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Evaluation of p53 expression in Oral Squamous Cell Carcinoma with and without tobacco habits. A comparative study

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ABSTRACT:

OBJECTIVES: Inactivation of the tumor suppressor gene like p53, plays a key role in tumor progression with high incidence of mutation in oral squamous cell carcinoma (OSCC). Usage of tobacco in various forms has proved to be a most common causative agent of OSCC. Interplay between tobacco and p53 mutation may lead to a path in understanding carcinogenesis. Present study aims to compare the levels of expression of p53 with respect to tobacco habituers and non habituers.

MATERIALS AND METHODS: 16 cases of OSCC were taken, among which eight were with tobacco habits and eight without. Formalin fixed paraffin embedded tissue blocks were analyzed for p53 expression immunohistochemically.

RESULTS: Expression of p53 was showed to be enhanced in tobacco habituers when compared to non-habituers.

CONCLUSION: These data suggest that induction of mutation in oncogenes in oral tumors may be associated with specific carcinogen exposure. The increased expression of p53 in tobacco habituers suggests a link between mutations in target genes like p53 and carcinogen metabolism in the process of carcinogenesis.

Key words: ***Oral Squamous cell carcinoma, Tobacco, p53.***

INTRODUCTION

Oral squamous cell carcinoma accounts for more than 90% of all oral cancers.[1] Oral cancer is one of the most common cancers representing 6% of all cancers in populations. In India it represents the commonest among males and third most common among females [2-4]

The crucial event in the transformation of a premalignant cell to a malignant cell is inactivation of cellular negative regulators, tumour suppressor genes and is regarded to be a major event leading to development of malignancy [5,6]

There has been much research on the tumour suppressor gene p53. The p53 protein blocks cell division at the G1 to S boundary, stimulates DNA repair after DNA damage, and also induces apoptosis. These functions are achieved by the ability of p53 to modulate the expression of several genes. Mutations of p53 occurs either as a point mutation, which results in a structurally altered protein that sequesters the wild- type protein to be a hereby inactivating its suppressor activity, or by deletion, which leads to a reduction or loss of p53 expression and protein function. The tumour suppressor gene p53 is known to be mutated in 70% of solid tumours. The mutations of p53 has been shown to be prevalent in most of the human cancers with varying frequencies.[9] The known classic risk factor of oral cancer is tobacco use, all forms of tobacco, cigarettes, pipes,cigars, and smokeless tobacco have been implicated in the development of OSCC While tobacco confers the highest risk for OC of the floor of the mouth and it is associated with an increased risk for all sites of OSCCC. Tobacco use is responsible for 90% of OC deaths in males and other etiological factors include alcohol, infections, dietary factors, chemical irritants.

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Tobacco products contain a large array of carcinogens, benzopyrene and other polycyclic aromatic carcinogens are most important carcinogenic agents in cigarette smoke, but in chewable tobacco nitrosamines are the strongest carcinogens. The metabolites of the above mentioned compounds are found in saliva of the oral cavity as well as in their body fluids. These agents are known to cause toxic effects particularly cancer and other cellular and DNA changes. The importance of p53 tumour suppressor gene in the process of carcinogenesis is well established. In particular the high incidence of p53 mutation has been demonstrated in tobacco related cancers. P53 mutations are etiologically associated with the development of oral Squamous cell carcinoma or are associated with the exposure to specific carcinogen.[7,8,10,11]

Mutation of the p53 gene has been linked to tobacco use in oral Squamous cell carcinoma. Present study assesses the correlation and association of p53 mutation and tobacco use in the complex process of carcinogenesis.

Materials and Methods: This study included blocks, taken from the archival collection of Coorg institute of dental sciences and Karnataka institute of oncology, Navnagar, Hubli. 16 cases of OSCC were selected, among which only eight were with tobacco habits. The case history of the patients was assessed to know the tobacco habitual history of the patients. Immunohistochemistry was performed on formalin fixed paraffin embedded tissue blocks and on lysine coated slides for analysis of p53 expression.

Immunohistochemical staining

Sections cut at 4 microns were floated on to Poly - L - Lysine coated slides and incubated 58^{0}C for overnight. The sections were then deparaffinized in two changes of xylene for 15 minutes each. Dexylinization were done by immersing the slides in two changes of absolute alcohol for 1 minute each. Sections ware alcoholized by immersing the slides in 90% and 70% alcohol for 1 minute each and then washed for 10 minutes and 5 minutes each in tap water and distilled water respectively.

Antigen retrieval was done by placing the sections in citrate buffer and then pressure cookerizing for 10 minutes. Pressure cooker was then cooled for 20 minutes in the sink with water. Sections were then rinsed with distilled water for 5 minutes and were then washed with two changes of TBS for 5 minutes each. To block the endogeneous peroxidase enzyme activity, the sections were treated with peroxidase block for 10- 15 minutes and then again washed with 3 changes of TBS for 5 minutes each. Sections were then treated with Power block for 15 minutes in order to block non- specific reaction with other antigens. Sections were then drained and covered with primary antibody against p53 for 1 hour to identify tumor markers by antigen - antibody reactions and again washed with TBS as described earlier. To enhance the reaction between primary and secondary antibodies, sections were then treated with Super Enhancer for 30 minutes. And again washed with TBS. Enzymes were labeled by treating the sections with Super sensitive poly - HRP secondary antibody and washed with TBS.

Chromogen was added to the sections for 5 minutes to give color to the antigens and sections were again washed with TBS. Sections were then washed with tap water for 5 minutes and were counterstained with haemotoxylin for 1 minute and washed in tap water, dried, cleared in xylene and mounted with DPX.

Breast cancer tissue section was taken as positive control for p53 expression and for negative control the primary antibody antibody was omitted during IHC staining.

Interpretation of IHC stains

Tumor markers - brown in color. (Localized to nucleus).

Assessment of p53 staining pattern: Samples were scored as negative (less than 10% of nuclei positive), positive (10 - 50% of nuclei positive) or highly positive (more than 50% of nuclei positive) and score was given as 0 for negative,1 for positive and 2 for highly positive depending on the intensity and number of positive cells. These percentages were grossly assessed by counting nuclei. All slides were assessed by two oral pathologists independently.

Results : Enhanced expression of p53 was found with tobacco habituers, showing positivity in four cases(50%) in OSCC of tobacco habituers and only one case (12.5%) showed positivity in tobacco non-habituers. Out of four positive expressions in OSCC of tobacco habituers two showed high positivity. The expression was showed as a brown colour localized to the nuclear area. Though there was a enhanced expression was found in tobacco habituers, the statistical significance was not obtained with the p value of 0.234.

Discussion

Oral carcinogenesis is a multistep process in which multiple genetic events occur that alter the normal functions of oncogenes and tumour suppressor genes. In combination with the loss of tumour suppressor activity, this leads to a cell phenotype capable of increased cell proliferation with loss of cell cohesion and the ability to infiltrate local tissue and spread to distant sites. It is known that cancer is caused by a series of genetic changes, each potentially leading to a clonal outgrowth of cells through a selective growth advantage. Determining the nature and timing of these changes in OSCC is critical to both a clinical and biological understanding of the disease. [5,8]

Data from previous Experiments suggests that specific mutagens in tobacco (benzo[a]pyrene and N-nitrosamines) may induces high frequencies of p53 mutations.[9,10] This association of tobacco and p53 mutation and progression has also been shown in malignancies of other parts of the body like lungs, bladder, colorectal cancers significant expression of p53 in oral carcinomas and adjacent histologically normal epithelium was there in tobacco habituers, where the findings was not present with tobacco nonhabituers p53 expression in normal epithelium which is not associated with tumors but has been exposed to tobacco would be significant, because it would indicate that this epithelium is at increased risk for transformation to carcinoma and indicating the association of p53 and tobacco in the process of carcinogenesis.[13]

The spectrum of p53 mutations in tumors may provide information about their cause.Study showed the highest incidence of mutations was associated with exposure to tobacco suggesting that tobacco may produce carcinogens that increase the frequency of such mutations and also a site specification, Study in Taiwan also showed the frequent association of p53 mutations in OSCC with tobacco users showing results in accordance with the present study.[14]

Advances in cancer research have provided abundant new knowledge about cellular processes and molecular biology in OSCC. Our knowledge of carcinogenesis, identification of biological markers, and molecularly targeted therapies is advancing through basic research, translational research and clinical trials, and ultimately analysis of factors specific to the individual and their tumor may result ineffective "personalized medicine." [15]

The main risk factor for oral cancer is exposure to exogenous carcinogens such as tobacco smoke and alcohol. Annually, it is estimated that 127,459 deaths are caused from oral cavity cancer worldwide, of which 96,720 occur in developing countries [16]

The aberrant Inactivation of tumor suppressor genes can occur via epigenetic or genetic mechanisms. The reasons underlying this choice of gene inactivation routes during tumorigenesis have not been clarified. Chemical carcinogens in tobacco smoke may contribute to the genetic mutations in TP53. The inactivation of the TP53 tumor suppressor signaling pathway is seen in most human cancers including OSCC .The aberrant p53 protein activity may be caused by mutations in the TP53 sequence producing truncated or inactive mutant proteins, or by aberrant production of other proteins that regulate p53 activity (such as gene amplification of MDM2 or viral proteins). Recent studies have also suggested that inherited genetic polymorphisms in the p53 pathway influence tumor formation, progression, and/or response to therapy.[17,18]

In present study, assessment has done on alterations of p53 in OSCC from patients with documented exposure to tobacco habits. The aim of the study was to determine alterations in these common genetic alteration in the same set of tumors and explore the possibility that exposure to different risk factors may change the profile of the genetic alterations in these tumors.

In this study, using a polyclonal rabbit antibody to human p53, we found that 50% of oral Squamous cell carcinomas in tobacco habituers showed nuclear immunoreactivity and 12.5% of the oral Squamous cell carcinoma in tobacco non-habituers suggesting the enhanced expression.

In summary, No good prognostic markers for SCCHN are available today and the heterogeneity of tumours within this group causes problems in finding such generalised markers, the present study investigated alterations of the p53 in OSCC related to tobacco use. Our results suggest that p53 mutations are common genetic events in this type of tumor. The mutations are shown to be enhanced in tobacco habituers when compared to non habituers suggesting tobacco specific carcinogens playing a main role in p53 mutations and progression in carcinogenesis. Further prospective studies are needed to confirm the data.

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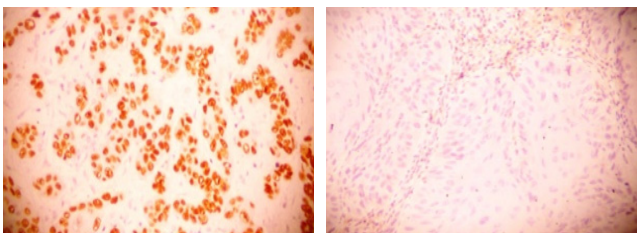
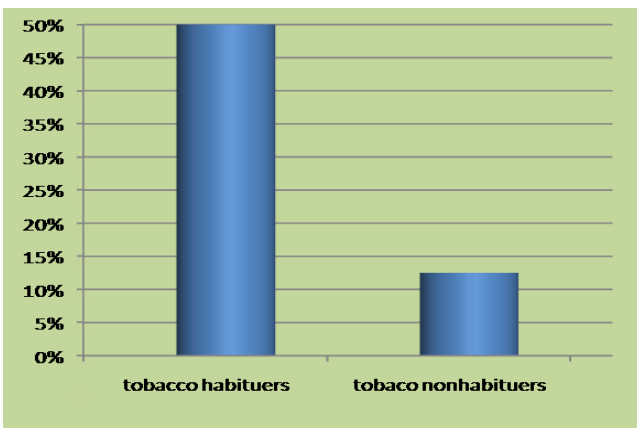


Fig.1: (a) - strongly positive for P53 (b) Negative p53 expression



Graph: Positive p53 expression in tobacco habituers and non-habituers