Original Article

Comparison of p53 Protein Expression between Oral Squamous Cell Carcinoma and Verrucous Carcinoma

Anil Singh[1], Shikha Saxena[2], Safia Siddiqui[3], Ruchita Bali[4], Priyanka Singh[5]

[1]Professor, Department of Dentistry, Government Medical College and Associated Hospital, Rajouri, Jammu and Kashmir; [2]Associate Professor, Department of Oral Pathology and Microbiology, RUHS College of Dental Sciences, Jaipur, Rajasthan; [3]Ex Associate Professor, Department of Dentistry, Integral Institute of Medical Sciences and Research, Lucknow; [4]Professor, Department of Oral Pathology and Microbiology, Shree Bankey Bihari Dental College, Ghaziabad; [5]Associate Professor, Department of Oral Pathology and Microbiology, King George's Medical University, Lucknow, Uttar Pradesh, India

DOI:10.37821/ruhsjhs.7.4.2022.433

This is an open-access article distributed under the terms of Creative Commons Attribution-Non-Commercial-No Derivatives 4.0 International License (CC BY-NC-ND) (http://creativecommons.org/licenses/by–nc-nd/4.0/)

ABSTRACT

Introduction: Deletion or mutation of tumor suppressor genes plays a significant role in cancer development. Mutation of the p53 tumor suppressor gene is the most common genetic alteration associated with oral cancer. The aim of this study was to compare the expression of p53 protein in oral squamous cell carcinoma and verrucous carcinoma.

Methodology: Immunohistochemical analysis of p53 was done using monoclonal anti- p53, clone BP53-12 antibody (SIGMA, USA); immune-stained cells were counted and intensity of immune-staining in nucleus was noted.

Results: Positive immunoreactivity for p53 oncoproteir was seen in 70.4% oral squamous cell carcinoma and 71% cases of verrucous carcinoma.

Conclusion: No statistical significance was seen between the number of positive staining cases and staining intensity of oral squamous cell carcinoma and verrucous carcinoma. However, statistical significance was observed on quantitative analysis between oral squamous cell carcinoma and verrucous carcinoma.

Keywords: Carcinoma cell; oral squamous cell; p53; verrucous carcinoma.

INTRODUCTION

The term 'oral cancer' includes a diverse group of tumors arising from the oral cavity.[1] More than 95% of the carcinomas of the oral cavity are of squamous cell type in nature.[2] Squamous cell carcinoma is defined as "a malignant epithelial neoplasm exhibiting squamous differentiation as characterized by the formation of keratin and/ or the presence of intercellular bridges."[3]

Multistep processes of genetic changes are involved in the development and progression of human cancers. The various changes in the DNA can progress from a normal keratinocyte to a premalignant or a potentially malignant keratinocyte that is characterized by an ability to proliferate in a less controlled fashion than normal.[4] Mutation in the TP53 gene is the most common genetic change found in squamous cell carcinoma and it is found in 40-50% of the oral squamous cell carcinoma cases (OSCC).[5] Oral verrucous carcinoma (VC) is an exophytic, proliferative growth of malignant epithelial cells and shows low degree of malignancy. It is characterized by large, heavily keratinized fronds with bulbous rete pegs. There is generally marked epithelial proliferation with down growth of epithelium into the connective tissue but without a pattern of true invasion. There are cleft like spaces having parakeratin plugging and occasional areas of degeneration.[6]

p53, so termed, is the product of p53 tumor suppressor gene and is a protein with a molecular weight of 53 kilo Daltons. This protein acts on nucleus and is thought to be involved in regulation of the multiplication of DNA.[7] Point mutations alter p53 from a recessive tumor suppressor gene to a dominant oncogene in that mutant p53 together with another activated oncogene can transform cells.[8] Thus, p53 acts as an oncogene in its mutant form and as a tumor suppressor in its normal form.

The aim of this study was to observe the expression of p53 protein in oral squamous cell carcinoma and verrucous

carcinoma and to compare the expression of p53 protein in oral squamous cell carcinoma and verrucous carcinoma.

METHODS

The study was done on buffered formalin fixed, paraffin embedded tissues of previously diagnosed cases of oral squamous cell carcinoma and verrucous carcinoma after taking approval from the institutional ethics committee. A total of 58 cases of oral carcinomas were assessed of which, twenty-seven cases were of oral squamous cell carcinoma and thirty-one cases were of oral verrucous carcinoma.

The assessment included the following:

1. Intensity of immunostaining in the nucleus of the malignant cell.
2. Counting of immunostained cells out of a total of 600 dysplastic cells from three different areas in the same section of the lesional tissue.

The immunohistochemical procedure was carried out using monoclonal anti-p53 clone BP53-12 antibody (SIGMA, USA), raised using recombinant human wild type p53 as immunogen. The p53 antibody used recognizes an epitope on mutant and wild type p53 between amino acids 32 and 79. The immunohistochemical detection kit used in this study was LSAB+ System (DAKO, USA) and immunostaining was performed using the recommended procedure of the manufacturer.

The presence of a red/ brown colored end product at the site of the target antigen was indicative of positive staining results. Cytoplasmic staining was not considered. Only nuclear stain was considered positive. Positively stained cells were analyzed quantitively by counting the total number of intact positively stained cells per high power field (40X) of light microscope. Images were captured by digital camera attached with light microscope and analyzed using image analysis software (ij152-win-jva8image J). Counting was done in three representative areas of epithelium. 200 cells were counted in per high power field and total 600 lesional cells were calculated. The average, in percentage of cells with positive p53 immunostaining was tabulated for each case. The fields were studied in a step ladder fashion and care was taken to prevent the overlapping of fields. Figures 1 and 2 show p53 positivity in OSCC and VC, respectively.

The tissue section staining was graded as either negative or positive. When there was no staining in the nuclei it was recorded as negative. The positive staining was graded on a three point scale as "+" when there was weak intensity, as "++" when staining was of moderate intensity, and as "+++" when the staining was intense. For statistical evaluation, the intensities were graded as 1, 2, and 3, for weak, moderate, and intense degree of staining respectively. To eliminate observer bias, the opinion of two other qualified observers was taken. The results obtained were statistically analyzed.

RESULTS

A comparison of p53 overexpression in cases of squamous cell carcinoma and verrucous carcinoma is shown in table 1. Chi square analysis shows p53 expression in the two groups to be 0.048. This table shows that there is no association between the two groups at 5% level of significance ($p>0.05$). In table 2, comparison of overall intensity (quantified) of p53 expression between cases of squamous cell carcinoma and verrucous carcinoma is seen at 5% level of significance ($p>0.05$). Table 3 shows the

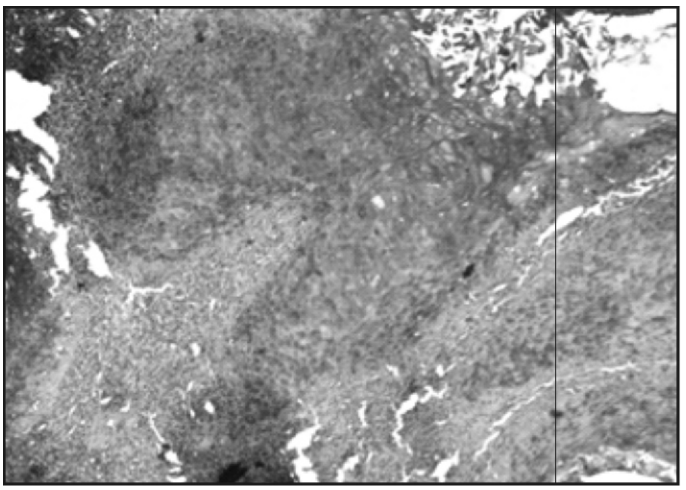

Figure 1: p53 positivity in oral squamous cell carcinoma.

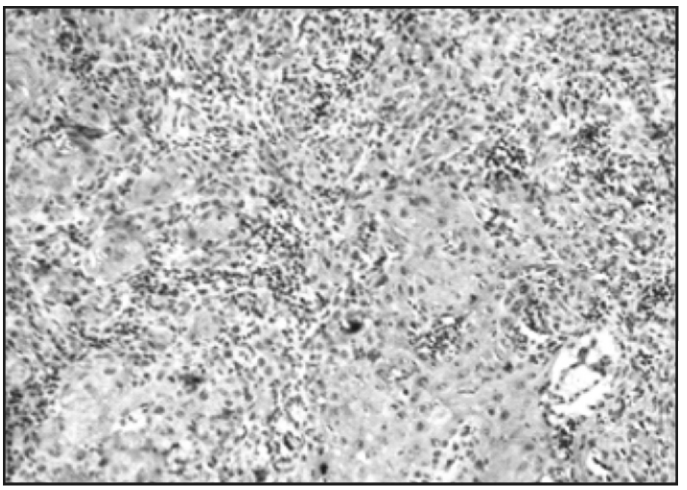

Figure 2: p53 positivity in verrucous carcinoma.

Table 1: Comparison of p53 overexpression in cases of oral squamous cell carcinoma (OSCC) and verrucous carcinoma (VC)

	OSCC	VC	Total
Positive	19	22	41
Negative	8	9	17
	27	31	58

Chi Square = 0.0048; $p > 0.05$; Non significant.

Table 2: Comparison of overall intensity (quantified) of p53 expression in oral squamous cell carcinoma (OSCC) and verrucous carcinoma (VC)

Type	Mean	Standard deviation
OSCC	1.4839	1.1216
VC	1.5926	1.2172

$t = 0.3518$; $p > 0.05$; Non significant.

Table 3: Comparison of quantitative expression for p53 between oral squamous cell carcinoma (OSCC) and verrucous carcinoma (VC)

Type	Mean	Standard deviation
OSCC	75.7546	21.8709
VC	61.0447	23.9566

$t = 2.0409$; $p < 0.05$; Statistically significant.

comparison of quantitative expression of p53 between squamous cell carcinoma and verrucous carcinoma. Association between the two groups is seen at 5% level of significance ($p>0.05$). p53 staining intensity was observed to be intense and moderate in 42.1% cases each and mild in 15.8% cases of squamous cell carcinoma cases. The positive cases of verrucous carcinoma showed p53 staining intensity to be intense in 27.3% cases, moderate in 54.5% cases, and mild in 18.2% cases.

DISCUSSION

p53 has a major impact on both fundamental and applied cancer biology. The mutation of p53 gene is crucial than the mutations seen in other tumor markers as it is a fundamental mechanism involved in disordered growth control and not a secondary consequence of the neoplastic phenotype and it is amazingly widespread, occurring in high frequency in tumors of cervix[9], larynx[10], breast[11], lungs[12], besides many others. The most common genetic abnormality associated with malignancy is the mutation of p53 gene. Studies suggested that p53 mutation arises relatively 'late' in neoplastic progression and the underlying genetic lesion-point mutation ceases the tumor suppressor activity of the p53 protein, and also stabilizes it so that it gets collected in the cell nucleus resulting in its detection by immunohistochemistry. p53 mutation is also called a gain of function mutation, as the mutant p53 protein not only looses the ability to act as growth suppressor but in many instances gains the ability to promote cellular proliferation and is considered to be a hallmark of p53 mutations.[12]

In the present study, 19 out of 27 (70.4%) OSCC expressed p53 protein, which is in accordance with many other studies of this tumor type with 94% positivity[13], 80% positivity[14], 73% positivity[15], 67% positivity[16], 64% positivity.[17] However, Warnakulasuriya et al[18] in their study found p53 positivity in only 35% cases of OSCC. Their data suggested that p53 gene mutations are commonly involved in oral cancer but are not crucial for the development of malignancy. Nevertheless, its presence can be used as a marker of risk in a high proportion of malignant and potentially malignant lesions. In the review of literature available on the expression of p53 in OSCC, we found that an average of around 64% positivity was seen in majority of cases and is in accordance with the present study. Poeta et al[19] also showed that TP53 mutations were found in 53.3% cases of OSCC. Liu et al[20] observed positivity in 81% of cases. Sa et al[21] reported that 76.8% lesions showed positivity for p53.

The intensity of staining was graded as mild, moderate, and intense in all the cases of positive expression, as done by earlier workers. In OSCC, an equal number of cases had intense (8/19) and moderate (8/19) staining grades, while 3 cases of mild staining were seen. Intensity could still be a highly subjective observation, though care is taken to reduce observer bias. Of the 19 positive cases of squamous cell carcinoma, p53 staining intensity was observed to be intense and moderate in 8 (42.1%) cases and mild in 3 (15.8%) cases.

Verrucous carcinoma of the oral cavity has a unique biologic behavior and is distinct from the more common squamous cell carcinoma of this area. It is basically an exophytic, squamous epithelial neoplasm with a very limited propensity to metastasize. In the present study, p53 over expression was seen in 71% (22/31) cases of oral verrucous carcinoma. The increase in positive cases in our research can be attributed to the wet heat antigen retrieval used which is supposed to be more effective for unmasking formaldehyde-fixed antigenic epitopes and to the highly sensitive monoclonal antibody BP53-12 done for detection of an epitope of mutant and wild type p53.[22]

The quantitative analysis of verrucous carcinoma revealed that the staining intensities of p53 positive lesions were mild in 4 (18.1%) cases, moderate in 12 (54.5%) cases and intense in 6 (27.2%) cases. The qualitative analysis of p53 positive cells in oral verrucous carcinoma (VC) in this study was 75.7%. In the only quantitative study done previously on oral VC positive cells were present at a frequency higher than 50%.[23]

The staining analysis of p53 staining in OSCC and VC reveals a similar percentage (70%) of p53 positive lesions. In a similar study conducted by Drachenberg et al[24], positive p53 expression was seen in 62.5% (5/8) cases of SCC and 50% (4/8) cases of VC. They concluded that the different levels of gene expression correlate with the different biology and prognosis of the tumors. As the number of cases observed in study were very less, the observations made by him lack sufficient statistical support. In SCC, the staining intensity of lesions was equal between intense and moderate grades, in VC there was a distinct predominance of moderate staining intensity (55%) suggesting a slightly slower proliferation rate of neoplastic cell and a lower stability of p53 protein.

In quantitative analysis of p53 positive cells from areas representative of the lesions histology, VC revealed a higher percentage 75.7% compared to 61% by SCC. This fact may be attributed to the extensive p53 positive areas observed in VC as compared to strands and sheets in SCC and the level of activation of p53 oncogene. Though no statistical significance was observed between the number of positive cases or staining intensities between SCC and VC, the quantitative analysis showed significance at the level of 5%. In a similar study by Saito et al[23] none of the 15 VCs contained p53 positive cells at a higher frequency then 50%, while half of the 44 SCCs contained p53 cells at a frequency of more than 70%. They concluded that the different expression pattern between the two lesions might be the result of differences in cell proliferation or the state of inactivation of p53 gene. The difference in quantitative expression of p53 between our study and Saito et al.[23] awaits further evaluation.

CONCLUSION

Quantitative analysis shows a higher percentage of p53 positive cells in VC when compared to OSCC which may be attributed to differences in cell proliferation and state of activation of p53 gene. There was a definite correlation for quantitative analysis of number of p53 positive cells between OSCC and VC cases.

REFERENCES

1. Khalili J. Oral cancer: Risk factors, prevention and diagnosis. *Exp Oncol*. 2008; 30(4): 259-64.
2. Mehrotra R, Yadav S. Oral squamous cell carcinoma: Etiology, pathogenesis and prognostic value of genomic alterations. *Ind J Can C*. 2006; 43(2): 60-66.
3. Gupta PC, Mehta FS, Daftary DK, Pindborg JJ, Bhonsle RB, Jalnawalla PN, et al. Incidence rates of oral cancer and natural history of oral precancerous lesions in a 10- year follow-up study of Indian villagers. *Community Dent Oral Epidemol*. 1980;8:287-333.
4. Scully C, Bagan J. Oral squamous cell carcinoma overview. *Oral Oncol*. 2009; 45: 301-08.
5. Nylander K, Dabelsteen E, Hall PA. The p53 molecule and its prognostic role in squamous cell carcinomas of the head and neck. *J Oral Pathol Med*. 2000; 29: 413-25.
6. Cutilli T, Leocata P, Dolo V, Altobelli E. p53 as a prognostic marker associated with the risk of mortality for oral squamous cell carcinoma. *Oncology Letters*. 2016; 12(2): 1046-50.
7. Leedy DA, Trune DR, Kronz JD. Tumor angiogenesis, the p53 antigen, and cervical metastasis in squamous carcinoma of the tongue. *Otolaryngeal Head Neck Surg*. 1994; 111(4): 417-22.

8. Giannondis A, Hernington CS. Differential expression of p53 and p21 in low grade cervical squamous intra-epithelial lesions. *Cancer*. 2000; 89(6): 1300-07.
9. Garima, Pandey S, Pandey LK, Saxena AK, Patel N. The role of p53 gene in cervical carcinogenesis. *J Obstet Gynaecol India*. 2016;66:383-88.
10. Cercelaru L, Stepan AE, Mărgăritescu C, Osman A, Popa IC, Simionescu CE, et al. p53 immunoexpression in laryngeal squamous cell carcinoma. *Curr Health Sci J*. 2017; 43(4): 325-29.
11. Gasco M, Shami S, Crook T. The p53 pathway in breast cancer. *Breast Cancer Res*. 2002;4(2):70-76.
12. Melhem M, Law JC, Srivastava S. Assessment of sensitivity and specificity of immunohistochemical staining of p53 in lung and head and neck cancers. *Am J Pathol*. 1995: 146(5); 1170-76.
13. Kerdpon D, Rich AM, Reade PC. Expression of p53 in oral mucosal hyperplasia, dysplasia and squamous cell carcinoma. *Oral Dis*. 1997; 3(2): 86-92.
14. Langdon JD, Patridge M. Expression of the tumor suppressor gene p53 in oral cancer. *Br J Oral MaxilIofac Surg*. 1992; 30(4): 214-20.
15. Ibrahim SO, Johannessen AC, Vasstrand EN. Immunohistochemical detection of p53 in archival formalin fixed tissues of lntraoral squamous cell carcinoma. *APMIS*. 1997; 105 (10): 757-64.
16. Trivedy C, Warnakulasuriya KA, Tavassoli M. p53 aberrations in oral submucous fibrosis and oral squamous cell carcinoma. *J Oral Pathol Med*. 1998; 27: 72-77.
17. Nylander K, Stenling R, Gustafsson H. p53 expression and cell proliferation in squamous cell carcinoma of the head and neck. *Cancer*. 1995; 75: 87-93.
18. Warnakulasuriya KA, Johnson NW. Expression of p53 mutant nuclear phosphoprotein in oral carcinoma and potentially malignant oral lesions. *J Oral Pathol Med*. 1992; 21:404-08.
19. Poeta ML, Manola J, Goldwasser MA. TP53 mutations and survival in squamous cell carcinoma of the head and neck. *N Engl J Med*. 2007: 357(2S): 2552-61.
20. Liu CJ, Chang KW, Chao SY, Kwan PC, Chang SM, Yen RY, et al. The molecular markers for prognostic evaluation of areca-associated buccal squamous cell carcinoma. *J Oral Pathol Med*. 2004; 33: 327-34.
21. Sa CT, Fonseca LMS, Cardoso SV, Aguiar MCF, Carmo MA. p53 immunoexpression in oral squamous cell carcinomas from different anatomical sites: A comparative study. *Int J Morphol*. 2006; 24(2): 231-38.
22. Regezi JA, Zarbo RJ, Regev E. p53 Protein expression in sequential biopsies of oral dysplasias and in situ carcinoma. *J Oral Pathol Med*. 1995; 24: 18-22.
23. Saito T, Nakajima T, Mogi K. Immunohistochemical analysis of cell cycle associated protein p16, pRb, p53, p27 and Ki67 in oral cancer and precancer in relation to verrucous carcinoma. *J Oral Pathol Med*. 1999; 28: 226-32.
24. Drachenberg CB, Blanchaert R, Loffe OB. Comparative study of invasive squamous cell carcinoma and verrucous carcinoma of the oral cavity: Expression of bcI-2, p53 and Her-2/neu and indexes of cell turnover. *Cancer Detect Prev*. 1997; 21 (6): 483-89.

Corresponding Author

Dr Shikha Saxena, Associate Professor, Department of Oral Pathology and Microbiology, RUHS College of Dental Sciences, Jaipur, Rajasthan.

email: drshikhasaxena29@gmail.com