinoma x200.

TABLE 3: COMPARISON OF FREQUENCIES OF p53 EXPRESSION IN OED AND OSCC

p53 protein expression	Oral epithelial dysplasia	Oral squamous cell carcinoma	Total
Positive	18	20	38
Negative	12	10	22
Total	30	30	60

Chi-square = 0.29, p = 0.59 (insignificant).

TABLE 4: FREQUENCIES OF p53 PROTEIN EXPRESSION IN ORAL DYSPLASIA AND ORAL SQUAMOUS CELL CARCINOMA CALCULATED ALONG WITH 95% CONFIDENCE INTERVAL

p53 protein expression	Positive cases	Frequency	95% confidence interval
Oral dysplasia	18/30	60%	42-77
Oral SCC	20/30	67%	49-83

DISCUSSION

Oral SCC is usually preceded by precancerous or dysplastic progenitor lesion.⁴ Several researches worldwide have proved that p53 tumour suppressor gene and its protein are altered very early in the process of carcinogenesis.¹¹ Oral epithelial dysplasia (clinically 'Leukoplakia') has shown varying range of malignant transformation worldwide, after follow-up period of several months or years.¹⁵

This study was thus designed to see protein p53 expression in dysplastic lesions, and comparing them with cancer.

Kannan et al¹⁶ conducted a study to see p53 expression in normal mucosa, dysplastic and malignant lesions of the oral mucosa. Overexpression of p53 was seen in 67% of OSCC which is comparable to our study. However, p53 positivity in OED was found in only 13% of cases, which is a low frequency compared to frequency in our study.

In a Pakistani study by Panjwani and Sadiq¹², p53 immunoeexpression was positive in 75% of OED and 76.8% of SCC. The frequencies are slightly higher but comparable to our results in both the lesions.

Ravi et al¹⁷ conducted a study on expression of p53 and bcl-2 proteins in hyperplastic mucosa, dysplastic mucosa and invasive oral carcinoma. All 46 cancer specimens i.e. 100% samples showed positive expression for p53, while all 11 dysplastic lesions (100%) were positive for p53, which is not comparable to this study.

Shoelch et al¹⁸ analyzed expression of cell cycle proteins (p53, Rb, p21, p27, Ki-67, Cyclin D1) in premalignant and malignant oral lesions. Twenty seven percent of dysplastic lesions showed positive expression for p53, while 52% SCC were positive for p53. Lower frequencies were observed as compared to this study, in both the lesions.

Dong Shin et al¹⁹ performed IHC analysis in patients with head and neck SCC. Forty five percent of dysplasia samples expressed p53 protein while 45% of SCC samples showed positive p53 staining. These frequencies are less than those found in the present study.

CONCLUSION

A significant number of patients had p53 expression in oral dysplastic lesions and oral squamous cell carcinomas. The frequencies of expression of p53 in oral dysplasia and oral squamous cell carcinoma were almost similar. The results signify the importance of early diagnosis and early treatment of dysplastic (pre-cancerous) lesions.

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