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Research Article

EXPRESSION OF p53 IN PRIMARY INVASIVE HEAD AND NECK SQUAMOUS CELL CARCINOMA AND ITS CORRELATION WITH CLINICOPATHOLOGICAL PARAMETERS

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A B S T R A C T

Dysfunction in the p53 tumor suppressor gene is implicated in many cancers, including head and neck cancer, and has received most attention. The present study was designed to evaluate the expression of p53 in primary squamous cell carcinoma of head and neck region, and also the relationship between p53 expression with clinicopathological parameters. 50 specimens of Head and Neck Squamous Cell Carcinoma (HNSCC) were subjected to staining by immunohistochemistry for p53 protein using monoclonal primary antibody. The study revealed 27/50 (54%) cases of HNSCC being positive for p53 protein. Peak incidence was found in the age group of 41-60 years, the most common site being hypopharynx. There was a significant correlation of p53 expression with lymph node metastasis (*p*-value=0.040). However, no significant correlation was found with the other clinicalpathological parameters.

INTRODUCTION

An emerging public health problem in India are the head and neck cancers. Squamous cell carcinomas comprise more than 95% of the head and neck region. Head and neck squamous cell carcinoma (HNSCC) arises from the mucosal linings of the upper aerodigestive tract, which comprises of 1) the nasal cavity and the paranasal sinuses, 2) the nasopharynx, 3) the hypopharynx, the larynx, and the trachea, and 4) the oral cavity and the oropharynx [1]. HNSCC is the 6th most common neoplasm in the world today [1]. Each year, approximately 560,000 new cases and 300,000 deaths are detected worldwide [2]. Incidence rates are higher in men than in women [3]. In India, prevalence of head and neck cancers is high accounting for 30-40% cancers at all sites, out of which oral cancers comprise about 9.4%. It is the sixth common cause of death in males and seventh in females. In North-east India, where chewing of tobacco is very common, incidence of tobacco related oral cancers is about 33% [4].

p53 gene mutation is the most common mutation in human cancer; 40-50% of HNSCC carry a p53 mutation. p53 alterations in head and neck carcinogenesis are very frequent and appear at early stages. They are of particular relevance as two major risk factors i.e tobacco carcinogens and HPV infection, appear to target p53. p53 alterations in HNSCC has been widely studied. Some of the technologies described for the identification of p53 alterations include immunohistochemistry, mutation screenings, antibody detection and functional tests in yeast [5]. The objective of this work is to assess the expression of p53 in HNSCC and also to find if any correlation exist between the expression of p53 and the other clinicopathological parameters.

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MATERIALS AND METHODS

The study was conducted in the Department of Pathology, Assam Medical College, Dibrugarh over a duration of one year from July 2013- June 2014. It was a hospital based cross sectional study. 50 cases of HNSCC diagnosed by histopathology were included in the study.

Inclusion criteria: The patients diagnosed histopathologically as invasive squamous cell carcinoma of the head and neck region.

Exclusion criteria: Secondary cases of squamous cell carcinoma, any metastatic lesion, primary squamous cell carcinoma of other sites and all carcinoma in situ of head and neck were excluded from the study.

Ethical clearance was taken from the Institutional ethical committee before starting the study.

Representative sections were taken and fixed in 10% buffered formalin for histopathological examination. Sections were stained with the routine haematoxylin and eosin stain using standard protocol. The histological diagnosis was as certained and the grade of differentiation was categorized according to

the WHO guidelines 2005 into well, moderate and poorly differentiated carcinoma [6].

Immunohistochemistry was done on all the 50 paraffin embedded blocks to look for the expression of p53 staining.
For positive control a colorectal neoplasm with a high level of nuclear p53 immunoreactivity was used. Negative control slides were processed with each slide run by excluding the primary antibody but including all other steps of the procedure [7].

For reporting of p53, all the stained slides were examined under light microscope with a magnification of 400x. A total of 500 cells were counted in atleast 5 histological fields, randomly chosen for each case in the most representative areas of tumour. Of the 500 cells, the number of cells which had taken up the stain were counted and the percentage of stained cells was calculated from it. Presence of p53 protein was confirmed by the appearance of a brown precipitate in the nucleus of the tumour cells. The samples were then evaluated on a 4-point scale based on the percentage of the cells showing p53 staining [8]. They can be graded as follows;

Negative- if no cell showed positive stain or <10% cell showed positive stain.
Positive stains are divided into the following grade
Grade 1+ if 10-30% tumour cells showed positive stain
Grade 2+ if 30-50% tumour cells showed positive stain
Grade 3+ if > 50% tumour cells showed positive stain.

For statistical analysis, we divided the 50 cases into two groups, p53 positive and p53 negative groups. The p53 positive group comprised of those cases in which >10% malignant cells showed nuclear staining. The p53 negative group was considered as those cases where <10% tumour cells showed nuclear staining [9,10,11].

RESULTS

Of the 50 cases, 36 cases were male and 14 were female. There was a clear male preponderance with male:female ratio of 2.57:1. The mean age of presentation was 54.74 years with age groups ranging from 31-80 years. Peak incidence of HNSCC was found in the age group of 41-60 years. The most common site of HNSCC was hypopharynx (34%), followed by oral cavity (30%), oropharynx (22%) and larynx (14%). 24/50 (48%) of the cases were well differentiated tumours, 23/50 (46%) were moderately differentiated and 3/50 (6%) were poorly differentiated tumours. 16/50 (32%) of the cases had lymph node metastasis at the time of presentation.

24/50 (48%) of the cases showed <10% staining of tumour cells with p53 whereas 26/50 (52%) of the cases showed >10% staining. (Table 1) Out of the 26 cases of positive p53 staining majority showed Grade 3+ staining 15/50 (30%) followed by Grade 2+ staining 10/50 (20%) and Grade 1+ staining 1/50 (2%). (Table 2) There was a significant correlation of p53 expression of HNSCC with lymph node metastasis (p-value=0.0354).

Table 1 Percentage of p53 expression of the HNSCC cases

% of tumour cells showing p53 expression	No of cases	Percentage
Negative (<10%)	24	48%
Positive (>10%)	26	52%

Table 2 Grading of HNSCC cases showing percentage of p53 expression

Grade of p53 staining	No of cases	Percentage of cases
Negative	24	48%
Grade 1+	01	02%
Grade 2+	10	20%
Grade 3+	15	30%

However, no significant correlation of p53 expression of HNSCC was found with the other clinicopathological parameters such as age, gender, site and histological differentiation. (Table 3)

Table 3 Expression of p53 and the clinicopathological features of the HNSCC included in the study.

Parameter		P53 expression <10%	P53 expression >10%	p-value
Age	<50	13	08	0.862
	>50	11	18	
Gender	Male	15	21	0.150
	Female	09	05	
Tumour site	Oral cavity	08	O7	0.746
	Oropharynx	07	04	
	Hypopharynx	06	11	
	Larynx	03	04	
Histological differentiation	Well differentiated	10	14	0.55
	Moderately differentiated	13	10	
	Poorly differentiated	01	02	
Lymph node metastasis	Present	04	12	0.0354
	Absent	20	14	

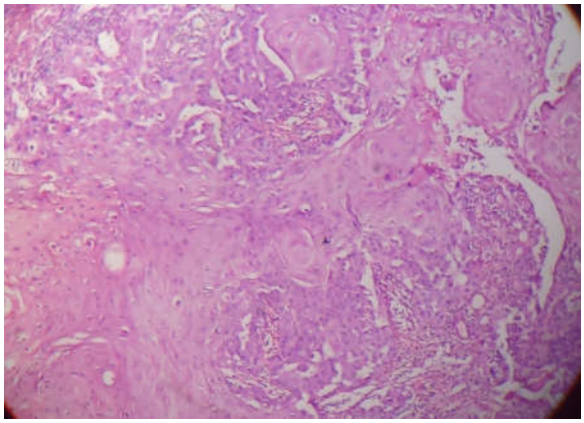

Figure 1 Well differentiated squamous cell carcinoma (H & E 100X)

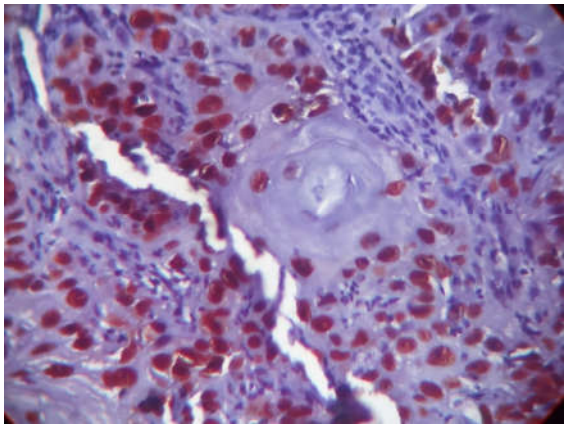

Figure 2 p53 positive staining Grade 3+, well differentiated squamous cell carcinoma (IHC 400X)

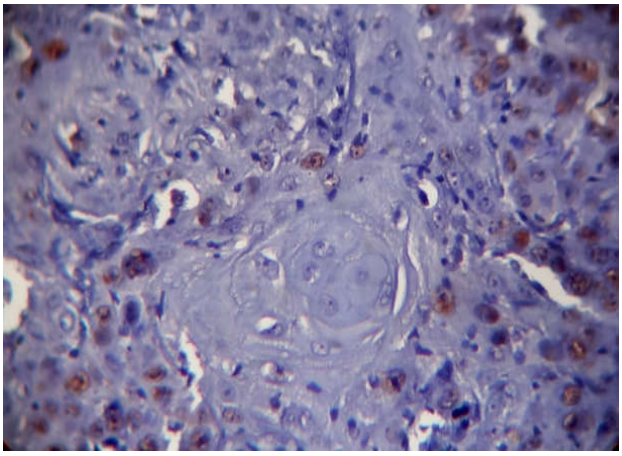

Figure 3 p53 positive staining Grade 2+, well differentiated squamous cell carcinoma (IHC 400X)

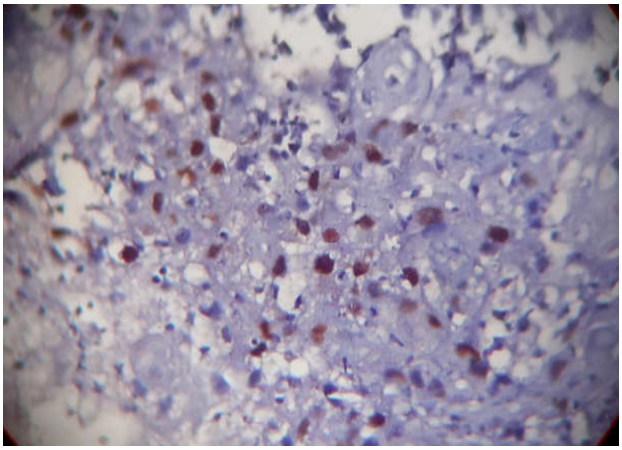

Figure 4 p53 positive staining Grade 1+, moderately differentiated squamous cell carcinoma (IHC 400X)

DISCUSSION

The head and neck cancers largely arise due to environmental insults, because it occurs among those with prolonged exposure to smoking, betel quid, tobacco chewing and alcohol consumption. HNSCC is a heterogenous group of cancer, with variable outcome, but usually a poor prognosis in the patient. The mortality of head and neck cancer has not changed much over the last few decades, despite advanced treatment modalities. It has been suggested that carcinogenesis of aerodigestive tract epithelium involves a complex multistep process [12]. The studies on the carcinogenesis in HNSCC will help to understand the biological behavior and throw light on the underlying mechanism of cancer progression and therapy resistance [13]. Among the genes related to head and neck cancer p53 has been most frequently studied. In normal squamous mucosa p53 overexpression is extremely uncommon [14,15]. The steady concentration of p53 is low, and half life of normal (wild type) p53 is short (four to five minutes) [16]. In contrast, if p53 gene is mutated, the genetic product is often present in high concentration with a long half life around 6 hours [17].

In this study, we found a significant correlation between positive immunoexpression of p53 with lymph node metastasis cases. (P- value=0.0354) but there was no correlation with the other clinicopathological parameters. Motta *et al*, 2009 [18], found a correlation between lymph node status and p53 expression (p-value=0.023). Jain *et al*, 2007 [19], found that cases presenting with lymph node metastasis were 100% positive with a mean p53 positivity of 51.6% ±13.2, in contrast to those without metastasis(20%± 19.5 positivity). The difference between p53 positivity was found to be statistically significant (p-value= <0.001). Kim *et al*, 1997 [20] also found a positive relationship between p53 expression and lymph node metastasis; where p53 expression was 69.2% in metastasis group and 16.7% in non metastasis group. The author concluded that p53 expression in squamous cell carcinoma was related to tumour progression and metastasis. Ashraf *et al*, 2010 [21] also found significant correlation of p53 expression with tumour location (p-value=0.0040, tumour stage (p-value=0.020 and lymph node involvement (p-value=0.008).

There is no distinct site specific histopathological differences in squamous cell carcinoma for the various intraoral sites. In general, tumours of the oral cavity and oropharynx having a larger size are more prone to metastasis. Risk of developing metastasis for tumors of size less than 3 cm is low, the risk from tumours that are 3 to 4 cm is intermediate, and the risk from tumors more than 4 cm is the highest [22]. The presence of neck lymph node metastases with extranodal extension of carcinoma is considered the most reliable prognostic factor in head and neck cancer [23].

CONCLUSION

Squamous cell carcinoma of the head and neck region with advanced primary lesions, with or without regional lymph node metastases, are challenging to treat effectively while maintaining the function of vital healthy structures. The outcome of patients with advanced stage of the disease still remains poor. p53 is a prime target for anticancer therapy and is beneficial when p53 inhibitor treatment is combined with radiotherapy to treat locally advanced head and neck cancer. In this study a significant correlation was found between the percentage of tumour cells stained by p53 with tumour that presented with lymph node metastasis. Hence, expression of this protein can be used as a marker for targeted therapy especially in patients with locally advanced tumours. It may help in determining the prognosis as well as play a role in gene therapy for treatment of head and neck squamous cell carcinoma. However, long term studies with large sample size would further help to assess the significance of the protein in head and neck carcinoma.

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