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Original Article

p53 Expression in Oral cancer: A study of 50 cases

Ghanghoria S[1], Ghanghoria A[2], Shukla A[1]

[1]*Department of Pathology & Department of Surgery, Mahatma Gandhi Memorial Medical College, Madhya Pradesh, India*
[2]*Department of Surgery Mahatma Gandhi Memorial Medical College, Madhya Pradesh, India.*

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ABSTRACT

Background: Oral cancer is the sixth most common cancer in the world. P53 mutations are associated with the development of oral squamous cell carcinomas. This study is to determine the presence of p53 oncogene expression in cases of oral malignant, premalignant and benign lesions and to show association of p53 oncogene and lymph node enlargement in malignant lesion.

Materials and methods: Four to five micron-thick sections of formalin fixed, paraffin embedded biopsy material from various intra-oral sites of 50 patients were collected, in the series of 50 cases, 35 oral squamous cell carcinoma, 10 dysplastic lesions and 05 hyperplastic lesions were assessed for p53 expression. The tissue sections were immunohistochemically analyzed for the expression of p53 gene.

Results: Out of 50; 22/35 (63%) cases of squamous cell carcinoma, 02/10 (20%) cases of dysplasia (20%), were positive for p53. Five hyperplastic lesions were negative for p53. The P53 protein was not identified in benign lesion.

Conclusion: Results indicate that p53 over-expression is seen in oral squamous cell carcinomas. It is a significant marker of carcinogenesis and can be considered as an important marker for clinical evaluation, diagnosis as well as prognosis of disease.

INTRODUCTION

Oral cancer is the sixth most common cancer in the world.[1] p53 mutations are associated with the development of oral squamous cell carcinomas (OSCCs).[2] Oropharyngeal cancers are amongst the most common cancers in the world, and account for up to 40% of all malignancies in India and South East Asian Countries.[3,4]

Premalignant oral lesions are clinically distinct in Indian population, and 5-10% progress toward carcinoma.[5] Molecular genetics of formation of the malignant tumor constitute, tumor suppressor gene, which promote tumor development, when it is inactivated and oncogene, which

Correspondence:
Dr Shikha Ghanghoria
Asso. Prof, Dept of pathology, D-1, MGM Medical College Campus,
A B Road, Aman Diagnostic Indore MP India,
E-mail: amandiagnostic@gmail.com

promote tumor development when it is activated.

Mutation of the tumor suppressor gene p53 confers the single greatest selective advantage favoring cancer formation.[6] The p53 gene located on chromosome 17p 13.1 encodes a 53-kDa, 393 amino acid nuclear phosphoprotein known to regulate cell growth and proliferation.[7] Alteration of this gene or inactivation of the wild type of gene product is thought to play an important role in multistep carcinogenesis.[8] Normal p53 protein has a very short half-life 6-20 minute making it hard to be detected in normal tissues. But an altered protein has a half-life of about 6 hours so that it can be detected in premalignant and malignant tissue through immunohistochemistry.[9]

In this study, we examined p53 expression in primary oral squamous cell carcinomas by immunohistochemistry. We have also included dysplastic and hyperplastic lesion, for assessment of the lesions which are known to have a potential, for progressing to malignancy.

MATERIALS AND METHODS

This retrospective study was carried out in department of pathology, Mahatma Gandhi

Memorial Medical College Indore Madhya Pradesh after all the approval of institute.

Four to five micron-thick sections of formalin fixed, paraffin embedded biopsy material from various intra-oral sites of 50 patients were collected and used for the study. All cases had their histopathological diagnosis using tissue sections adjacent to those used for the study. Out of 50 cases, 35 comprised oral squamous cell carcinomas, while 10 were dysplastic lesions and 05 cases of hyperplastic lesion included. The ages of the patients ranged from 33 to 85 years.

For immunohistochemistry, the tissue sections were first deparaffinized with xylene, then rehydrated with grades of ethanol, and treated with 3% H2O2 in methanol for 10 minutes to quench endogenous peroxidase activity. After blocking nonspecific antibody binding with 1% bovine serum albumin diluted in phosphate buffered saline for 20 minutes, sections were incubated with mouse anti-p53 monoclonal antibody PAb 240 at a dilution of 1:50 for 2 hours at 37°C. After being washed with PBS, the sections were treated with rabbit anti-mouse IgG conjugated with horseradish peroxidase for half an hour at room temperature. Colour was developed using Sigma fast diaminobenzidine tablets. A brown precipitate in the nucleus confirmed the presence of p53 protein. The slides were lightly counter-stained with Mayer's haematoxylin, mounted in DPX, and examined by light microscopy. Primary antibody was omitted from the negative control, and a breast carcinoma known to express high levels of p53 served as the positive control. In this study, only the percentage of positive cells and not the intensity of staining were taken into account as suggested by Hall and Lane.[10]

The proportion of positively stained cells out of all cancer cells (in %), was determined and recorded as follows:

+++ = >50% cells positive,

++ = 26 to 50%cells positive,

+ = 5 to 25% cells positive,

- = <5%cells positive. In oral dysplastic and hyperplastic lesions, sections with less than

1% positive cells were considered negative.

Table1: Various lesions in oral cavity and it's p53 staining pattern

Type of lesion/ Grade	No. of cases	p53+ cases (%)
Oral squamous cell carcinoma	35	22 63%
Grade 3-	14	14(100%)
Grade2-	16	07
Grade 1-	05	01
Dysplastic lesions	10	02 (20%)
Severe-	03	02
Moderate-	05	00
Mild-	02	00
Hyperplastic lesions	05	00

RESULTS

A total of 50 cases were investigated for p53 expression. Table 1 shows various lesions in oral cavity and it's p53 staining pattern. Among the 35 cases of oral squamous cell carcinoma 22 (63%) showed positive nuclear staining, while 13 cases

(37%) did not show p53 staining. Out of the positive samples, 14 cases (40%) showed 5 to 25% nuclear staining (+) ,07 cases (20%) showed 25-50 % nuclear staining(++) and in 01 cases (3.0%), more than 50% of the tumour cells were p53 positive (+++). Among the 10 cases of oral dysplastic lesions, only 2(20%) were positive. Staining was again confined to basal and parabasal layers of the epithelium in one of the sample. None of the 5 cases of hyperplasia showed any evidence of p53 positive nuclear staining (-).

Expression of p53 depends on grading and expression increases with higher grade. Five year survival in carcinoma with various p53 positivity is showin in table 2. Out of 5 cases of well differentiated squamous cell carcinoma 03 cases survived 05 year follow up. Similarly 7 of 16 cases with moderately differentiated carcinoma survived on

Table 2: 5 year follow up of patients with diagnosed Oral squamous cell carcinoma

Grade	No. of cases(total 35)	P53 positivity	(Follow up) 5 yr survival
Well differentiated	05	01	03(60%)
Moderately differentiated	16	07	07(43%)
Poorly differentiated	14	14	03(21%)

05 year follow up. Of 14 cases of poorly differentiated squamous cell carcinoma all show p53 positivity and of them only 03 cases survived more than 05 years.

DISCUSSION

In the present series of primary oral squamous cell carcinomas, p53 phosphoprotein was detectable in 63% of the cases. The prevalence of p53 positive OSCCs in this study was compared by other investigators, wide range of variations are noticed in the percentage of p53 over expression in oral carcinomas by immunohistochemistry.

Diversity of risk habits may contribute to the wide range of prevalence reported also variation in techniques employed, reagents and antibodies used, and methods of pretreatment done may also account for some of the discrepancies. Xie et al. in their research work evaluated p53 expression in tongue carcinoma and found that 61% cases expressed detectable levels of P53.[11] Lazarus et al. stated that incidence of p53 mutations have been observed in around 63% of OSCC.[12] The above findings matches our finding of 63% positivity of p53. On the contrary, other workers found the lower frequency of p53 immunoexpression, that is 46% by Shiraki et al[13] and 43% by Siegelmann-Danieli et al.[14] Wong et al. suggested that p53 has been shown to interact with the oncogenic protein E6 of the human papilloma virus, which results in the rapid degradation of the p53 protein by the ubiquitin-mediated proteolysis system.[15] p53 expression increased linearly from the control group through various grades of OSCC in the resent study. In pairwise comparison, a significant difference was obtained between normal mucosa against WDSCC, MDSCC, and PDSCC ($P < 0.001$).

The results were in accordance with Kannan et al[16] and Xu et al[17] who showed a significant difference between the expression pattern of normal mucosa and OSCC.

Ruchita et al[18] found p53 expression in 66.6% cases of squamous cell carcinoma.

Hsieh and Wang et al[19] found the mutation in p53 is found in 48% of tumour samples. In 23% of infiltrating squamous cell carcinoma, over expression of p53 was also observed in the adjacent nonmalignant epithelium. This could indicate histologically undetectable epithelial premalignant change with p53 aberration[20] while we found p53 positivity in 63%. p53 is generally considered to be a nuclear protein[21] but significant cytoplasmic staining of a few samples (n=2/50; 4%) was observed in the study. In these samples, nuclei were not stained. Cytoplasmic staining of p53 has also been reported by other authors.[22,23] According to Pinhasi-Kimhi et al[24], binding of mutant forms of p53 to heat shock proteins may be responsible for the cytoplasmic location of p53. Jansson et al[25] found that tumours with p53 accumulation in both the nucleus and cytoplasm demonstrated a higher mutation rate. Since there is some conflict of opinion regarding the cytoplasmic expression of P53, in this study, only nuclear staining was taken into consideration. In the present study, p53 over expression was observed in 02 out of 10 (20%) dysplastic lesions. As in the case of OSCCs, there has been a wide range of variations in the percentage of p53 positivity in oral premalignant lesions. The same reasons for the wide range of variations in carcinomas could be ascribed to this. Cheng and Yang [26] reported 90% of oral dysplastic lesions to be positive, 64% as per Hogmo et al[27], while Ibrahim et al[28] reported that none of the premalignant oral lesions from

Sudanese snuff dippers and non-snuff dippers expressed p53 protein. The accumulation of p53 protein could be due to a number of factors, such as mutation in the TP53 gene accumulation of wild type p53 protein as a result of a defect in the degradation pathway, or binding of wild type protein to other proteins. Therefore, the p53 protein would most likely become non-functional. In the present study, though only 20% of the cases were positive; the fact that adjacent non-tumourous epithelium of 23% infiltrating squamous cell carcinomas showed positive staining for p53 in the progenitor compartment of the epithelium indicates that p53 immunoreactivity could be used to detect early tumours as well. It has also been reported that p53 immunodetection in normal looking areas of tumour bearing epithelium, in pre-neoplastic and pre-invasive lesions, suggests that p53 accumulation represents both an early event in oral carcinogenesis and a marker of field cancerisation.[29,30] Out of the two positive cases of dysplasia, in one sample, the staining was confined to the basal and parabasal layers, but in other, suprabasal staining pattern was observed. A recent study by Cruz et al[31] reported that p53 expression in the suprabasal layers in the oral mucosa is an early event of malignant transformation and has predictive value for developing oral squamous cell carcinoma. The suprabasal cell layers of the normal epithelium have lost the capacity to divide, and hence p53 is not expected to be accumulated in these cells.

Suprabasal expression is, therefore, likely to reflect the presence of mutant protein, which due to its decreased turn over, persists for longer periods of time. Alternatively, it could indicate the presence of proliferating cells with DNA damage in the superficial compartments of the epithelium

showing dysplasia, or in other words, epithelium larger than normal is dividing. None of the benign hyperplastic lesions showed detectable levels of p53, probably because the levels are too low to be detected by immunohistochemistry. But the proportion of positive cases with p53 over expression increased from normal and hyperplastic lesions, to dysplasia and oral squamous cell carcinoma, indicating an involvement of p53 in neoplastic transformation and proliferative events.

CONCLUSION

p53 act as molecular policeman, and links cell damage with DNA repair by causing G1 arrest. In oral squamous cell carcinoma expression of p53 is increased and its intensity of expression is related with clinical severity of the disease. In preneoplastic conditions also expression of p53 is found raised. None of the hyperplastic condition show increased expression supporting the fact that expression increases only in malignant cases. Expression of p53 depends on grading and expression increases with higher grade.

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