Immunohistochemical Overexpression of p53 in Head and Neck Squamous Cell Carcinoma; Clinico-Pathological Correlation

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ABSTRACT

BACKGROUND

Amongst all the malignancies that can develop in our body, Head & Neck Squamous Cell Carcinoma (HNSCC) is one which has a very high incidence with approximately 6,00,000 new cases diagnosed annually having a mortality rate of fifty percent.[1] Over expression of p53 is considered to be a marker that is associated with poor prognosis & hence a predictor of outcome of the disease.[2] The aim of the study therefore was to evaluate the over expression of p53 in HNSCC with its association to survival & various clinico pathological features.

METHODS

This study is a prospective study at PRM Medical College & Hospital, Baripada, Odisha. Cases diagnosed to be HNSCC in routine histopathology were taken up for the study using the paraffin blocks for IHC to study the expression of p53 and correlate with the histologic grade & outcome of the disease.

RESULTS

The study included 50 cases of HNSCC in which p53 over expression was seen in 64% of the cases with a higher expression in cancers of buccal mucosa. There was a significant difference in relation to histologic grade of the disease. Majority of the cases diagnosed belonged to Grade I & II and p53 expression was noted in 32/50 cases (64%), 63.3% of Grade I tumour expressed p53, whereas 73.68% cases of Grade-II tumours expressed the same. There was no significant gender bias.

CONCLUSIONS

p53 can be used as a prognostic indicator to comment upon eventual aggressiveness and overall survival.

KEYWORDS

P53, Head & Neck Squamous Cell Carcinoma, Tobacco

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BACKGROUND

Head & Neck Squamous Cell Carcinoma still remains a significant cause of morbidity & mortality despite all the advances made in the treatment of cancer.[3,4] HNSCC has multifactorial aetiology like tobacco smoking, betel quid chewing, alcohol intake, poor oral hygiene and oncogenic virus such as HPV 16.[5] Though these risk factors are common, still patients with little or no exposure to risk factors can develop HNSCC. Approximately half of all patients with HNSCC have an advanced stage at the time of diagnosis, with an expected 5 yrs. survival of 10-40%.[6] Prognosis depends largely on the stage of disease i.e. presence of neck node metastasis and positive resection margin status which reduces long term survival by 50%.[7] Oral carcinogenesis is a multi-step process involving multiple proto-oncogenes including p16, Cyclin D1, p53 and EGFR. p53 is a tumour suppressor gene located on chromosome 17p. Mutation of p53 gene is one of the most common events in human carcinogenesis and as mutated protein is not easily digestible, therefore it accumulates inside the cancer cell leading to immunohistochemical over expression which is considered a marker of poor prognosis. Moreover, p53 over expression may result in decreased sensitivity of tumour cells to chemotherapeutic drugs.[8] Therefore this study was aimed to evaluate immunohistochemical over-expression of p53 in HNSCC and its association with various clinico pathological features and survival so as to provide an insight to use of therapeutic protocols devised for loco-regional population.

We wanted to study the immunohistochemical over expression of p53 in HNSCC and correlate it with clinical features, histologic grade and prognosis.

METHODS

The present study is a prospective study carried out all PRM Medical College & Hospital for a period of one year i.e. November 2018 to November 2019 in collaboration with SCB Dental College. This study has been approved by Institutional Ethics Committee. Informed written consent was taken from all of the patients.

Inclusion Criteria

Cases histologically diagnosed to have HNSCC at the institute pertaining to Oral Cavity, Oropharynx, hypopharynx & larynx. 5 specimens of normal mucosal area from these cases were taken as control.

Exclusion Criteria

Histologic diagnosis limited to dysplasia & recurrent case of HNSCC.

H & E Stained section of all the cases & paraffin blocks of 50 cases were recruited & new section were cut when felt necessary. Slides of all the cases were re-evaluated by two senior histopathologist independently and pathologic characteristic like tumour type, grade, tumour stage, nodal spread, lymphovascular & perineural invasion were interpreted. Clinical details pertaining to age, sex duration of tobacco use were obtained from the records.

Immunohistochemistry

The antibodies and chemicals were from Biogenex, USA, Mouse monoclonal antibody to p53 (clone BP53-12-1; catalog no. Am 195-2) were used for evaluation. External positive & negative control slides were used with each batch of staining. Positive controls were prepared from carcinoma breast & Negative control slides were prepared from the same tumour block under study. For assessment of staining, five different areas were evaluated and the immune expression limit was set at 25%. i.e. p53 gene mutation was considered positive if 25% or more than 25% of tumour cells showed nuclear staining for p53 protein. Further grading was done according to the number of cells that showed positivity for nuclear staining. The most densely stained area was considered for grading. Such as Grade I -25% positive, Grade-II - 25-50%. Grade-III 50.75% & Grade IV- 75 to 100%. The relationship between qualitative parameters was determined using the chi-square test. Statistical significance was defined as $p<0.05$ (SPSS software).

RESULTS

The study included of 50 cases Head & Neck squamous cell carcinoma out of which Grade I tumour consisted of 60%, Grade-II 38% and 2% were of grade III. (Table-IV). The age range of patients were from 26-83 yrs. & age with mean age 52.94 yrs. Age group 50 to 70 had the highest number of cases accounting for about 52%. Lowest number was in the early age group of 20-29 yrs.

Age Group in Yrs.	No. (n)	(%)
20-29	2	6%
30-39	8	16%
40-49	9	18%
50-59	13	26%
60-69	13	26%
>70	5	10%
Total	50	100%

Table 1. Age Distribution of HNSCC Cases (n=50)

Site	No. (n)	(%)	Ratio
Male	31	62%	
Female	19	38%	
Total	50	100	1.63:1

Table 2. Gender Distribution of HNSCC Case (n=50)

Site	No. (n)	(%)
Tongue	18	36
Buccal Mucosa	20	40
Lower Alveolar Sulcus	09	18
Lip, Larynx & Tonsil	03	6
Total	50	100

Table 3. Tumour Distribution in Head & Neck Region (n=50)

Tumour Grade	No. of Cases	(%)	p53 Expression Positive
Grade-I	30	60%	19/30 (63.3%)
Grade-II	19	38%	14/10 (73.68%)
Grade-III	01	02%	1/1 (100%)

Table 4. Histologic Grade of HNSCC (n=50) & p53 Expression (n=50)

Out of 50 cases, 39 were male & 19 were female: the ratio is affection was found to be 1:63:1. The maximum site predilection was buccal mucosa followed by the tongue other site involved were lower alveolar sulcus, lip, larynx & tonsil.

LN Metastasis	p53 Expression	
	Positive	Negative
Negative (19)	10 (52.63%)	9 (47.36%)
Positive (31)	21(67.74%)	10(32.25%)

Table 5. Association of p53 Expression and Nodal Metastasis

Risk Factor	Site of Predilection	Grade Histologic Grade	Associated with Depth of Invasion	Nodal Status Association
Tobacco 70%	Buccal mucosa	Grade-II 73.68% Grade III 100%	Absent	Significant association absent

Table 6. Association between Risk Factors, Site, Tumour Grade Depth of Invasion and Nodal Status

DISCUSSION

In the present study p53 positively was found in 32 out of 50 cases accounting for 64 percent of cases. Similar results have been found in study done by Kaur J et al[2] who reported positivity in 64% case. Another study done by Dragmir et al[9] found a positivity of 82.3%. Tuen PW et al[10] reported an expression in 52% of cases conducted in a study done on 87 cases. Even though the results were variable p53 expression was noted in more than 50% of cases in all these studies. P53 positivity was seen in 66.6% i.e.- 20 out of 31 male & 12 case out of 19(63.11%) in females: indicating no significance gender association as regards p53 expression. P53 over expression in HNSCC varies in different parts of the world possibly due to divergent risk factors and pathogenesis of disease. A positive association of alcohol use with p53 over-expression & negative association with betel nut & tobacco use was reported by Kerdpon et al.[11]

Gonzalez-Moles revealed 57.7% expression of p53,[12] on the other hand 63.3% expression was noted in research conducted in Brazilian population,[13] where as an expression as low as 28.5% was noted in a study conducted in Iran.[14] This varied expression in different studies showing expression ranges from 25 to 90% may be due to use of different techniques, methods of interpretation or due to ethnicity & risk factors associated with HNSCC pathogenesis.

Many studies have proved the prognostic significance of p53 in HNSCC, owing to association of p53 over expression with overall survival, recurrence and high tumour grade,[15] however in our study, cases are being followed up for further analysis. Conversely a meta-analysis involving 174 studies reveal no association of p53 over expression as a marker of poor prognosis.[16] Due to significant difference in different studies conducted worldwide, further study involving a large sample size to evaluate the prognostic significance of p53 over expression HNSCC and the association with disease free survival will be very helpful.

CONCLUSIONS

p53 can be used as a prognostic indicator to comment upon eventual aggressiveness and overall survival. Further studies with larger sample of moderately differentiated and high grade tumours are needed to evaluate the role of p53 as a prognostic marker and evaluate its possible role in targeted therapy of the disease.

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