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P53 Expression in oral squamous cell carcinoma as a predictor of high grade malignancy and regional metastasis – an experience from a tertiary care hospital

Jasmeet Kaur[1], Rahul Mannan[2,*], Mridu Manjari[3], Tejinder Singh Bhasin[4], Sonam Sharma[5]

[1]Resident, [2,3,4]Professor, SGRD, Amritsar, Punjab, [5]Assistant Professor, Dept. of Pathology, Kalpana Chawla Govt. Medical College, Karnal, Haryana

***Corresponding Author:**
Email: rahulmannan@gmail.com

Abstract

Introduction: Oral squamous cell carcinoma (OSCC) is the sixth most common cancer in the world. It usually develops in males in the 6th and 7th decade and is caused by tobacco and alcohol consumption.

Materials and Method: This study was performed on 40 histopathologically proven cases of squamous cell carcinoma of oral region in Department of Pathology SGRDIMSR, Amritsar, Punjab. Tissue were stained for H & E and further immunostained for p53 receptor. p53 expression was correlated with histological grading, age, sex, vascular, perineural, muscle invasion and lymph node metastasis.

Results: In our study, maximum incidence was seen in age group of 41-60 years with a male:female ratio of 7:1. A statistically significant correlation between p53 positivity with grade of carcinoma and cases presenting with nodal metastasis was seen. However, no such correlation was seen with age, sex, vascular, neural and muscle invasion.

Conclusion: p53 positivity shows a direct correlation with grade and lymph node metastasis of SCC in head and neck. Thus, p53 has an influence on prognosis.

Keywords: Immunohistochemistry, Oral Squamous Cell carcinioma, p53, Punjab

Introduction

Incidence of oral squamous cell carcinoma (OSCC) is on an increase, with this being the 6th most common cancer in the world and the most common cancer in Indian males.[1] In India itself, it accounts for 40% of all malignancies.[2,3] Many factors are associated with oncogenesis of oral cancer which include tobacco and alcohol use and increased prevalence of high risk strains of HPV. Often it develops from a pre existing dysplastic lesion of which 5-10% progress eventually towards carcinoma.[4]

Various IHC markers are being increasingly used not only for diagnosis but also as research tools in cases of OSCC to predict prognosis and for therapeutic usage. Some of the molecules being used are - p53, Ki 67, CD 44, CD 133, and c- met.

p53 is a well-known tumor suppressor gene reported to have central role in various human neoplasia and also associated with Li–Fraumeni and related syndromes. p53 expression has been studied in various cancers by many researchers. A study done on immunoexpression of p53 as an indicator of invasiveness in esophageal cancer showed a significant correlation between p53 positivity and adventitial involvement.[5] Kaur H et al studied the immunoexpression of p53 in prostate carcinoma and found it to be 58% and also documented that with increase in grade of tumour, the number of cases showing p53 immunoexpression also increased.[6] p53, also known as TP53 or tumour protein is a gene encoding cell cycle regulator proteins and hence functions as a tumour suppressor gene.[7] p53 plays a key role in mediating cell response to various stresses, mainly by inducing or repressing a number of genes involved in cell cycle arrest, senescence, apoptosis, DNA repair and angiogenesis. p53 mutation has a strong association with the genesis of OSCC.

Although there are studies done in Indian sub-continent, describing the relationship between squamous cell carcinoma in oral cavity with p53, but very few have tried to investigate SCC with various clinicopathological parameters.

The present study was conducted in a tertiary care teaching hospital on north Indian Punjabi population predominantly rural based on histopathologically proven cases of squamous cell carcinoma of oral cavity. These lesions were classified and graded on histomorphology and expression of p53 was noted on immunohistochemical staining. The findings of the study were analyzed particularly in reference to p53 expression and their correlation with various epidemiological and histopathological parameters.

Materials and Method

The study was conducted on 40 cases of oral squamous cell carcinoma diagnosed in Pathology Department of Sri Guru Ram Das Institute Of Medical Sciences And Research, Vallah, Amritsar. Apart from the epidemiological data, clinical details were noted. In all the cases tissue were processed and 2-4 microns sections were cut and stained with Haematoxylin and Eosin stain. The slides were studied under light microscopy for histopathological grading and to document other pathological parameters (histological grading, vascular, perineural, muscle invasion, lymph node metastasis).

IHC was done on formalin-fixed sections, and paraffin embedded sections of representative blocks of each tumor mounted on poly-lysine pre-coated slides. Antigen retrieval was done in a pressure cooker using citrate buffer solution at pH 6.0. Peroxidase inhibition was then done, followed by washing in tris buffer saline and incubation in protein block. To evaluate p53 expression, p53 antibody (Diagnostic Biosystem) was used. Post primary block was then applied and incubation was done with DAB (3,3'diaminobenzidine). Sections were washed in deionised water followed by Haematoxylin counterstaining, dehydration and clearing of the sections in propanol and xylene respectively. Antigen thus expressed was visible under light microscopy as brown coloured nuclei of variable staining intensity.

Cases > 5% cells positive for p53 were included for final scoring. Positive control was colonic cancer cases which were positive for p53. Negative control section was provided by omission of primary antibody. Brown nuclei were taken as positive for p53. The intensity of p53 positivity was scored as – No staining (0), weak staining (+1), moderate staining (+2) and intense staining (+3).

Parameters included were age, sex, histological grading, vascular, perineural, muscle invasion and lymph node metastasis. The relationship between IHC expression and clinicopathological parameters was statistically analyzed using SPSS version 12 software. Chi-square test was used for data analysis. $P < 0.05$ was considered statistically significant.

Results

The maximum incidence was seen in age group of 41-60 years comprising 58% of the total. The incidence of OSCC was found to be higher in males with male: female (M:F) ratio being 7:1. Most of the patients presented with complaints of difficulty in speaking, dysphagia and complained of non-healing ulcer.

p53 Immunoexpression Rates: Overall, immunopositivity rate of p53 was 70% of all the OSCC cases. Percentage positive cells varied from 5-76% with mild, moderate and strong staining intensity.

Age Group Specific and Gender Specific P53 Immunoexpression Correlation: p53 positive cases had age variation from 32 -70 years. Most of the cases were in the age group of 41 to 60 years comprising 46% of the total p53 positive cases. However, no definite correlation of p53 expression with age was found. (p=0.071; chi square test) [Table 1]

Table 1: Correlation of p53 status with age

Age Group	p53 Expression		Total
	Positive	Negative	
21-40	06	00	06
41-60	13	10	23
61-80	09	02	11
Total	28	12	40

Correlation of p53 Immunoexpression and Tumour Grade: Most of the cases were moderately differentiated comprising 68% of the total cases, followed by well and poorly differentiated tumours comprising 17% and 15% respectively. Correlating tumour grade with p53 expression, it was noted that in well differentiated carcinomas the rate of expression was 14%, in moderately differentiated, the immunoexpression rate was higher (78%) and in poorly differentiated carcinomas, immunoexpression rate was highest (100%). Thus, an increase in p53 immunopositivity expression was observed with increase in tumour grade, and this was found to be statistically significant. (p = 0.001; chi square test) [Table 2]

Table 2: Correlation of p53 expression with grade of carcinoma

Grade	p53 Expression		Total
	Positive	Negative	
Well	01	06	07
Moderate	21	06	27
Poor	06	00	06
Total	28	12	40

Correlation of p53 expression with Vascular, Peri-neural and Muscle Invasion: p53 positivity was seen in 63% cases showing vascular invasion. Both the cases showing peri-neural invasion showed p53 positivity. 8 cases with muscle invasion showed p53 positivity (62%). No significant statistical correlation of p53 expression with vascular, peri-neural or vascular invasion was seen with p value being 1.000, 0.253 and 0.916 respectively. (Chi square test) [Table 3]

Table 3: Correlation of p53 expression with vascular, perineural and muscle invasion

p53 Expression	Invasion					
	Vascular		Peri-Neural		Muscle	
	Present	Absent	Present	Absent	Present	Absent
Positive	05	10	02	12	08	07
Negative	03	06	00	10	05	04
Total	08	16	02	22	13	11

Correlation of p53 expression with lymph node status: Metastatic deposits were seen in 13 cases of which 10 cases (77%) showed p53 positivity. A significant correlation between lymph node metastasis and p53 positivity was seen with p value of 0.041. (Chi square test) [Table 4]

Table 4: Correlation of p53 expression with lymph node status

p53 Expression	LN Status		Total
	Metastatic	Reactive	
Present	10	03	13
Absent	03	06	09
Total	13	09	22

Discussion

p53 has been described as "the guardian of the genome", referring to its role in conserving stability by preventing genome mutation.[8] p53 was identified in 1979 by Arnold Levine, David Lane and William Old, working at Princeton University, Dundee University and Sloan-Kettering Memorial Hospital respectively. The human p53 gene is located on the seventeenth chromosome (17p13.1).[(9)]

If p53 gene is damaged, tumour suppression is severely reduced. Defective p53 could allow abnormal cells to proliferate resulting in cancer. p53 gene mutations are involved in early events of multistage carcinogenesis of oral epithelium.

The purpose of present study was to determine the expression of p53 in cases of OSCC and to determine the relationship of p53 expression and intensity with various clinic-histomorphological parameters.

Most of the patients in the present study were in the age group of 41-60 years of age with higher male preponderance showing that OSCC usually affects elderly age group and is seen mainly in males. This is in corroboration with the work done by other reseachers.[(1,10)] However; no definite correlation of p53 expression with age and gender was found.

In the current series of 40 cases of OSCC, p53 immunopositivity was seen in 70% of the cases (percentage positive cells varying from 5-76% with mild, moderate and strong staining intensity and final score varying from 0 to 9). Many researchers such as Dragomir LP et al and Jain et al have also reported a high p53 expression in OSCC (82.3% and 74.3% respectively).[(1,11)]

Contrary to our findings, many researchers have found a lower p53 immunoexpression such as Shiraki et al and Ruchita et al who have reported them to be in the range of 46-60 %.[(12,13)]

In the present study, a correlation was seen between p53 expression and tumour grade. An increase in p53 expression was seen with increase in tumour grade (78% in moderately differentiated to 100% in poorly differentiated) and was found to be statistically significant (p = 0.001). In similar vein when the p53 immunointensity was correlated with tumour grade, it was found that as grade increases, intensity of p53 stain increased. This was also found to be statistically significant. (p value = 0.011, chi square test). This implied that both immunoexpression as well as intensity of staining are independent variables in predicting eventual tumour grading, as a non representative punch biopsy if exhibiting immunoexpression and increased intensity in dysplastic/in-situ component or in tumour tissues will be associated with a poor prognosis. These findings find a corroboration with the work done by various researchers.[(11)] However, many studies have shown no correlation of p53 with the degree of differentiation in oral cancers.[(1)]

p53 expression, was seen in 77% of cases in which metastatic deposits were seen. Thus an increase p53 expression was noted in those cases which showed positive lymph node deposits. This was also found to be statistically significant in present study in concordance with the work done by researchers like Jain A et al and Li Y.[(11,14)] The lymph node metastasis is the most important prognostic factor for overall survival in case of OSCC including head and (HNSCC) as about two-third of the patients with HNSCC who present with lymph node metastasis have a poor five year survival rates of 40-50%.[15] Thus, p53 expression can predict the overall prognostication in all patients of HNSCC and OSCC.

Although p53 immunoexpression was seen to be in significant numbers in cases having vascular, muscular and perineural invasion, no significant correlation could be elicited statistically. Similar results were obtained by researchers who did not find any correlation between p53 overexpression and vascular invasion and early local recurrence.[(16)]

Conclusion

It is thus recommended that p53 should be employed in all the cases of OSCC to predict prognosis and to comment upon eventual aggressiveness/tumor free survival. All the cases exhibiting p53 immunoexpression should be managed aggressively.

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