

Original Research Article

Histomorphological Correlation of Oropharyngolaryngeal Dysplasia and Squamous Cell Carcinoma with Special Reference to P53 and PCNA Expression

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ABSTRACT

Background: Despite advances in surgery, radiotherapy and chemotherapy over the past three decades, no significant improvement in the prognosis for oral cancer has been observed. This could change if the cancer can be detected at an early stage. Aim: To evaluate the p53 and PCNA (Proliferative cell nuclear antigen) expression as an indicator for clinical aggressiveness in dysplasia and squamous cell carcinoma (SCC) in oropharyngolaryngeal (OPL) region.

Materials and Methods: 50 histopathologically diagnosed patients were taken from the oral pathology department of our institute. It comprised of 15 dysplasia cases and 35 SCC cases of OPL region. Immunohistochemistry was done to investigate the expression of p53 and PCNA and scored based on percentage of positive tumor cells and the staining intensity.

Results: The expression of p53 and PCNA was detected in 34(68%) and 50(100%) cases respectively. There was significant increase in staining intensity and percentage of tumor cells expressing p53 and PCNA from dysplasia to different grades of SCC.

Conclusion: Present study concluded that p53 and PCNA expression can be used to assess the potential for malignant transformation in dysplasia and aggressiveness in different grades of SCC of OPL region.

Keywords: Dysplasia, Squamous cell carcinoma, Oropharyngolaryngeal region, p53, PCNA

INTRODUCTION

It is already established that carcinomas of oropharyngolaryngeal (OPL) region which includes oral cavity, oropharynx, hypopharynx and larynx constitute 40-47% of total carcinomas affecting the body. [1] Oral squamous cell carcinoma (OSCC) is the sixth most common malignancy and is a major cause for morbidity and mortality in developing nations. [1] Spectrums of epithelial alterations range from hyperplasia, atypical hyperplasia, dysplasia, carcinoma in situ to invasive carcinoma. As the degree of

dysplasia increases from mild to severe, so does the risk of malignant transformation. [2]

Relevant biomarkers hold more promise as early prognostic markers. [3]

Among these, the p53 tumour suppressor gene (TP53) product, p53, deserves particular attention, not only because of its central role in genomic stability and cell cycle regulation, but also because its function is abrogated in most human cancers, in the case of oral mucosa in preinvasive stages. [3]

Proliferating cell nuclear antigen (PCNA) is a cell-cycle-specific protein and well correlated with cell proliferation.

p53 may act as a complimentary marker to PCNA given that PCNA reactivity defines the growth fraction of a tumour and p53 reactivity demonstrate the irreversible malignant change having occurred in the molecular fraction. [4]

Therefore, this study is taken up to assess the immunohistochemical expression of p53 and PCNA in OPL tumorogenesis and its degree of expression from dysplasia to carcinoma, which is also important for prognostication.

MATERIALS AND METHODS

This 2 year prospective study was performed on 50 histopathologically proven squamous (intraepithelial and invasive) lesions of OPL region (15 dysplastic lesions and 35 SCCs) during the study period, November'15-October'17. The sample for this study was obtained from the archives of the Department of Pathology, Hi Tech Medical College, Utkal University, Bhubaneswar, Odisha, India.

From the paraffin-embedded blocks, two sections of 3µm were taken on poly-L-lysine-coated glass slides for immunohistochemical staining of p53 and PCNA respectively. The immunostaining was carried out using avidin biotin peroxidase technique.

Heat-mediated antigen retrieval was performed using citrate buffer in the microwave oven set at 540° for 30 minutes. Slides were then incubated in 3% hydrogen peroxide to block endogenous peroxidase activity. Ready-to-use mouse IgG-1 anti p53 (DO-7) monoclonal antibody (BioGenex, Milmont drive, Fremont, California- 94538 USA) and mouse IgG anti PCNA (PC-10) monoclonal antibody (BioGenex, Milmont drive, Fremont, California- 94538, USA) were used as primary antibodies and were incubated for 60 min, followed by super enhancer for 25 min. For secondary antibody application, slides were incubated with polymer-

horseradish-peroxidase reagent (antimouse and antirabbit IgG labeled with enzyme polymer in phosphate-buffered saline) for 30 min. 3,3'- diaminobenzidine (DAB) (DAB tetrahydrochloride) solution was used as the chromogen and counterstaining was done with Mayer's hematoxylin.

When a distinct brown staining was confined to the nuclei, p53 expression was considered positive. Similarly, tumor cells were considered positive for PCNA if there was intranuclear light brown granular staining.

The positive control was taken from previously diagnosed case of SCC. The negative control consisted of the replacement of the primary antibody for 1% bovine serum albumin, diluted in phosphate saline solution (TRIS).

The percentage of positive cells was scored according to the method of Nakagawa et al. [5] as follows: (++++) = strong staining (more than 50% stained); (++) = moderate staining (25 –50% stained); (+) = weak staining (5 – 25%); 0 =negative (less than 5% stained).

STATISTICAL ANALYSIS was made considering the clinical, histopathological and immunohistochemical data. Then transformed to a master chart by using Microsoft excel sheet, which was then subjected to statistical analysis using chi square test by using SPSS, version 20. Analysis was done in the form of percentage and represented as tables and figures where necessary. P value of ≤ 0.05 is considered as statistically significant.

RESULT

In the study group, out of 50 histologically diagnosed cases, 15 (30%) were dysplastic lesions and 35(70%) were squamous cell carcinoma (SCC). In accordance to their histopathological characteristics dysplasia were classified as follows: 6 (40%) were grade I, 6 (40%) were grade II and 3(20%) were grade III lesions. Among SCCs, 20(56.5%) were WD SCC, 11(30%) were MD SCC, and 4 (11.4%) were PD SCC.

The distribution with respect to anatomical regions was: 28(56%) in the tongue, 11(22%) in the buccal mucosa, 5(10%) in the larynx, and 6(12%) in pharynx.

2 out of total 6(33.3%) cases of mild dysplasia, 4 out of 6(66.7%) cases of moderate dysplasia, and all 3 (100%) cases of severe dysplasia showed suprabasal p53 expression. Progressive increase in percentage of p53 positive tumor cells and

immunostaining intensity were observed with progression of lesion from mild to severe dysplasia (fig 1). This association was statistically highly significant ($P = 0.001$). All 15 (100%) cases of dysplasia showed suprabasal PCNA expression, increasing in intensity with progression of the lesion. This association was also statistically highly significant ($P = 0.0019$).

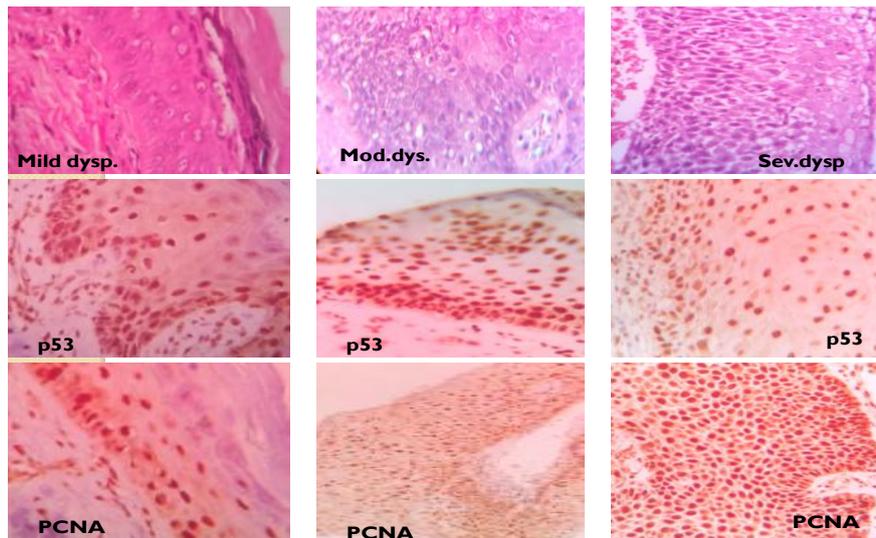


Fig 1: p53 & PCNA staining intensity in different grades of dysplasia

In SCC 25 (71.4%) cases expressed p53 in invasive cell nests, islands and cords of proliferative neoplastic epithelial cells; and 16 (32%) didn't express., p53 expression was seen in 13 (65 %) cases of WD SCC, 9 (81.8%) cases of MD SCC and 3 (75%) cases of PD SCC. Percentage of p53 +ve tumor cells increased gradually from WD SCC to MD SCC and decreased with

progression of lesion from MD SCC to PD SCC (Fig2). This association was statistically highly significant ($P = 0.001$). PCNA expression was seen in all grades of SCCs increasing in intensity with progression of the lesion. This association was also statistically highly significant ($P = 0.0019$).

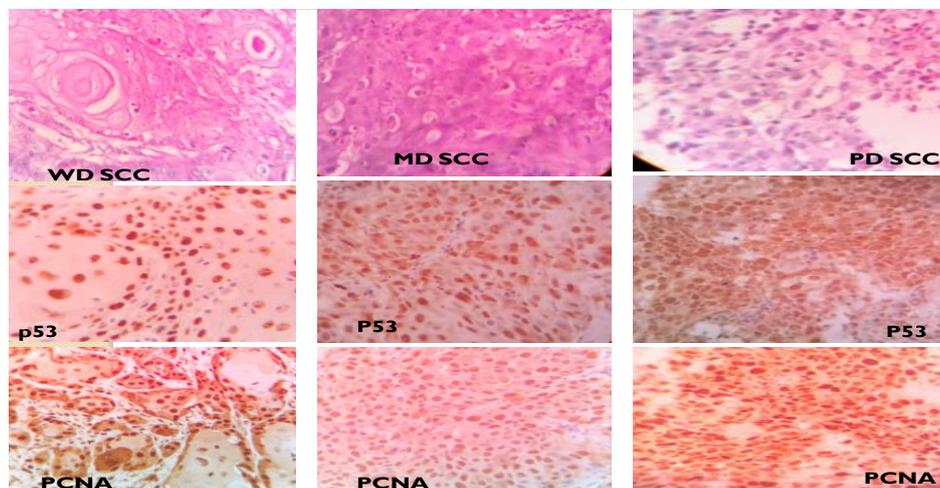


Fig 2:p53 and PCNA staining intensity in different grades of SCCs

DISCUSSION

Present study was performed on archival tissues diagnosed as OPL dysplasia and SCC with differing histological grades. Identifying the diverse cell populations and analysing their rate of proliferation may help in predicting the prognosis of a particular patient. [6] Thus, we investigated the histopathological correlation of different grades of OPL dysplasias and SCCs with varying expression of p53 and PCNA.

In the present study, p53 positivity was seen in 60% of dysplastic lesions and the number of p53 positive cell increased gradually with progression of lesion from grade I to grade III. Similar observation was noted in Sauter et al [7] and A Jain et al [8] study. These investigators also found that the p53 expression pattern was significantly related to the development of carcinoma.

In the present study, p53 positivity was seen in 71.4% of the SCC cases and negative in 28.6%. Similar observation was noted in P Baweja et al [9] study. 28.6% cases didn't express p53, but showed high PCNA expression in our study, suggesting that p53 mutation is not essential for tumor transformation. This might be interpreted as most of the mutation in these cases is truncating mutations, which may lead to less protein production and absence of its reactivity in nucleus, which in turn indicate an aggressive nature of p53 negative SCC. [10] The data in present study shows that there was progressive increase in percentage of p53 positivity with progression of lesion from WD SCC to MD SCC. The maximum numbers of p53 positive cases were seen in MD SCC. Similar finding was seen in P Baweja et al [9] and Juan et al [11] study. Decrease in percentage of p53 +ve tumor cells, with progression of lesion from MD SCC to PD SCC could be due to presence of tumor cells with nonsense or frameshift mutations, resulting in unstable, truncated proteins which are p53-ve, [12] and indicates aggressive nature of p53-ve SCCs.

Rüdiger G Steinbeck et al [13] analysed varying degrees of atypia in squamous cell carcinomas located in the

oral cavity, by means of PCNA immunoreactivity. Focal immunoreactivity of PCNA was noticed in well differentiated squamous cell carcinoma and strongly elevated proliferation; aneuploidy was seen in the entire tumour mass of poorly differentiated squamous cell carcinoma. Our study also showed higher PCNA expression in poorly differentiated oral squamous cell carcinoma compared to well differentiated oral squamous cell carcinoma.

The generally similar immunolocalisation of p53 and PCNA staining in suprabasal cell layers in dysplasia and at the tumour invasive front in SCCs appear to suggest that p53 protein expression is found in areas with proliferative activity and might indicate the involvement of the mutated form of the p53 protein in the alteration of the cell cycle regulation process. [4] Therefore it has been suggested that p53 may act as a complementary marker to PCNA, since PCNA reactivity defines the growth fraction of tumour and p53 reactivity demonstrates the irreversible malignant change having occurred inside this fraction. [13]

CONCLUSION

Gradual increase in p53 and PCNA staining intensity was observed with progression of lesion from mild to severe dysplasia as well as from WD SCC to PD SCC. Thus, it is feasible to use these markers in future studies as prognostic indicators. With such patterns, a comparison of the survival patterns between those with higher and lower p53 and PCNA expressions at the tumour invasive front can further indicate its possible clinical significance.

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