Original Article

Expression Of P63 In Oral Dysplastic And Malignant Squamous Cell Carcinoma

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Abstract

Introduction: Oral cancer is one of the most commonly found cancers in the general population with variations in incidence rates in different countries. Squamous cell carcinoma(SCC) is the commonest carcinoma of the oral cavity, constituting 90 percent. A large number of OSCC are followed by the premalignant lesion. P63, which is a part of the p53 family protein, is a known basal cell immunohistochemical marker. Overexpression of P63 is found in many tumors including oral SCC. By changing stem cell function in the basal layer P63 may have its contribution to the development of epithelial dysplasia and this may play role in oral carcinogenesis.

Objective: The aim was to determine the P63 IHC marker expression in oral epithelial dysplasia and Squamous cell carcinoma (SCC) biopsy specimens.

Methods: Present study included ninety-five cases, included 41 of dysplasia and 54 of SCC that were evaluated for P63 immunohistochemical expression and correlated this expression on different grades of dysplasia and SCC.

Results: P63 positivity was found in 100% cases of dysplasia and 96.3% cases of SCC. A highly significant P-value was observed between the immunoreactive score for P63 staining in various grades of dysplasia and SCC.

Conclusion: The current study found increased P63 positivity in increasing severity of dysplasia and SCC. Increase P63 positivity in increasing grades of dysplasia and SCC may reflect the major role of this protein in the development of OSCC, and might be a useful indicator of dysplastic change. On the basis of these results, P63 might be a beneficial marker for improved prognosis of dysplasia and SCC.

Keywords Premalignant disorders, Squamous cell carcinoma, Immunomarker

Introduction

Oral cancer is one of the most commonly found cancer in the general population with variations in incidence rates in different countries. India having a high occurrence rate of oral cavity cancer.[1]This cancer is dominant among all cancers in Pakistani male population. It is found among women too, however, men having a higher percentage due to their smoking and tobacco chewing habits. [2] SCC is the commonest carcinoma of the oral cavity, constituting 90 percent.[3] Many genetic events take part in mouth cancer by altering the normal functioning of both tumor-promoting

and inhibiting genes.[4]This genetic alteration leads to the formation of protein that promote the growth of oncogenes and loss of tumor suppressor genes. A large number of OSCC are followed by premalignant lesions, among them, leukoplakia is the most common one, showing different degrees of dysplasia ranging from mild to severe. The possibility of happening an invasive tumor rises with increasing grade of dysplasia.[5] The prediction of malignant transformation of the premalignant lesion is very difficult, so the better method for early diagnosis would be to develop immunomarkers, which helps to identify initial genetic changes in these lesions and may promote the identification of those lesions which have likely to develop into OSCC.[5,6]

P63,which is a part of p53 family protein , present on chromosome $3q^{27-2}$, is a known basal cell immunohistochemical marker. Alternative splicing in the molecular structure, results in the formation of multiple variants isoforms, like trans-activation p63 and delta negative p63. It is believed that the TAp63variant has essential involvement in tumor suppression and the ΔNp63 variant behaves like oncogenes.[1] Overexpression of P63 is found in multiple tumors, especially in SCC of the lung, skin, cervix, and head and neck. Many of them overexpress the ΔNp63 isoforms.[7] Increase P63 expression is also seen in invasive urothelial carcinoma, Intestinal-type gastric cancer, and adenocarcinoma of pancreas. [8] By changing stem cell function in the basal layer this protein may have contribution to the development of epithelial dysplasia. This results in an increased number and disturbed distribution of proliferating cells in basal and suprabasal layers within oral epithelial dysplasia.[6]

The disordering in the distribution of p63-positive cells in the upper layers of oral epithelial dysplasia alter with varying grades, thus it may play role in oral carcinogenesis.[9]. The present study aims to determine the P63 expression in biopsy specimens of dysplasia and SCC.

METHODOLOGY

This was a retrospective cross-sectional study involving histopathologically diagnosed cases of oral dysplasia and SCC, that were retrieved from the histopathology department of BMSI, JPMC Karachi from 2018 - 2021. The study was approved by the ethical review board (ERB) of JPMC. Total 41 cases of dysplasia and 54 cases of SCC were evaluated for P63 expression by using non-probability convenience sampling. World Health Organization grading system as mild, moderate and severe for dysplasia [10] and well-differentiated (WD) , moderately differentiated (MD), and poorly differentiated (PD) for SCC was done.[11]

Immunochemistry

The P63 staining was done on sections mounted on slides coated with poly-L-Lysine. The procedure for the marker was performed on the basis of recommendations provided by the manufacturer. Dewaxing and rehydration of tissue sections were done by xylene changes and decreasing concentration of alcohol. Antigen unmasking was performed by using tris Hcl buffer PH 9.0 in a steamer. After blocking peroxidase the sections were stained with primary monoclonal anti-Human, P63 protein(Clone DAK-P63 from Dako). After incubation with secondary antibody, sections were treated with Di-aminobenzidine chromogen for visualization. Sections of normal skin tissue were taken as positive control. Brown nuclear staining of P63 in lining epithelium in dysplasia. [6] and in tumor cells in SCC.[11] was indicated as positive. The positive P63 staining was graded on the basis of prominent staining intensity as (+) weak, (++) moderate, and (+++) strong staining.[6] All slides were reviewed by 02 senior pathologists.

Data analysis

The data was collected and statistically analyzed by using SPSS 21 version. Statistical significant was considered as $P \leq 0.05$ by using the chi-square test .

RESULTS

Total 95 cases were selected in present study for P63 immunostaining. Distribution of cases are illustrated in table 1.

Table: 1 Grade wise distribution of selected cases of dysplasia and SCC

Morphological grade	cases number (no=95)	%
Dysplasia		
Mild	13	13.7
Moderate	15	15.8
Severe	13	13.7
Total	41	43.2
SCC		
WD	17	17.9
MD	17	17.9
PD	20	21.9
Total	54	56.8

P63 positivity was found in 100% cases of dysplasia and 96.3% cases of SCC. In a total of 13 mild dysplasia, the majority, 10(76.9%), showed weak (+) nuclear positivity. Among the 15 moderate dysplasia, most of them, 8(53.3%), showed moderate (++) immuno positivity. Among the 13 severe dysplasia, bulk of them, 8(61.5%), showe strong (+++) immunopositivit .Table 2

Table :2 P63 immunoreactivity in selected cases of dysplasia

Morphology	Grade	P63 immunopositivity				Total number	P-value
		0	+	++	+++		
Dysplasia	Mild	0(0%)	10(76.9%)	3(23.1%)	0(0%)	13(100%)	<0.001
	Moderate	0(0%)	2(13.3%)	8(53.3%)	5(33.3%)	15(100%)	
	Severe	0(0%)	2(15.4%)	3(23.1%)	8(61.5%)	13(100%)	
	Total	0(0%)	14(34.1%)	14(34.1%)	13(31.7%)	41(100%)	

< 0.05 was considered statistically significant p-value

Table: 3 P63 immunoreactivity in selected cases of SCC

Morphology	Grade	P63 immunopositivity				Total number	P-value
		0	+	++	+++		
SCC	WD	0(0%)	4(23.5%)	5(29.4%)	8(47.1%)	17(100%)	0.003
	MD	2(11.8%)	1(5.9%)	4(23.5%)	10(58.8%)	17(100%)	
	PD	0(%)	3(15%)	1(5%)	16(80%)	20(100%)	
	Total	2(3.7%)	8(14.8%)	10(18.5%)	34(63%)	54(100%)	

< 0.05 was considered statistically significant p-value

Among SCC, 8(47.1%) WD tumor , 10(58.10%) MD tumor and 16(80%) PD tumor showed strong (+++) P63 immunoreactivity. Table 3.

Discussion

A considerable proportion of cancer cases in Pakistan are of oral cavity. By applying immunomarkers, the identification of mouth tumors in the initial stage provides a perspective of preventive treatment by repressing the disease in initial stage. Present work utilized P63 monoclonal antibody to clarify the role of this tumor suppressor gene in the pathogenesis of oral dysplastic and malignant SCC.

Studies have shown that an increase in the severity of dysplasia increases the risk of development of carcinoma .[10]

P63 is a nuclear stain in normally stratified squamous epithelium, presence of this marker is restricted to the basal cell layers. It have its contribution in the development of dysplasia by altering stem cell function in the basal layer .[5]

In present study among 41 cases of dysplasia all the cases (100%) displayed positive nuclear P63 immunoreactivity. Increase expression of P63 was found with increasing severity of dysplasia. Present findings are comparable with Patel et al. who observed, All(100%)of the cases displayed P63 positivity, among them, most of mild dysplasia cases showed weak expression, while most of severe dysplasia showed strong P63positivity.[6]

Weak and moderate expression of P63 in mild and moderate dysplasia, and intense P63 expression in severe dysplasia was reported by Ramasubramanian et al. which is similar to the current study. Increased P63 expression in dysplastic epithelium, when compared to normal mucosa, was also reported by Sinha et al. [8,12]

Bavle et al. observed that high-grade cases of all Potentially malignant disorders showed P63 positive cells involving the upper part of the epithelium as compared to low-grade cases.[5]

Current study results found p63 immunopositivity increases with increasing grades of dysplasia, this may well be a prediction of any preceding malignant transformation and may thus act as biomarkers for oral cancer progression

In current study 52(96.3%) cases of SCC displayed positive P63 immunoreactivity. Dayakar et al. observed smilar results that is 96.15% cases showed positive P63 expression Moergel et al. and Patel et al. found 100% of cases displayed P63 immunopositivity which are also closely similar to current study. In present study 8(47.1%) WD tumor, 10(58.10%) MD tumor and 16(80%) PD tumor showed strong positive immunoreactivity for P63. [1,6,13]

Convincing differences between P63 expression and different grades of SCC are also observed by Sanghravanian et al. and Sinha et al.[11,12]

2(11.8%) cases of MD tumors in present study, showed negative immunoreactivity for P63.

Negative results were also reported by Dayakar et al. and Shetty et al. in their study regarding P63 expression , they suggested that these observations are due to dedifferentiation of the tumor cells resulting in absence of P63 expression.[1,14]

In present study and all of above studies, increase P63 expression with increasing severity of dysplasia and SCC may reflect major role of this protein in the development of OSCC.

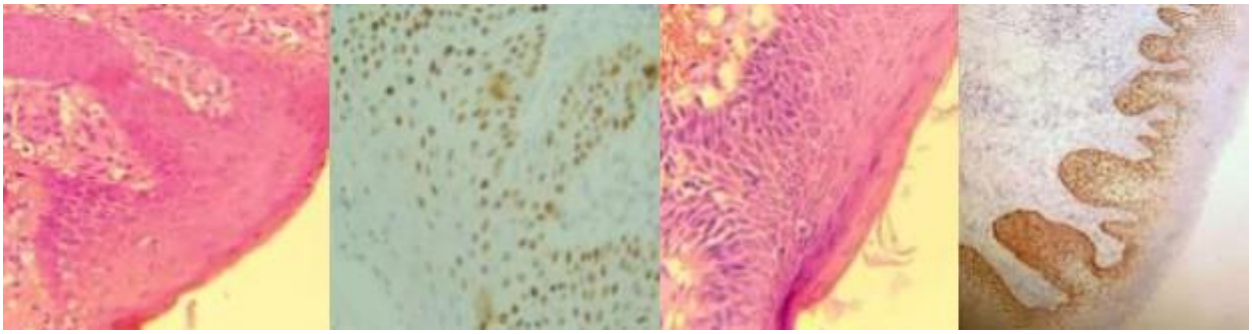
Conclusion

This study found that the expression of p63 correlates with high histological grades of dysplasia and SCC.

In current study, P63 expression with rising severity of dysplasia may be a possible sign of malignant transformation in dysplastic lesions and may serve as a prognostic biomarker for oral SCC.

These results can possibly help in deciding the modalities of treatment planning for oral squamous cell carcinoma patients.

Similar studies with large sample size will provide additional insight into oral carcinogenesis and the role of P63.

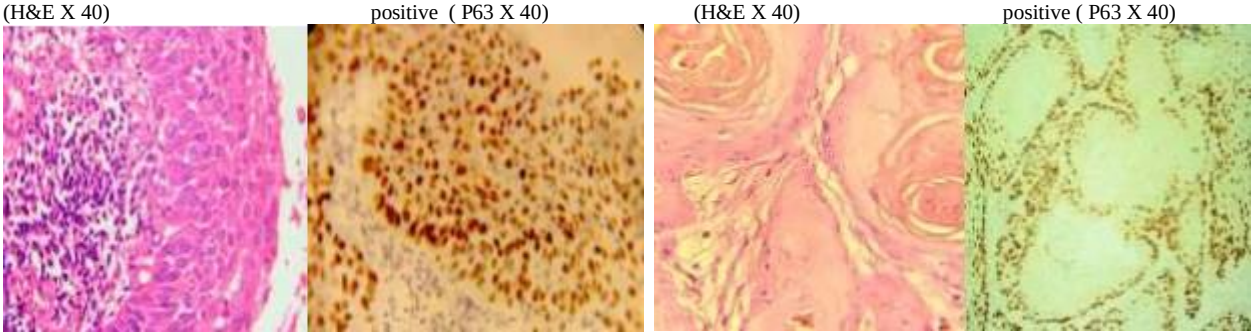


Pic 1: Mild dysplasia (H&E X 40)

Pic 2: Mild dysplasia positive (P63 X 40)

Pic 3: Moderate dysplasia (H&E X 40)

Pic 4: Moderate dysplasia positive (P63 X 40)

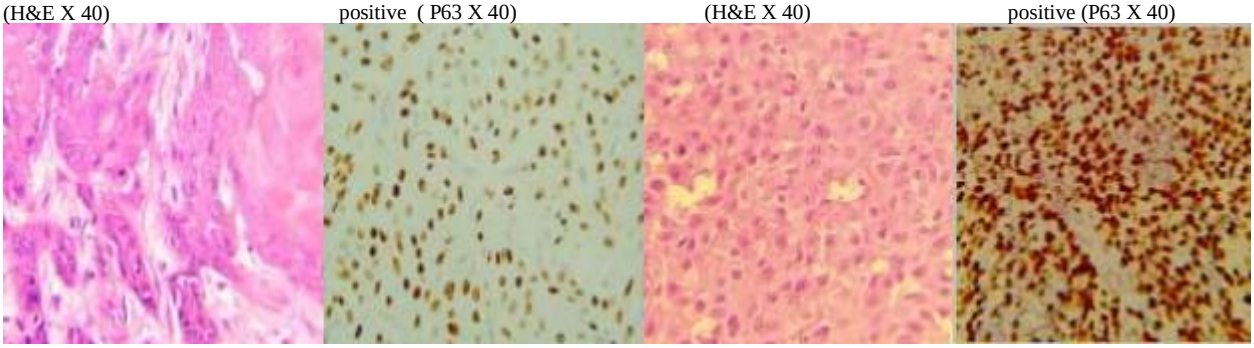


Pic 5:Severe dysplasia (H&E X 40)

Pic 6: Severe dysplasia positive (P63 X 40)

Pic 7: Well differentiated SCC (H&E X 40)

Pic 8: Well differentiated SCC positive (P63 X 40)



Pic.9: Moderately differentiated SCC(H&E X 40)

Pic.10 Moderately differentiated SCC positive (P63 X 40)

Pic.11: Poorly differentiated SCC (H&E X 40)

Pic.12: Poorly differentiated SCC positive (P63 X 40)

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