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p53 Staining Pattern in Benign, Premalignant and Malignant Oral Lesions

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Abstract

Introduction: Recent advance in the field of tumour suppressor genes and oncogenes have provided the tool for the study the genetic changes occurring at different stages of carcinogenesis including transition from premalignant to malignant. *Methodology:* Histopathologically diagnosed benign lesions, premalignant lesions & SCC were included in the study which consist of 33 cases of oral squamous cell carcinaoma (of which14 were well differentiated, 14 moderately differentiated and 02 poorly differentiated) and 03 were verrucous carcinoma. 11 premalignant lesions and 08 were benign lesions of the oral cavity. These cases were subjected to routine H&E staining and p53 immunohistochemical staining. *Results:* In this study, 4 out of 7(57.14%) cases of hyperplasia showed basal p53 positivity whereas the only cases of squamous papilloma was p53 negative. It was observed that 69% of malignant and 63% of premalignant lesions showed suprabasal p53 positivity whereas only one case of benign lesions showed the above pattern of staining. *Conclusion:* In conclusion, expression of p53 above the basal layer is an early event in oral carcinogenesis and an indicator of a developing carcinoma preceding morphological alterations

Keywords: p53 Staining; Benign; Premalignant; Malignant Oral Lesions.

Introduction

Head and neck cancers is the 6th leading carcinoma worldwide with >50,000 cases diagnosed every year. Vast majority of oral carcinomas are head and neck cancers are the oral squmaous cell carcinoma arising from the epithelial lining of oral cavity including tongue and lips [1].

Several risk factors are related to oral squamous cell carcinoma with main being tobacco, alcohol consumption & infection by high risk genotype of human papilloma virus [2].

In India the incidence is high, that is, 20 per 100,000 population and accounts for over 30% of all cancers in the country [3].

Oral squamous cell carcinoma usually preceded clinically by evident precancerous lesions. It is a multistep process requiring accumulation of multiple genetic alteration influenced by environmental influences like tobacco, alcohol or viral infection [4].

Recent advance in the field of tumour suppressor genes and oncogenes have provided the tool for the study the genetic changes occurring at different stages of carcinogenesis including transition from premalignant to malignant [5].

Among the gene associated with oral cancer p53 is a well known tumour suppresser 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.

Cells with loss of p53 function & 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 [6].

Therefore, the study was performed to assess the biological role of p53 in different types of premalignant and malignant lesion of oral mucosal & evaluate if

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any of these markers have value in early detection and prognostication of oral cancers.

Methodology

This study was a prospective study carried out in the department of pathology over a period of 24 months from September 2013 to September 2015.

All the patients referred to the department of pathology with oral lesions forhistopathological examination from the department of surgery and ENT were included in our study. The detailed history including the duration, site, onset, progression, personal habits and duration of those habits like smoking, beetle quid chewing were noted.

Histopathologically diagnosed benign lesions, premalignant lesions & SCC were included in the study which consist of 33 cases of oral squamous cell carcinoma(of which14 were well differentiated, 14 moderately differentiated and 02 poorly differentiated) and 03 were verrucous carcinoma. 11 premalignant lesions and 08 were benign lesions of the oral cavity. These cases were subjected to routine H&E staining and p53 immunohistochemicalstaining.

Compiling the clinical history and diagnosis we got different types or oral growth in our department i.e Exophytic, endophytic, leukoplakic, erythroplakic and erythroluekoplakic.

Histopathological examinations were done from tissue samples obtained by excisional, incisional and punch biopsy. Histopathological sections were routinely stained with haematoxylin an eosin [H&E] and also p53 was done on same specimens.

Criteria to Define p53 Positivity

The presence of Brown Precipitate seen with in the was considered as positive immunoreactive for p53.

Positive and Negative Controls

The positive control was taken was Adenocarcinoma colon slide staining. For the negative **control** addition of primary antibody supplemented by adding buffer to p53 staining slides. Immuno-histochemistry was scored by its extent and intensity.

Evluation and Interpretation

The slides stained for p53 were observed under light microscope with a magnification of 400X. The tissue sample were thoroughly examined and the total number of 500 cells were counted. Then among the 500 cells the number of cells which had taken up the stain was counted and the scoring was done.

Scoring

The fraction of stained cells was scored according to following criteria.

Score 0: 0 or <10% positive cancer cells.

Score1: 11-50% positive cancer cells.

Score 2: 51-80% positive cancer cells.

Score 3: >80% positive cancer cells.

The Staining Intensity on 4 Step Scale

Depending upon the staining intensity of the p53 positivity is graded as according to Ching et al 2013

0- No staining.

1+ - Weak staining.

2+ - Moderate staining.

3+- Strong staining.

Three Categories Were Identified

Depending upon the localization of staining it is catogerised into following category.

Category 0- No p53 staining inany epithelial layer.

Category 01- Nuclear staining confined to basal cell layer.

Category 03- Clear suprabasal nuclear staining.

The ultimate score attributed to the lesion was always the highest score found in several regions analyzed. The consecutive H&E stained slides were evaluated by two pathologists without knowing the previous histopathological diagnosis or the p53 staining pattern.

Subsequently the p53 stained slides were compared with the H&E stained slides to establish a relationship between p53 stained areas and the respective histopathological diagnosis.

Results

The table above shows 4 out of 7(57.14%) cases of hyperplasia showed basal p53 positivity whereas the only cases of squamous papilloma was p53 negative.

As above show, 5out of 6 (83.33) cases of severe dysplasia, all 2(100%) cases of verrucous hyperplasia showed suprabasal p53 positivity. As for a mild

dysplasia 2out of 2 (100%) was p53 negative.

In the malignant category 78% of well differentiated 64% of moderately differentiated and 50% of poorly differentiated squamous cell carcinoma, as well as all 77% cases odd verrucouscarcinoma showed suprabasal p53 positivity ranging from moderate to strong in the majority of cases. On the other hand it was observed that 7/33malignant cases were negative for p53 expression.

Immunohistochemical staining for p53, when observed, was found exclusively in the nuclei of the epithelial cells.

Table 1: P53 staining pattern in Benign lesions (n=08) (P=0.513)

Histopathological Diagnosis	Negative	Basal	Supra Basal	Total
Hyperplasia	02	04	01	07
Without dysplasia	28%	57%	15%	
Squammous	01	00	00	01
papilloma	100%			

Table 2: P53 staining pattern in Pre malignant lesions

Histopathological Diagnosis	Negative	Basal	Suprabasal	Total no
Mild	02	00	00	02
Dyplasia	100%			
Moderate	01	00	00	01
Dysplasia				
Severe	00	01	05	06
Dysplasia		17%	83%	
Verrucos	00	00	02	02
Hyperplasia			100%	

Table 3: Staining pattern in various grades of squamous cell carcinoma (n=33)

Histopathological Diagnosis	Negative	Basal	Suprabasal	Total
Well Diffrentiated Squamous Cell	02	01	11	14
Carcinoma	14%	2%	78%	
Moderately Differentiated	03	02	09	14
Carcinoma	21%	14%	64%	
Poorly Differentiated Carcinoma	01	00	01	02
	50%		50%	
Verrucous	01	00	02	03
Carcinoma	33%		77%	

Table 4: Distribution of oral epithelial lesions

Epithelial Lesions	No of cases	%
Papilloma	01	1.9
Dysplasia		
Mild	02	3.8
Moderate	01	1.9
Severe	06	11.5
Verrucous hyperplasia	02	3.8
Malignant lesions SCC	33	63.4

Table 5: P53 Staining PATTERN in Benign, premalignant &Malignant lesions (p=0.201)

	P53			
	Negative	Basal	Suprabasal	Total No
Benign	03	04	01	08
	37%	50%	13%	
Premalignant	03	01	07	11
	27%	09%	63%	
Malignant	07	03	23	33
	22%	09%	69%	

It was observed that 69% of malignant and 63% of premalignant lesions showed suprabasal p53 positivity whereas only one case of benign lesions showed the above pattern of staining.

Discussion

Mutation in p53 gene are the most common genetic basis for human carcinogenesis. These mutations lead to uncontrolled cell proliferation resulting in futher genetic abnormalities and finally malignancy [7].

P53 mutation usually shows clonality in cancer therefore it has occurred in early stage of carcinogenesis in oral squmaous cell carcinoma [8].

In the present study most common oral epithelial lesion found is OSCC(63%) (n=33). Malignant lesions are common in > 40 years of age group. Benign lesions are common in lower age group i.e 20-40 years(n=5). Moreover it was found that malignant SCC carcinoma found in 59.6% of cases were in 40 years or above (n=31).

Premalignant lesions found in 11 cases (21.1)(n=11). This is same as compared to most of the other studies [9,10,11].

SuchitaPanjawani reported 34% of cases of SCC were below the age of 40 years and 1.9% patients were below the age of 20 years this could be attributed to the fact that children come in contact with paan and tobacco specially in lower socioeconomic group at very young age so that exposure to carcinogens starts at early age [12]. According to Llewellyn et al SCC is not frequent in young patient[D7]. Only 1 to 6% of SCC cases occur in patients under age of 40 years indicating that the occurance in children and adolescent it is extremely rare [13,14].

In our study we have found 2 cases of SCC below the age of 30 years i.e 3.8% which is comparable with the above study and its is due to alcohol intake & smoking habit growing in younger adults.

In the present study males were affected with premalignant lesions more than females i.e 3:1 it is because males are more likely to display oral habits such as tobacco, smoking, betel quid chewing, ghuthka chewing. It can be compared with the study in Greek & Brazilian population where a higher ratio of 9.2:1 and 4.8:1 was found 15,16 respectively.

In the persent study the most common location of oral lesion is toungue 48%, of which squmous cell carcinoma comprises of 42% cases (n=14) this is same as compared to most other studies [9,10]. Well differentiated and moderately differentiated SCC found in equal no (n=14) in our study this finding is in contrast to Haq ME et al whome found that poorly differentiated SCC was tha most prevalent histological variant [17]. Zedanetalreported WDSCC as the most common histological type [9].

Out of 62 cases studied 8 are benign lesions and 11 are premalignant lesions.Premalignant lesions showing different grades of dysplasia.Similar comparative study of p53 expression done in hyperplastic & dysplastic epithelium and oral SCC done by SuchithaBansal et al [18].

Tumour suppressor gene is the most common identified mutated gene in diverse type of human cancers. P53 tumour suppressor gene is thought to be an important component of oral carcinogenesis. Identification of mutant p53 gene but IHC staining gives important information of tumour progression from benign to malignant lesions.

Our attempt to analyze p53 expression showed uniform pattern and intensity of expression in all staining batches which composed of benign premalignant & malignant lesions suggesting that the Immunohistocemical procedure utilized is standardized & hence the results can be consider variable.

In our study 33.3% of benign lesions shows negativity with p53 (n=3). 12.5% of benign lesions show suprabasal positivity and the rest 50% of the lesions showed basal positivity.

In Smitha et al study all cases of normal mucosa showed negativity with p53 this is in accordance with many other studies suggesting its short half life & lack of stabilization in oral mucosa.

In our study p53 was not done over normal mucosa. P53 nucleus staining confined to basal and parabasal layer in 66.6% of cases(n=5). Similar findings have been reported in oral buccal mucosa, skin &larungeal epithelia.

Oral epithelial dysplastic cases showed varing proportion of p53 staining with 3 cases being negative. There is no statistical significance in p53 expression in different grades of dysplasia which is concordance with the study of Abbas et al who also could not find any clear correlation between grades of dysplasia and percentage of p53 positive cells in oral premalignant lesions. An increased positivity of p53 staining (72.7) (n=8) in premalignant oral lesions indicate that altered p53 expression may be an early event in pathogenesis of oral neoplasia. However, the probable reason for p53 to be undetectable in premalignant & malignant oral lesions might be that the cells with frame shift or non sensemutation in coding sequence of the gene

result in absence, truncated or unstable protein.

Conclusion

Since immunohistochemistry cannot always detect changes in p53 expression in lesions preceding carcinoma, p53 immunohistochemical analysis is strongly recommended in conjunction with histological parameters, which will increase the sensitivity of detection of cases that will progress to carcinoma.

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