Original Article

p53 EXPRESSION IN BENIGN, DYSPLASTIC AND MALIGNANT ORAL SQUAMOUS EPITHELIAL LESIONS

Suchita Panjwani[1], Saleem Sadiq[2]

ABSTRACT

Objective: To observe the frequency of benign, dysplastic and malignant oral squamous epithelial lesions and to calculate p53 expression in these lesions.

Methodology: This was a retrospective study. All the oral biopsies received during the period 1st July 2000 to 30th June 2005 were reviewed. Histopathological parameters were noted. Immunohistochemical staining was performed to see the p53 expression in these lesions.

Results: There was a single case of benign lesion/papilloma, which showed basal p53 immunoexpression. The dysplastic lesions account for 10 cases. p53 immunoexpression was positive in 75% cases of the dysplastic lesions. Squamous cell carcnoma was found to be the commonest malignant oral epithelial lesions accounting for 412 cases. Malignancy grading was also performed which showed maximum number of cases between score of 9-12. p53 immunoexpression was found positive in 76.8% cases of squamous cell carcinoma.

Conclusion: Squamous Cell Carcinoma (SCC) was found to be the commonest oral malignant epithelial lesion. p53 immunoexpression was found in 76.8% cases of oral squamous cell carcinoma. The p53 immunopositivity was increasing as the grade score was rising but decreasing further as the tumor became poorly differentiated. Combining histological analysis with p53 immunoexpression, evaluation of dysplastic lesions could be improved.

KEY WORDS: Squamous cell carcinoma, p53.

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INTRODUCTION

The concept of a step wise development of cancer in the oral mucosa, i.e., the initial presence of a precursor (pre-malignant, pre-cancerous) lesion subsequently developing into cancer is well established. The term 'epithelial dysplasia' is assigned to histopathological changes associated with an increased risk of progressing to malignant stage (i.e, squamous cell carcinoma). The presence of epithelial dysplasia may be even more important in predicting malignant development than the clinical characterstics.[1]

The mutations in the p53 (also known as Tp53) gene and its alteration in the p53 protein resulting in its accumulations in cells may play critical role in tumorigenesis.[2] It has been reported that immunoreactivity for p53 protein can be detected in benign tumors and premalignant lesions, including dysplasia of the oral mucosa.[3] p53 alterations can occur early in carcinogenesis and that these alterations are maintained upon progression to overt malignancy.[4] Infact oral cancer may provide an even better model for the study of multistage tumor igenesis because the lesions are easily detected early, are frequently diagnosed at the pre

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invasive stage, are accessible to biopsy and are amenable to accurate follow up evaluation.[5,6]

MATERIALS AND METHODS

This was a retrospective study carried out in the Department of Pathology, Basic Medical Sciences Institute, Jinnah Postgraduate Medical Center, Karachi. All the oral biopsies received over a period of five years 1st July 2000 to 30th June 2005 were reviewed. All the neoplastic oral epithelial lesions were identified. Modified malignancy grading of squamous cell carcinomas was performed on the invasive front.[7] The malignant lesions showing only tumor tissue were excluded for grading purpose.

Immunohisto chemistry: Section of 5um were cut from formalin fixed, paraffin embedded tissues and were mounted on silane coated slides. Antigen retrieval was performed in citrate by pressure cooker technique. A mouse monoclonal antibody recognizing both wild and mutant type p53 (Ready to use clone DO-7, Labvision, USA) was used as the primary antibody. Sections where the primary antibody had been omitted served as negative controls and known p53 positive colon cancer was used as the positive control.

Quantification of Immunohistochemistry results: For benign and dysplastic lesions three categories were defined, namely:

1. Negative, no nuclear p53 staining detected in any epithelial cell.
2. Basal, nuclear p53 staining confined to the basal epithelial layer.
3. Suprabasal, nuclear p53 staining in (basal and) suprabasal epithelial layers.

For malignant lesions also three categories were defined, namely:

1. Negative, no nuclear staining in any tumor cell.
2. +, nuclear p53 staining in less than 25% tumor cells.
3. ++, nuclear p53 staining in more than 25% tumor cells.

RESULTS

In our series there were 412 malignant oral epithelial lesions followed by 10 dysplastic lesions and a single case of a benign lesion (Table-I). Oral epithelial lesions were common in 5th decade of life in males and 4th decade in females. In most of the cases site of the lesion was not specified. Excluding that tongue was the common site of lesion.

There was only a single case of benign lesion i.e. verruca vulgaris (Figure-1), which showed basal p53 immunoexpression (Table-II). However, dysplastic lesion accounts for 10 cases. Out of these three exhibit morphologically papillary pattern with keratosis, parakeratosis and dysplasia while five cases show simple kerato-

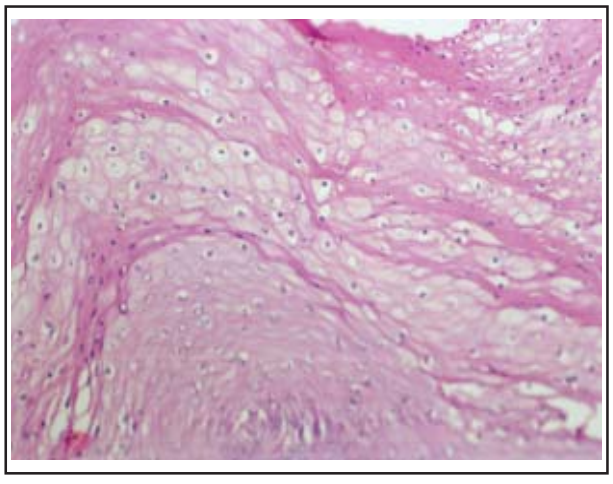

Figure-1: Photomicrograph of verruca vulgaris H& E x 200.

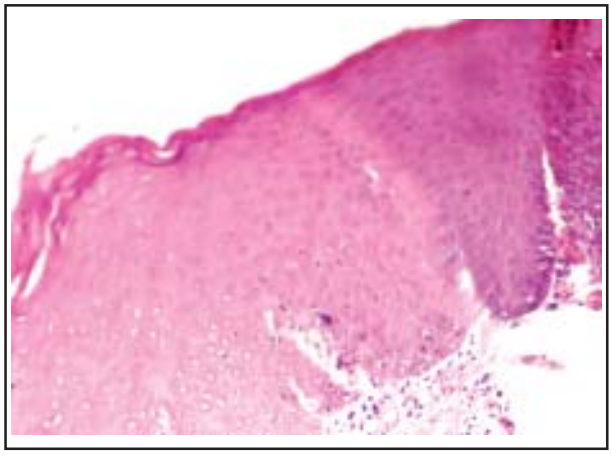

Figure-2: Photomicrograph of verrucous hyperplasia (showing acanthotic epithelium superficial to the margin) H& E 100.

Table-I: Distribution of oral epithelial lesions

Epithelial lesions	*No. of cases*	*%*
Benign lesions		
Papilloma/ verruca vulgaris	01	0.2
Dysplasia		
Mild	10	2.4
Moderate	-	-
Severe	-	-
Carcinoma in situ	-	-
Malignant lesions		
Squamous Cell Carcinoma	412	97.3
Total	423	

sis with mild dysplasia. There were two cases showing features of verrucous hyperplasia (Figure-2). p53 immunopositivity was seen in 8 cases of dysplasia, which showed 75% immunopositivity.

Squamous cell carcinoma (Figure-3) was found to be the commonest neoplastic oral squamous epithelial lesion. There were 379 (92%) cases SCC, NOS. Its variants accounts for 30(7.2%) cases of verrucous carcinoma. One (0.2%) case each of basaloid, papillary and acantholytic SCC was seen (Table-III). Squamous Cell Carcinoma (SCC) was predominantly seen in males. The male to female ratio in our study was 1.19:1. The mean age of the patients was 49.9 years. The peak malignancy score that showed maximum number of cases expressing p53 immunopositivity was 9-12.

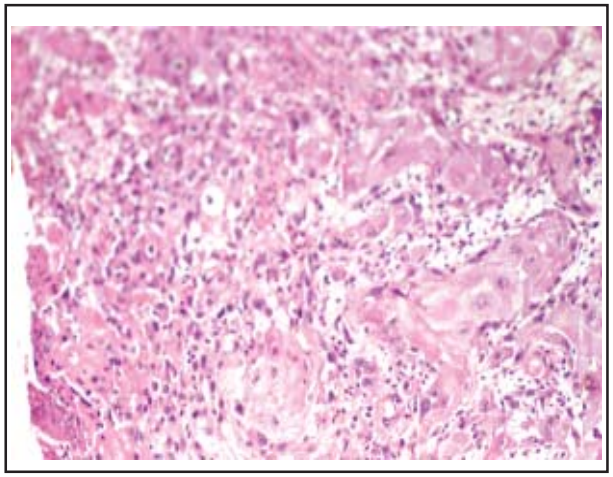

Figure-3: Photomicrograph of squamous cell carcinoma H&E x100.

In our study the p53 immunoexpression by IHC analysis in SCC was seen in 76.8% cases. There were 147(46.5%) cases showing ++ p53 immunopositivity (Fig-4), 93(29.4%) were + positive while 76(24%) cases stained negative for p53 (Table-IV).

DISCUSSION

In our series benign lesions are rare, as only a single case of verruca vulgaris was seen. The p53 immunoexpression in the benign lesion showed basal positivity. No study was found for comparison. However p53 localization was seen in the 75% of the dysplastic lesions. p53 overexpression has been reported with considerable variation in patient population with oral dysplastic lesions from as low as 0% by Ogden et al[8] to a high 55% by Kerdpon et al[9] Ravi et al[10] reported p53 expression in 82% cases with minimal dysplasia whereas Kaur et al[3] reported p53 over expression in 55% cases of dysplasia.

Oral leukoplakia is a premalignant lesion that has been considered to confer increased risk for development of oral cancer.[11,12] It should be emphasized that leukoplakia is a clinical term and its use carries no implication with regard to the histological findings. However, it is recommended that a histological report should always include a statement on the presence or absence of epithelial dysplasia and if present, the assessment of its severity.[13] There were two

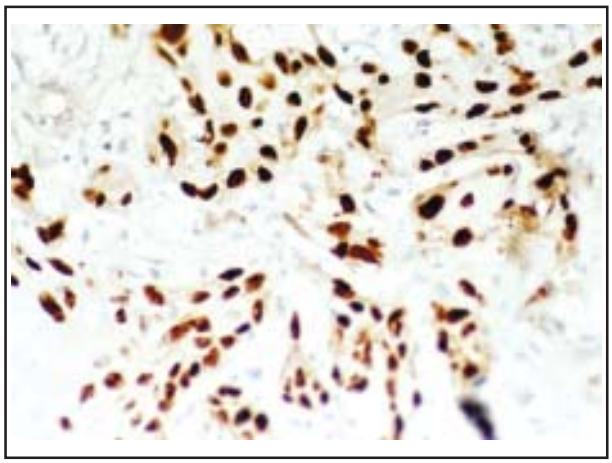

Figure-4: Photomicrograph of squamous cell carcinoma (showing ++ p53 immunoexpression) IHC x200.

Table-II: p53 Expression in benign and dysplastic oral epithelial lesions

Histology	*P53 Immunoexpression*			*Total*	*Percentage %*
	Suprabasal	*Basal*	*Negative*		
Benign lesions					
Verruca vulgaris	-	1	-	1	11.1
Dysplasia					
Mild	2	4	2	8	88.8
Moderate	-	-	-	-	
Severe	-	-	-	-	
Carcinoma in situ	-	-	-	-	
Total	02	05	02	09	

cases showing features of verrucous hyperplasia. Verrucous hyperplasia is the forerunner of verrucous carcinoma and the transition is so consistent that the hyperplasia, once diagnosed should be treated like verrucous carcinoma.[13] The most common neoplastic oral epithelial lesion in our study was squamous cell carcinoma. The male to female distribution in our study is 1.19:1 that is in accordance by Pinholt et al.[14] However the studies in Greek and Brazilian population show quiet a higher ratio of 9.2:1 and 4.8:1 respectively.[15,16] The average age was 49.9 years that was lower than the one reported by Gervasio et al.[16] i.e. 58.6 years and Mirza et al[17] i.e. 54.3%.

Jafarey and Zaidi[18] reported 13% of the cases below the age of 40 years and 0.5 % of the cases were below the age of 20 years. Comparing these results after three decades to our study there is significant rise in oral cancer i.e. 34 % of the cases were below the age of 40 years and 1.9 percent patients were below the age of 20 years. This could be attributed to the fact that children come in contact with pan and tobacco (supari) especially in lower socioeconomic group at very young age so that exposure to carcinogens starts at early age.

Epidemiological studies have shown that the site of occurrence for oral cancer differs widely. Tongue, lip and floor of mouth are the most frequent sites of lesions of squamous cell carcinoma in the oral cavity.[16] In our study excluding the cases in which site was not specified, the tongue is the most commonest site of lesion. There is a slight male predominance in our study, which is in accordance to the other studies in Pakistan and Western countries.[19,20]

We also attempted to do the histopathologic grading of squamous cell carcinoma, which is a modification of a method recommended by Anneroth et al.[21] The cases were separated into three groups which had significantly different prognosis: score 5-8, 9-12 and 13-20 is the total malignancy score representing 19.6%,

Table-III: Malignancy score according to histologic types

Malignant oral epithelial lesions	*Malignancy Score*			*Total*	*Percentage %*
	5-8	9-12	13-20		
Squamous cell carcinoma, NOS	68	187	124	379	92.2
— Verrucous Ca	19	11	-	30	7.2
— Basaloid SCC	-	1	-	1	0.2
— Papillary SCC	-	-	1	1	0.2
— Spindle cell Ca	-	-	-	-	-
— Acantholytic SCC	-	1	-	1	0.2
— Adenosquamous Ca	-	-	-	-	-
— Carcinoma cuniculatum	-	-	-	-	-
Lymphoepithelial Carcinoma	-	-	-	-	-
Total	87	200	125	412	

Table-IV: p53 Immunoexpression in relation to malignancy score.

MalignancyScore	*P53 Immunoexpression*			*Total*	*Percentage %*
	++	+	_		
5-8	26(17.7%)	16(17.2%)	20(26.3%)	62	19.6
9-12	72(49.0%)	51(54.8%)	32(42.1%)	155	49.0
13-20	49(33.3%)	26(27.0%)	24(31.6%)	99	31.3
Total	147	93	76	316	100
Chi-square	32.39	31.4	4.42	-	-
P-value	0.001**	0.001**	0.11*	-	-

*Non-significant , ** Significant

49% and 31.3% which varies markedly from that reported by Bryne et al[7] who found malignancy score representing 82%, 26% and 16% respectively in their study.

In our study the expression of p53 by IHC analysis in SCC was seen in 76.8% cases. Our findings are in agreement with Kaur[3] and Mirza[17] who found p53 immunoexpression in 75% and 73.8% oral squamous cell carcinomas. Langdon and Partridge[5] showed 80% of oral cancer patients from United Kingdom with p53 expression. However other workers found lower frequency of p53 immunoexpression i.e. 45% by Yan et al.[22] 46% by Saranath el at.[23] 36% by Pillay et al.[24] Our findings show that site, age and sex did not influence p53 expression that is in accordance with other studies.[5,19]

There was increase in p53 immunoexpression as the malignancy score rises from 5-8 to 9-12, however this is further followed by decrease in p53 expression as the score rises further i.e. 13-20 showing statistically significant P value.

CONCLUSION

Squamous cell carcinoma was the commonest malignant oral epithelial lesion. Malignancy scoring should be performed at the invasive front of the carcinoma so that histological parameters may properly be assessed. p53 immunoexpression was present in 76% cases of squamous cell carcinoma. No correlation of p53 immunoreactivity with age, sex and site was found. The immunopositivity was increasing as the malignancy score was raising but decreasing further as the tumor became poorly differentiated. Dysplastic lesions also showed p53 immunoexpression in 75% cases. Based on these findings combining histological analysis with p53 immunoexpression, evaluation of dysplastic lesions could be improved.

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