P53 EXPRESSION IN BENIGN, PREMALIGNANT AND MALIGNANT LESIONS OF ORAL CAVITY

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ABSTRACT

BACKGROUND

Oral carcinogenesis is a multistep process. The premalignant oral lesions, which develop in the epithelial surface due to chronic exposure to various carcinogens such as tobacco, alcohol, betel chewing and HPV infections get transformed to squamous cell carcinoma in genetically predisposed person. Genetic alterations most commonly associated with oral carcinogenesis is p53 tumour suppressor gene mutation.

The objectives of this study are,

- To find out the incidence of various oral squamous epithelial lesions and to evaluate and compare immunohistochemical expression of p53.
- To assess the degree of p53 staining intensity from dysplasia to malignancy in differentiating benign and malignant lesions.

MATERIALS AND METHODS

In this prospective study, histopathological examination and immunohistochemical staining was performed to see the p53 expression in these oral epithelial lesions.

RESULTS

Immunohistochemical expression of p53 observed in 52 cases of oral epithelial lesions consisting of 8 benign, 11 pre-malignant and 33 squamous cell carcinomas. Out of all, 39 cases were showed p53 positivity. The dysplastic lesions account for 11 cases of which p53 positivity seen in 8 (72.72%) cases; p53 immunoexpression was found positive in 26 (78.78%) cases of Squamous Cell Carcinoma (SCC). The staining intensity increased from basal to suprabasal region with increase in grade of dysplasias.

CONCLUSION

Squamous cell carcinoma was found to be the commonest oral malignant epithelial lesion, in which p53 immunoexpression was found in 78.78% cases. Combined histological analysis with p53 immunoexpression could be a useful and simple molecular marker to detect the possibility of transformation from a premalignant to malignant lesion of oral epithelium.

KEYWORDS

Oral Squamous Cell Carcinoma, P53, Immunohistochemistry.

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BACKGROUND

Head and neck cancers is the 6th leading cancer worldwide, with more than 500000 cases diagnosed every year. The vast majority of head and neck cancers are Oral Squamous Cell Carcinomas (OSCCs) that is arising from the epithelial lining of oral cavity including tongue and lips.[1]

In India, the incidence is 20 per 100,000 population and accounts for over 30% of all cancers in the country.[2] Oral squamous cell carcinoma is considered as the result of a multistep process involving a number of aberrant genetic events. Multiple oncogenes, regulatory factors and tumour suppressor genes play a role in development and progression.[3,4] Genetically, predisposed persons are influenced by environmental risk factors like tobacco, alcohol, chronic inflammation and viral infection.[5]

Among the gene associated with oral cancer, p53 is a well-known tumour suppressor gene that is believed to serve as gate keeper against carcinogenesis. Under normal circumstance, the function of p53 protein is to prevent the propagation of genetically damaged cells.[6,7]

Cells with loss of p53 function and abnormal expression of p53 are speculated to undergo malignant transformation. Till date, mutation of p53 gene is one of the most common event in human cancer including oral SCC.[8]

Therefore, the study was performed to assess the immunohistochemical expression of p53 in oral tumorogenesis and to assess the degree of p53 expression form of dysplasia to malignancy.

MATERIALS AND METHODS

The present study is a prospective study carried out in the Department of Pathology, M.K.C.G. Medical College, Berhampur, Odisha, over a period of 24 months from September 2013 to August 2015 with the approval of Institutional Ethics Committee. Patients with oral lesions

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referred to the Department of Pathology from the Departments of Surgery and E.N.T., were included in our study. The detailed history including duration, site, onset, progression, personal habits like smoking, betel chewing were noted. The clinical diagnosis was recorded. Total number of 52 cases of histologically diagnosed benign, dysplastic and malignant lesions were included in our study.

Mechanical or thermal injury, drug induced oral ulcers, Vit. B12 deficiency oral ulcers and iron deficiency anaemic lesions were excluded from our study. Histopathological examination were done from tissue samples obtained by excisional, incisional or punch biopsy. Histosections were routinely stained with H and E, p53 immunostaining were also done on the same specimens. Immunohistochemical technique was performed using the DAKO cytochromatin Kit. Section of known p53 positive colon cancer was used as positive control and sections, where the primary antibody had been omitted served as negative control.

Depending upon Staining Intensity, p53 Positivity is Graded According to Cheng et al 2013[9]

- 0 = No staining.
- 1+ = Weak staining.
- 2+ = Moderate staining.
- 3+ = Strong staining.

The Fraction of Stained Cells was Scored According to Following Criteria

- Score 0 (0 or < 10% Positive cancer cells)
- Score 1 (11 - 50% Positive cancer cells)
- Score 2 (51 - 80% Positive cancer cells)
- Score 3 (> 80% Positive cancer cells)

The ultimate score attributed to the lesion was always the highest score found in several regions analysed. The consecutive H and E stained slides were evaluated by two pathologists without knowing the previous histopathological diagnosis or p53 pattern. Subsequently, p53 stained slides were compared with H and E stained slides to establish a relationship between p53 stained areas and respective histopathological diagnosis.

Pearson Chi-square test was applied to calculate P value and considered. X^2 ($P < 0.05$) being statistically significant.

RESULTS

Observation

The performed study includes 52 cases of oral lesions. Most of the benign lesions were present in between 20 - 40 years of age, whereas malignant lesions were being diagnosed in between 50 - 60 years of age (Table 1).

Age Group in Years	Benign	Premalignant	Malignant	Total
20 - 30	02	00	02	04
30 - 40	03	04	07	14
40 - 50	01	04	06	11
50 - 60	02	02	12	16
60 - 70	00	01	04	05
70 - 80	00	00	02	02
Total	8	11	33	52

Table 1. Oral Lesions According to Age Group

Epithelial Lesions	Male	Female	Total	Percentage
Benign Lesions				
Hyperplasia without Dysplasia	05	02	07	13.46
Squamous Cell Papilloma	01	00	01	1.92
Dysplasia				
Mild	01	01	02	3.80
Moderate	01	00	01	1.92
Severe	04	02	06	11.50
Carcinoma in situ	01	01	02	3.80
Malignant Lesions (Squamous Cell Carcinoma)	28	05	33	63.40

Table 2. Distribution of Oral Epithelial Lesions (n = 52)

In our series, there were 33 (63.4%) cases of malignant epithelial lesions followed by 11 (21.15%) cases of premalignant lesions and 8 (15.38%) cases of benign lesions. Malignant lesions are common in males than females (Table 2).

Histopathological Diagnosis	Negative	Basal	Supra basal	Total
Benign Lesions				
Hyperplasia without Dysplasia	2 (28%)	4(57%)	1 (15%)	7
Squamous Cell Papilloma	1 (100%)	0	0	01
Dysplasia				
Mild	2 (100%)	0	0	02
Moderate	1 (100%)	0	0	01
Severe	0	1 (17%)	5(83%)	06
Carcinoma in situ	0	0	2(100%)	02
Squamous Cell Carcinoma				
Well Differentiated	02 (14%)	01(2%)	11 (78%)	14
Moderately Differentiated	03 (21%)	02 (14%)	09 (64%)	14
Poorly Differentiated	01 (50%)	0	01 (50%)	02
Verrucous Carcinoma	01 (33%)	0	02 (77%)	03

Table 3. P53 Staining Pattern in Oral Epithelial Lesions

In our study, p53 immunoexpression by IHC analysis in oral lesion show suprabasal positivity in 31 (59.62%) cases, basal in 8 (15.38) cases and negative in 13 (25.0%) cases.

Epithelial Lesions	Intensity			Total
	Weak	Moderate	Strong	
Benign	2 (40%)	2 (40%)	1 (20%)	5
Premalignant	0	3 (38%)	5 (62.5%)	8
Malignant	2 (8%)	5 (19%)	19 (73.07%)	26

Table 4. P53 Staining Intensity in Benign, Premalignant and Malignant Lesions (Ching et al, 2013)

It was found that 73.07% malignant and 62.5% premalignant lesions showed strong p53 positivity including 1/5 (20%) of benign lesion.

DISCUSSION

Mutations in p53 gene are the most common genetic basis of human carcinogenesis. These mutations lead to uncontrolled cell proliferation resulting in further genetic abnormalities and finally malignancy.[10] P53 mutation usually shows clonality in cancer, therefore it has occurred in early stages of carcinogenesis in Oral Squamous Cell Carcinoma (OSCC).[11]

In the present study, most common oral epithelial lesion found was OSCC 63.4% (n = 33). Malignant lesions were common in more than 40 years of age group, whereas benign lesions were common in 20 - 40 years of age (n = 5). The average age was 50 years that was lower than authors reported by Gervasio et al[12] i.e. 58.6 years and Mirza et al i.e. 54.3%.[13] The male and female distribution in our study was 3.7:1. In accordance by Pinholt et al,[14] the male and female ratio is 1.19 : 1. However, in studies of Greek and Brazilian population show quite a higher ratio of 9.2 : 1 to 4.8 : 1 respectively.[15,16]

Epidemiological studies have shown that the site of occurrence of oral S.C.C. differs widely. Tongue, lip and floor of the mouth are the common sites of lesions of S.C.C. In the present study, most common location of oral lesions was tongue, i.e. 48% of which S.C.C. comprised of 42% (14 cases). This was compatible as compared to most other studies.[16,17,18] The well-differentiated and moderately-differentiated S.C.C. were found in equal number in our study, which contrasts with Haq ME et al who found that poorly differentiated S.C.C. was most prevalent histological variant. Zedan et al reported WDSCC as the most common histological type.[16]

Our attempt to analyse p53 expression showed uniform pattern and intensity of expression in all batches compared at benign, premalignant and malignant lesions suggesting that immunohistochemical procedure utilised was standardised.

In our study 33.3% of the benign lesions showed p53 negativity (n = 3), 12.5% of benign lesions showed suprabasal positivity and 50% cases of basal positivity. The positive p53 staining in the hyperplastic tissue was likely to be due to in part to the microwave antigen retrieval technique, which has been shown to reduce p53 detection thresholds[19] due to detection of wild type p53.[20] Another possible explanation for the number of positive cases of hyperplasia in present study may be due to proliferation activity in these tissue, as there is a positive relation between p53 expression and cellular proliferation.[21,22,23]

In 11 premalignant cases, p53 positivity was found in 72.72% cases with an increase in suprabasal positivity as the grade of dysplasia increased. Such pattern of staining was also observed by Cruz et al,[24,25] Kerdpon et al,[26] Vered et al[27] and Nasser et al.[28] These investigators also found that the p53 expression pattern was significantly related to the development of carcinoma. No statistical significance was found between histological grades, possibly due to small number of cases studied.

In our study, 26 (78.78%) cases out of 33 cases of OSCC expressed p53 protein. Our results were similar as reported by Kaur et al.[29] An interesting observation noted in this study was that well-differentiated tumours had a high p53 immunostaining, while poorly-differentiated SCC showed significant weaker IHC p53 expression in comparison with well-differentiated SCC. These results were consistent with other studies, who have demonstrated statistically significant correlation between histological grade and p53 expression.[13,30,31]

This might be interpreted as most of the mutation in PDSCC 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 PDSCC.[32,33]

CONCLUSION

The most common oral epithelial lesion found in our study is SCC. P53 immunoreactivity is seen in 62.5% of benign, 72.72% of premalignant and 78.78% of squamous cell carcinoma. P53 immunoreactivity has no relation with age, sex and site of lesion. The immunoexpression of p53 increased with increasing grades of dysplasia indicating that they may be used as predictive markers in oral cancer development. Based on these findings, combined histological analysis with p53 immunoexpression, evaluation of premalignant lesions could be improved.

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