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Original Article

Association of p53 and Bax in Oral Epithelial Dysplasia and Oral Squamous Cell Carcinoma – An Immunohistochemical study

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***ABSTRACT*:**
Aim: The aim of the study was to analyze the immunoexpression of p53 and Bax and to investigate the relationship of p53andBax in Oral Epithelial Dysplasia (OED) and Oral Squamous Cell Carcinoma (Oral SCC). **Material and Methods:** This study was performed on histopathologically confirmed samples of 90 OED (30 mild, 30 moderate and 30 severe dysplasia), 90 samples of Oral SCC (30 well differentiated, 30 moderately differentiated and 30 poorly differentiated SCC) and 30 oral epithelium for p53 and Bax protein expressions by immunohistochemical (IHC) method. The data were analyzed using chi-square and Kruskal-Wallis tests. **Result:** The quantification of IHC study result revealed that the expression of p53protein was confined to the basal layer but, Bax was expressed in both basal and suprabasal layers in oral epithelium. Whereas, p53 and Bax were expressed in basal, suprabasal layers in OED and Oral SCC. The expression of p53 and Bax was 16.7% and 56.7% in oral epithelium respectively. The p53 expression in mild, moderate and severe dysplasia was 76.7%, 83.3% and 93.3% respectively, the Bax expression was 86.7%, 60% and 50%.The p53 expression in well, moderate and poorly differentiated Oral SCC was 90%, 60% and 40% respectively,Bax expression was 73.3%,40% and 26.7%. The comparison between oral epithelium, OED and Oral SCC showed statistically significant for p53 (p-0.000) and Bax(p-0.028). The association between p53 and Bax expression was not significant in oral epithelium (p-0.568) and OED (p-0.174) while, it was significant in Oral SCC (p-0.000). **Conclusion**: The immunoexpression of p53 and Bax showed significant among oral epithelium, OED and Oral SCC. The significant association of p53 and Bax in Oral SCC suggested that the influence of p53 on Bax induced apoptosis probably played a role in malignancy.
Key words: Oral epithelial dysplasia, oral squamous cell carcinoma, pro apoptotic protein, tumor suppressor.

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INTRODUCTION

Oral SCC is an epithelial neoplasm and sixth most common malignancy worldwide and third most common cancer in South- East and Central Asian region of all cancers. Globally, around 500,000 new oropharyngeal growths are analyzed every year, and 75% of these are from the developing countries with a high rate of morality.[1, 2] The highest incidence of Oral SCC are found in the Indian subcontinent, reported as 12.6% per 10,000 populations with increased risk attributed by the habits of chewing tobacco, betel quid and areca-nut.[3-4]

The development of oral cancer is a multistep process comprising genetic mutations and chromosomal abnormalities. The stepwise transition from normal oral epithelium to oral dysplasia and oral cancer are the consequences of several genetic and epigenetic changes.[5] The imbalance in harmony between proto-oncogenes and tumor suppressor genes plays a crucial role in triggering and promoting cancerous growth in Oral SCC.[6]

When the cellular and molecular alteration restricted to the surface epithelial layers, it is termed oral epithelial

dysplasia which has an unpredictable course of malignant transformation.[7,8] There are complex molecular mechanisms involved in transition from oral leukoplakia to Oral SCC and numerous biomarkers can be used to predict the disease progression.[9] Our study was aimed to evaluate the association of p53 on Bax in OED and Oral SCC.

MATERIAL AND METHOD:

Specimen collection

This retrospective study performed on 10% neutral buffered formalin-fixed, paraffin embedded specimens, were retrieved from the archives of Department of Oral Pathology and Microbiology, Vinayaka Mission's Sankarachariyar Dental College, Salem from the period of February 2010 to March 2018 (VMSDC/IEC/Approval No.071).

Based on world Health Organization, the specimens were segregated according to their histological grades.[10,11] 90 samples of OED (30 mild, 30 moderate and 30 severe dysplasia), 90 samples of Oral SCC (30well differentiated, 30 moderately differentiated and30 poorly differentiated SCC) and 30 samples of oral epithelium were included for p53 and Bax protein expressions by IHC method.

Immunohistochemical procedure for p53 and Bax

The primary antibodies used were the Mouse Monoclonal p53 (Clone BP -53-12, 1: 100 dilution; Path in Situ, US) and the Rabbit Monoclonal Bax (Clone E63, pre-dilution form- Bio SB, CA) in a pre-diluted form. All retrieved paraffin-embedded tissues were cut into 3 to 5 µm thickness and placed over positive charged slides (Path in Situ). Then, slides were deparaffinized using xylene and rehydrated with graded alcohol. Immunohistochemical technique was performed according to the supplier's data sheet. Trisodium citrate solution was used for antigen retrieval (pH 7.4- 7.8 for p53 and pH 6 for Bax), then incubated in 6% peroxidase blocker. The sections with primary antibody for p53 and Bax were incubated at room temperature for 2hours and 3 hours respectively. The antibodies were detected using DAB chromogen and counterstained with Mayer's hematoxylin.

Interpretation of immunohistochemistry

Presence of brown colored end product at the target site antigen (nucleus for p53 and cytoplasm for Bax) was considered as a positive reactivity. The results were expressed in positive percentage of cells after counting 100 cells in 10 consecutive high power fields. The percentage of positive cells was assigned according to the grading system given by Kowichi Nakagawa et al.[12]

Negative : 0-5% of positive cells
Weakly positive : 5% to 25% of positive cells
Moderately positive : 25% to 50% of positive cells
Strongly positive : >50% of positive cells

Statistical analysis

The data were analyzed using IBM SPSS version 21.0statistical software. Kruskal-Wallis test was applied to compare the expression between oral epithelium, OED and Oral SCC along with their histopathological grades. Chi -square test was applied to evaluate the association between p53 and Bax expression in all groups.

RESULTS

Clinical distributions of samples

The clinical characters (sex and site distribution) of selected samples were summarized (Table I).

Table I: Clinical details of the sample

Clinical Data		Oral Epithelium (n=30)	Oral Epithelial Dysplasia (n=90)			Oral Squamous Cell Carcinoma (n=90)		
			Mild Dysplasia(n=30)	Moderate Dysplasia (n=30)	Severe Dysplasia (n=30)	WDSCC (n=30)	MDSS (n=30)	PDSCC (n=30)
Gender	Male	17(56.7%)	21(70%)	21(70%)	23(76.7%)	19(63.3%)	22(73.3%)	16(53.3%)
	Female	13(43.3%)	09(30%)	09(30%)	07(23.3%)	11(36.7)	08(26.7%)	14(46.7%)
Site	Buccal mucosa	14(46.6%)	26(86.7%)	26(86.7%)	29(96.7%)	09(30%)	09(30%)	15(50%)
	Gingiva	10(3.3%)	03(10%)	01(3.3%)	0	09(30%)	09(30%)	05(16.7%)
	Floor of the mouth	0	0	02(6.7%)	01(3.3%)	03(10%)	04(13.3%)	02(6.7%)
	Tongue	0	01(3.3%)	01(3.3%)	0	05(16.7%)	03 (10.0%)	07(23.3%)
	Palate	06(20%)	0	0	0	04(13.3%)	05(16.7%)	01(3.3%)

WDSCC: Well differentiated squamous cell carcinoma; MDSCC: Moderately differentiated squamous cell carcinoma; PDSCC: Poorly differentiated squamous cell carcinoma

Table II: Expression and comparison of p53 and Bax in oral epithelium, oral epithelial dysplasia and oral squamous cell carcinoma.

Oral epithelial dysplasia	Percentage of positive cells	p53	Bax	p-value
Oral epithelium (n=30)	0-5%	25(83.3%)	13(43.3%)	0.568
	5-25%	04(13.3%)	06(20.0%)	
	25-50%	01(03.3%)	03(10.0%)	
	>50%	0	08(26.7%)	
Oral epithelial dysplasia (n=90)	0-5%	14(15.6 %)	31(34.4%)	0.174
	5-25%	21(23.3%)	08(8.9%)	
	25-50%	24(26.7%)	13(14.4%)	
	>50%	31(34.4%)	38(42.2%)	
Oral squamous cell carcinoma (n=90)	0-5%	33(36.7%)	48(53.3%)	0.000*
	5-25%	12(13.3%)	06(06.7%)	
	25-50%	24(26.7%)	12(13.3%)	
	>50%	21(23.3%)	24(26.7%)	

* p value < 0.001 : Highly significant

Detection of p53 and Bax in Oral epithelium, OED, and Oral SCC

The expression of p53 was confined in basal /parabasal layers in oral epithelium but Bax was expressed in basal and suprabasal layers in oral epithelium. Whereas, p53 and Bax were expressed in basal, suprabasal layers in OED and Oral SCC. The result of immunoexpression of p53 and Bax in the oral epithelium, OED and Oral SCC was summarized in table II.

Comparison of p53 and Bax expression

The comparison between oral epithelium, OED and Oral SCC showed statistically significant positive cells for p53 and Bax. The expression of positive cells was also significant between histological grades OED and Oral SCC. The association between p53 and Bax expression was not significant in oral epithelium (p-0.568) and OED (p-0.174) whereas, it was significant in Oral SCC (p-0.000) (Table 2).

DISCUSSION

Normal epithelium constitutes proliferative compartment of basal/ parabasal layers and differentiating compartment of superficial layers. Usually immuno expression of p53 in the normal epithelium is unappreciated in basal layer. In case, p53 expression is positive, it is confined to the basal layer. This might have occurred due to the normal physiological response of cells to genotoxic stress (physical, chemical or microbiological).[13,14] However, wild-type p53 never accumulate in supra basal layer because of its short half - life and putative DNA got repaired prior to its replication.[15] Whereas, Bax expression was greater in suprabasal layers than the basal layer.[16,17] In our study also, p53 expression limited to basal layer, but Bax expression was greater in suprabasal layer.

In oral epithelial dysplasia, p53 expression was detected in basal and supra-basal layer of the epithelium. This expression pattern of p53 is likely to reflect the presence of mutant protein and diminished turnover of cells will persist for a longer period of time. It also indicated the presence of damaged DNA cells in differentiating compartments which have withdrawn from the cell cycle.[13] In contrast, reduced Bax expression in supra – basal layer indicateda deregulation of the apoptosis mechanism, preventing the death of genetically damaged cell.[18,19] In our study result also similar expressions were reflected in oral epithelial dysplasia groups.

The p53 positive cells were significantly increasing between histological grades of OED which coincides with Woods *et al* [13] and dissimilar with Regezi*et al* [20]. The Bax expression pattern showed a significant association which coincides with a study by Sousa *et al.*[21]. Strikingly, expression of p53 and Bax proteins were inversely proportioned in OED. This pattern of expression in literature reflects an irreversible process of the carcinogenic pathway, hence this might be considered as a predictive maker for malignant transformation.[13,20]

In this study, expression of p53 and Bax proteins were significantly associated with histological gradesin Oral SCC. It also revealed that greater number of positive samples present in well differentiated squamous cell carcinoma than moderately differentiated squamous cell carcinoma and poorly differentiated squamous cell carcinoma. The similar findings were observed in literatures.[22-24] But, p53 and Bax expressions showed a greater number of >50% positive cells in poorly differentiated squamous cell carcinoma and well differentiated squamous cell carcinoma respectively. These phenotypic expressions may indicate the difference in biological activity. This observation might have occurred due to an amount of more stable transcribed

protein existent in the dysplastic epithelial islands of Oral SCC.[25]

The study inference suggest, the expressions of p53 and Bax proteins were inversely proportioned in OED, which might indicate their role in early stages of oral carcinogenesis. Whereas in Oral SCC, the pattern of expression was greater in highly differentiated tumor than less differentiated tumor, suggest a good prognostic role of Bax and p53 proteins. But, the reduced p53 and Bax expressions in less differentiated tumors proposed a loss of its apoptotic potential and high proliferation of cancer cells. The down regulation of p53 and Bax proteins in less differentiated tumors might lead to increased cell proliferation and cease of apoptosis, resulting in development of a more aggressive tumor with the poor prognosis. [19,26]

In this study, the association p53 and Bax protein showed a statistically significant in Oral SCC. This corresponds with results of Rehab et al [27] and he stated that p53 may induce apoptotic cell death by down-regulation of Bcl-2 and up regulation of Bax expression.[28] The inference suggested that p53 mediated apoptosis through Bax presumably occurred in Oral SCC.

CONCLUSION

The immunohistochemical study of p53 and Bax indicated the significant pattern of expression among oral epithelium, OED and Oral SCC. The significant association of p53 and Bax in Oral SCC suggested that the influence of p53 on Bax induced apoptosis probably played a role in malignancy. Further challenge is to elucidate mutant p53 from wild-type and analyze p53 linked Bax mediated apoptosis with other signaling pathways might contribute to ascertain the direct relation of p53 mediated apoptosis in oral malignant tumors.

REFERENCES:

1. Jatin K Nagpal, Bibhu R Das. Oral cancer: reviewing the present understanding of its molecular mechanism and exploring the future directions for its effective management. Oral Oncology. 2003;39(3):213-221.
2. Liviu Feller, Johan Lemmer. Oral Squamous Cell Carcinoma: Epidemiology, Clinical Presentation and Treatment. Journal of Cancer Therapy 2012;3:263-268.
3. Petersen PE. Strengthening the prevention of oral cancer: the WHO perspective Community Dent Oral Epidemiol. .2005;33(6):397-9.
4. Dundy G, Kumar H, Singh A, Chandrakanta. p53 immunohistochemical staining patterns in oral squamous cell carcinoma. Journal of Pathology of Nepal 2016;6:1013-1017.
5. Patrick K. Ha , Steven S. Chang , Chad A. Glazer , Joseph A. Califano , David Sidransky, Molecular techniques and genetic alterations in head and neck cancer, Oral Oncology. 2009;45:335–339.
6. F.DostB ,K.Lê Cao, P.J.Ford, C.Ades,C.S.Farah, Malignant transformation of oral epithelial dysplasia: a real-world evaluation of histopathologic grading. Oral Surg Oral Med Oral Pathol Oral RadiolEndod. 2014;117(3):343-352.
7. Jaffer A. Shariff and Athanasios I. Zavras, Malignant Transformation Rate in Patients Presenting Oral Epithelial Dysplasia: Systematic Review and Meta-Analysis, Journal of Oral Diseases. 2015; Article ID 854636,10 pages
8. Ioanina PARLATESCU, Carmen GHEORGHE, Elena COCULESCU, and Serban TOVARU. Oral Leukoplakia – an Update, Maedica (Buchar). 2014;9(1):88–93.
9. Basu A, and Haldar S. The relationship between Bcl2, Bax and P53: consequences for cell cycle progression and cell death. Molecular Human Reproduction. 1998;4(12):1099-1109.
10. Hartwell,L.H and Kastan ,M.B, cell cycle control and cancer , Science, 1994,266:1821-1828.
11. Barnes, L., Eveson, J.W., Reichart, P., Sidransky, D. World Health Organization Classification of Tumours. Pathology and Genetics of Head and Neck Tumours. Lyon: IARCPress; 2005. 430p
12. Nakagawa K, Yamamura K, Maeda S, chihashi M.bcl-2 expression in epidermal keratinocytic disease. Cancer 1994;74:1720-124.
13. Abbas NF, Labib El-Sharkawy S, Abbas EA, Abdel Monem El-Shaer M. Immunohistochemical study of p53 and angiogenesis in benign and preneoplastic oral lesions and oral squamous cell carcinoma. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2007;103(3):385-90.
14. Hall PA, McKee PH, Menage HP, Dover R, Lane DP. High levels of p53 protein in UV irradiated normal human skin. Oncogene 1993;8:203–207.
15. Suni Ann Thomas and Sethupathy. S. Expression of apoptotic markers in patients with oral squamous cell carcinoma (OSCC). IOSR Journal of Dental and Medical Sciences. 2014;13(11):78-81.
16. Zhang YY, Deng T, Hu ZF, Zhang QP, Zhang J, Jiang H. Mechanisms of inhibiting proliferation and inducing apoptosis of human gastric cancer cell line SGC7901 by ursolic acid. Ai Zheng. 2006;25:432-437.
17. Piattelli A, Rubini C, Fioroni M, Iezzi G, Santinelli A. prevalence of p53, bcl-2, and Ki-67 immunreactivity and of apoptosis in normal oral epithelium and in premalignant and malignant lesions of the oral cavity. J Oral MaxillofacSurg 2002;60(5):532-40.
18. Abrahao AC, Bonelli BV, Nunes FD, Dias EP, Cabral MG. Immunohistochemical expression of p53, p16 and hTERT in oral squamous cell carcinoma and potentially malignant disorders. Braz Oral Res. 2011;25(1):34-41.
19. Fernando A.C.G. de sousa, Thais C. Paradella, YasminR.Carvalho and LuizE.B.Rosa. Immunohistochemical expression of PCNA, p53, bax and bcl-2 in oral lichen planus and epithelial dysplasia. Journal of Oral Science 2009;51:117-121.
20. Regezi JA, Zarbo RJ, Regev E, Pisanty S, Silverman S, Gazit D. p53 expression in sequential oral dysplasias and in situ carcinomas. J Oral Pathol Med 1995;24:18–22.
21. KhorGootHeah, Mohamed Ibrahim Abu Hassan, Siar Chong uat. P53 expression as a marker of Microinvasion in Oral squamous cell Carcinoma. Asian Pasific Journal of Cancer Prevntion 2011;12:1017-1022.
22. Van der Toorn , Veltman J, Bot F, et al. Mapping of resection margins of oral cancer for p53 overexpression and chromosome instability to detect residual (pre) malignant cells. J Pathol 2001;193:66-72.
23. Van Oijen M, Slootweg P. oral field cancerization: carcinogen-induced independent events or micrometastatic deposits? Cancer Epidemiol Biomarkers Prev. 2000;9:249-56.
24. ShimaNafarzadesh, Sina Jafari, Ali Bijani. Assessment of Bax and cl-2 immunoexpression in patients with Oral lichen Planus and Oral Squamous Cell Carcinoma. Int J Mol Cell summer. 2013;2:136-142.

25. ThanaaEl.A. Helal, Mona T.adel, Abdalla K. EL-Thobbani, AmiraM.l-Sarhi. Immunoexpression of p53 and hMSH2 in oral squamous cell carcinoma and Oral dysplastic lesion in Yemen: Relationship top oral risk habits and prognosic factors. Oral oncology 2012;48:120-124.
26. Iamaroon A, Khemaleelakul U, Pongsiriwet S, Pintong J. Co-expression of p53 and Ki67 and lack of EBV expression in oral squamous cell carcinoma. J Oral Pathol Med.2004;33(1):30-36.
27. Rehab FawziKasem , Dina SolimanKhater, Ghada A. Abdel-Latif, OlfatGamil Shaker. Expression of miR-34a/p53 and Their Apoptotic Target Bax in Oral Squamous Cell Carcinoma. International Journal of Cancer Research. 2018;14(1):32-38.
28. Miyashita T, Krajewski S, Krajewska M, Wang HG, Lin HK, Liebermann DA, Hoffman B, Reed JC. Tumor suppressor p53 is a regulator of bcl-2 and bax gene expression in vitro and in vivo. Oncogene.1994;9(6):1799-805.

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