

Study of p53 gene over expression as a prognostic marker in squamous cell carcinoma of the oral cavity

Ruchee Khandelwal¹, Rohit Sharma², Deepti Agarwal³, Aakriti Baijal^{4,*}

¹Associate Professor, ²Professor and HOD, ³Assistant Professor, ⁴Junior Resident, ^{1,3,4}Dept. of Pathology, ²Dept. of ENT, Sri Ram Murti Smarak Institute of Medical Sciences, Bareilly, Uttar Pradesh, India

***Corresponding Author:**

Email: aakritibaijal111@gmail.com

Received: 20th February, 2018

Accepted: 3rd March, 2018

Abstract

The present study entitled "Study of p53 gene expression as a prognostic marker in squamous cell carcinoma of oral cavity" was conducted in the department of pathology SRMSIMS Bareilly, 66 cases diagnosed on histopathology as squamous cell carcinoma of oral cavity from Jan 2016 to Jan 2017 were included in the study. Various parameters analysed for each case included age, gender, site, history of tobacco chewing, smoking &, alcohol consumption, histopathological grade of tumor and p53 expression. The patients age ranged from 24 to 85 years with mean age; 52.09 years and median age; 53 years. Maximum no of patients were male 54(81.8%) and 12 (18.2%) were female. Male: female ratio was 9:2. The most common site of presentation was lateral border of tongue (34.8). In the present study 89.4% were tobacco chewers, 72.7% were alcoholics and 57.6% were smokers. 34 cases (51.5%) were diagnosed as well differentiated, 26 cases(39.4%) as moderately differentiated and 6 cases (9.1)% as poorly differentiated squamous cell carcinoma. The p53 expression was observed in 41(62.1%) of cases. The expression of p53 increased with the grade of tumor. The positivity being 17(50%) in cases of well differentiated, 19(73%) in moderately differentiated and 5(83%) in poorly differentiated squamous cell carcinoma. In present study p53 expression is found to be significantly related with tobacco chewing (p value= 0.034), smoking (p value= 0.0046) and tumor grade (p value=0.030). However no association was found with alcohol consumption (p value=0.917) the increasing prevalence of p53 expression with increasing grade of tumor suggests that it can be used as a prognostic indicator in oral cavity cancers.

Keywords: Squamous cell carcinoma, p53 expression, Prognostic indicator, Oral cancer.

Introduction

Worldwide, oral cancer is one of the most prevalent cancers and is one of the 10 most common causes of death. Oral cancer accounts for 2% of cancer deaths in males and 1% in females.¹

Oral cancer is a disease of increasing age. Approximately 95% occur in people older than 40 years, with the average age at diagnosis of approximately 60. The increasing incidence of oral cancer reflect declining immune surveillance with age and the duration of exposure to chemical irritants, physical irritants, viral infection, hormonal effects, cellular aging and decreased immunologic surveillance. Evidence from long term follow-up of immunosuppressed patients following organ and bone marrow transplant shows that immunosuppression increases the risk of the development of squamous cell carcinoma.

It is important to note that 75% of oral squamous cell carcinomas have been reported to arise in an area that comprises the floor of the mouth and adjacent lingual mucosa, sublingual sulcus and retromolar region.²

The cause of oral squamous cell carcinoma is multifactorial. Extrinsic factors like exposure to tobacco, smoke, alcohol, syphilis and sunlight seem to work together with several intrinsic factors which include systemic or generalized states, such as general malnutrition or iron –deficiency anemia. Heredity does

not appear to play a major causative role in oral carcinoma. Optimal therapy and survival from oral cancer depend on adequate diagnosis and assessment of the primary tumor and its clinical extent. Few prognostic markers of oral cavity carcinomas are site³⁻⁸ stage,⁹ grade,^{10,11} depth of invasion,¹²⁻¹⁴ tumor size,¹⁵ tissue eosinophilia¹⁶ and lymphnode involvement.¹⁷ Tumor size and nodal status are the most significant prognostic factors.¹⁸ Histological grade correlates poorly with patient outcome^{19,20}

P53 is a proto-oncogene and has its role involved in controlling the cell death cycle, consequently in genomic stabilization. Mutated genes become oncogenes and lose their property of removing an altered cell from cell cycle, repair it and take it back to the cycle or lead it to programmed cell death, thus causing carcinogenesis.

In normal cells, wild type p53 protein has a very short half life (6-2 min) and is present in such small quantities that it cannot be detected by immunohistochemical methods. However, missense mutations in the p53 gene often result in a more stable gene product and prolong the half life of the p53 protein, causing it to accumulate within the cell nuclei to the extent that it can be easily detected by means of immunohistochemistry.²¹

The p53 detection by IHC may be one of the important tumor markers which can help not only in determining the prognosis but also have a key role in

gene therapy in future. Thus this study was undertaken to study p53 expression in squamous cell carcinoma of oral cavity and to see if there was any correlation with tumor grade.

Materials and Methods

This prospective study was carried out in department of pathology, Sri Ram Murti Smarak Institute of Medical Sciences, Bareilly Uttar Pradesh, India.

A total of 66 cases, diagnosed as oral cavity cancers on histopathology from January 2016 to December 2017 were included in the study.

The specimens received were fixed in 10% formalin and embedded in paraffin to make blocks. One section from each block was routinely stained with H&E for light microscopy. The squamous cell carcinoma was graded using Broder's classification and one section was subjected to immunohistochemical staining for p53 antibody.

For immunohistochemistry, the tissue sections were first deparaffinized with xylene, then rehydrated with decreasing grades of alcohol. Antigen retrieval and treatment with peroxidase for 15 minutes to block nonspecific reaction with other tissue antigens was done. Sections were incubated with mouse anti-p53 monoclonal antibody PAB 240 at a dilution of 1:50 for 2hr at 37 degree celsius. After being washed with PBS; the sections were treated with rabbit antimouse IgG conjugated with horse-radish peroxidase for half an hour at room temperature. Color was developed using DAB working solution for 5-8 minutes. A brown precipitate in the nucleus confirmed the presence of p53 protein. The slides were counterstained with Harris hematoxylin, mounted in DPX and examined by light microscopy.

All slides were evaluated for immunostaining without the knowledge of the histological diagnosis. Cases with p53 expression in more than 5% cells were considered positive for p53 expression. P53 expression was graded using the following grading system in the present study:

1. (+++) When greater than 50% of cells staining positive.
2. (++) When 26%-50% of cells staining positive.
3. (+) When 5-25% of cells staining positive.
4. (-) When <5% of cells staining positive.

Association/correlation of p53 expression with independent variables such as histological grades of squamous cell carcinomas, alcohol consumption, tobacco chewing and smoking, was analysed by applying Pearson's Chi Square test and p-value of less than 0.05 was considered statistically significant at 95% level of significance. All results and data were expressed as tables.

Results

A total of 66 cases were investigated for p53 expression. Table 1 shows age and sex distribution. The

age of patients in present study ranged from 24-85 years and the median age of patients was 53 years. The male: female ratio observed was (9:2). Table 2 shows site distribution, the most common site of presentation was lateral border of tongue (23%) followed by buccal mucosa (21%).

In present study, 34(51.5%) were well differentiated, 26(39.4%) were moderately differentiated and only 6(9%) were poorly differentiated squamous cell carcinoma. The p53 expression in well, moderately and poorly differentiated squamous cell carcinoma was seen in 50%, 75% and 83% cases respectively. The intensity of p53 staining also increased with grade as shown in tables 3 & 4.

The p53 expression was found to be significantly associated with habits of smoking and tobacco consumption. Whereas no association was seen with alcohol consumption as shown in table 5. In present study a significant co-relation was found between p53 expression and tumor grade as shown in table 6.

Table 1: Age and sex distribution

Age	Male	Female
21-30	3	2
31-40	10	1
41-50	9	2
51-60	22	3
61-70	6	4
71-80	3	0
81-90	1	0
Total	54	12

Median age of presentation in our study= 53.

Male: Female Ratio= 9:2.

Table 2: Site distribution

Site	No. of patient
Lateral border of tongue.	23
Buccal mucosa.	21
Hard palate.	10
Lower lip.	2
Ventralsurface of tongue	3
Gingiva.	4
Dorsum of tongue	3
Total	66

Most frequent site was lateral border of tongue n=23(34.8%)

Table 3: P53 grading

P53 grading	Frequency	Percent
-	25	37.9
+	15	22.7
++	10	15.2
+++	16	24.2
Total	66	100.0

P53 expression was positive in 41(62.1%) of cases

Table 4: Frequency distribution of p53 expression with respect t to various grades of SCC

Grade	Well diff scc		Mod diff scc.		Poorly diff scc.	
	n	%	n	%	n	%
-	17	50%	7	27%	1	16.66%
+	9	26%	5	19%	1	16.66%
++	6	18%	3	12%	1	16.66%
+++	2	6%	11	42%	3	50%
Total	34	100%	26	100%	6	100%

P53 expression increased with the grade of the tumor

Table 5: Statistical analysis (co-relation) of P53 with different variables

P53 Grading	Alcohol Consumption		Tobacco Consumption.		Smoking	
	Yes	No	Yes	No	Yes	No
Negative	18	7	22	3	14	11
Positive	30	11	37	4	24	17
P value.	0.917		0.034		0.046	

P53 expression was found to be significantly associated with habits of smoking and tobacco consumption whereas no association was seen with alcohol consumption.

Table 6:

P53 Grading	Histological Grading			Chi-Sq = 14.596, DF = 2, P-Value = 0.030
	Well diff scc.	Mod diff scc.	Poorly diff scc.	
Negative	17	7	1	
Positive	17	19	5	

Hence the relationship between histological grading and p53 is significant by the probability value

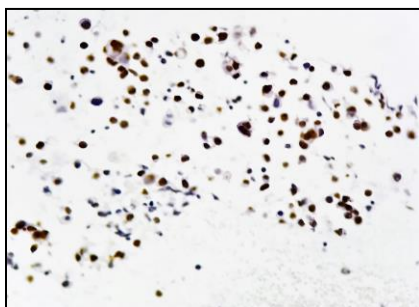


Fig. 1: Photomicrograph1 showing p53 expression, (+) intensity, when 5-25% of cells staining were considered positive

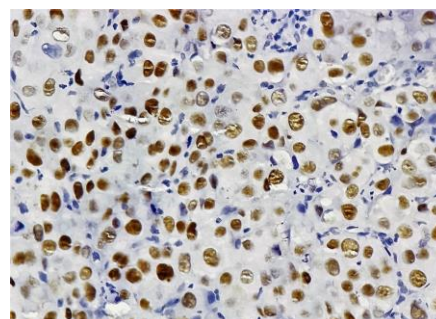


Fig. 3: Photomicrograph3 showing p53 expression,(+++intensity, when >50% of cells staining were considered positive

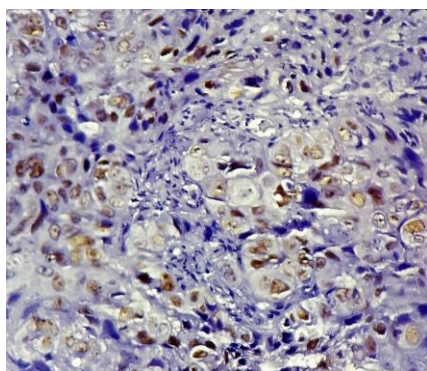


Fig. 2: Photomicrograph 2 showing p53 expression, (++) intensity, when 25-50% of cells were considered positive

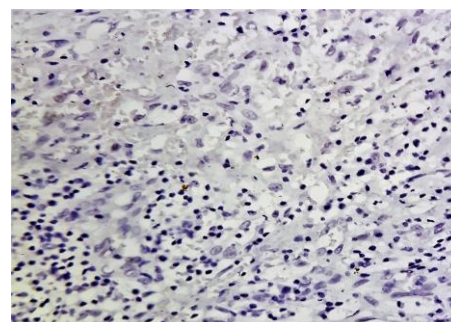


Fig. 4: Photomicrograph 4 showing p53 expression, (-) intensity when <5% of cells staining were considered positive (40x)

Discussion

Oral cancer is the sixth most common cancer in the world. Squamous cell carcinoma is the most common malignant neoplasm of the oral mucosa, representing 90% of oral malignant tumors.

A high prevalence of mutation in p53 tumor suppressor genes have been reported in head and neck carcinomas. The immunohistochemical detection of p53 in biopsy is of immense interest, as potential tumor marker; since it is the most commonly identified and mutated gene in diverse types of human cancers.

The age of patients in the present study ranged from 24 years to 85 years, which was similar to that found in studies done by Po Wing et al²² (20-85 yrs) and Nighat Ara et al²³ (21-86yrs).

The mean age of patients observed was 52.09yrs, similar to that reported by Jaun C et al²⁴ (56.5 yrs) and the median age of patients was 53 yrs which was similar to that reported by Po Wing et al²² (57 yrs).

In the present study male: female ratio observed was (9:2) similar to that observed by Rafeal Da et al²⁵ (6:1). Lower male : female ratio were reported by Po Wing et al²² (1.9:1), Faris Foco et al²⁶ (2.1:1), Nighat Ara et al²³ (1.7:1), Shinohara S et al²⁷ (3.8:1) and Jaun C et al²⁴ (0.1:1). This difference is probably due to variation in sample size.

In the present study the most common site of presentation was lateral border of tongue, (23%) followed by buccal mucosa (22%), which is similar to that observed by Jaun C et al²⁴ where the commonest site of presentation was tongue (27.4%), followed by buccal mucosa (20.1%), whereas Nighat Ara et al²³ reported the commonest site as buccal mucosa (43.3%) followed by tongue (23.3%).

In the present study out of 66 cases of oral cavity squamous cell carcinoma, 34 (51.5%) were well differentiated, 26 (39.4%) moderately differentiated and only 6 (9.1%) were poorly differentiated squamous cell carcinoma.

Present study was in concordance with the study done by Nighat Ara et al²³ and Jaun C et al²⁴, whereas study by A Jain et al²⁸ showed slightly lower percentage 18 (36%) of well differentiated squamous cell carcinoma.

In the present study p53 immunoexpression was noted in 62.1% of cases. The present study which was similar to the observations by Ghanghoria S et al²⁹ Umaswaminathan et al³⁰ Nighat Ara et al²³ where p53 expression was 63%, 65% and 67% respectively, but expression of p53 was 20% higher in Nishat Sultana et al³¹ where it was seen in 80% of cases.

Raefael Da et al²⁵ and (Po winfg et al)²² have reported p53 overexpression in lower no of cases, i.e 50%. This difference may be due to lack of standardization of IHC studies leading to heterogenous results, different antibodies used, methodological aspects and variability of interpretation of the results.

In present study a statistically significant relationship was noted between p53 expression and smoking (p-value 0.0046) and tobacco consumption.

(p-value 0.034). It was in agreement with studies done by (Efrain et al)³² and (Shinohara S et al)²⁷. This data suggests the induction of mutation in specific tumor suppressor gene in oral cavity tumors may be associated with specific carcinogen exposure.

No significant relationship was seen between p53 expression and alcohol consumption similar to that observed by (Faris Foco et al)²⁶.

In present study a significant co- relation was found between p53 expression and tumor grade (p value-0.030) which was in agreement with (A Jain et al)³⁵.

This study emphasizes the role of p53 mutation in carcinogenesis and tumor progression in oral cavity squamous cell carcinoma, and p53 targeted therapies may have a role in treatment of oral cavity squamous cell carcinoma.

Conclusion

We conclude that p53 overexpression seen in oral squamous cell carcinomas is significantly correlated to tumor grade and thus it can be used as an independent prognostic marker.

References

1. Boring CC, Squires TS, Tong T. Cancer statistics 1991. *Ca*. 1991;41:19-36.
2. Moore C, Catlin D (1967). Anatomic origins and locations of oral cancer. *Am J Surg* 114: 510-513.
3. Bryne M, Thrane PS, Dabelsteen E. Loss of expression of blood group antigen H is associated with cellular invasion and spread of oral squamous cell carcinomas. *Cancer* 1991;67:613-618.
4. Frierson HF Jr, Cooper PH. Prognostic factors in squamous cell carcinoma of lower lip. *Hum Pathol* 1986, 17:346-354.
5. Givens CD Jr, Johns ME, Cantrell RW. Carcinoma of the tonsil. Analysis of 162 cases. *Arch otolaryngol* 1981, 107:730-734.
6. Ildstad ST, Bigelow ME, Remensnyder JP. Intra-oral cancer at the Massachusetts General Hospital. Squamous cell carcinoma of the floor of the mouth. *Ann Surg* 1983;197:34-41.
7. Ildstad ST, Bigelow ME, Remensnyder JP. Squamous cell carcinoma of the alveolar ridge and palate. A 15 year survey. *Ann Surg* 1984;199:445-453.
8. Marks JE, Smith PG, and Sessions PG. Pharyngeal wall cancer reappraisal after comparison of treatment methods. *Arch otolaryngeal* 1985;111:79-85.
9. IroH, Waldfahrer F. Evaluation of the newly updated TNM classification of head and neck carcinoma with data from 3247 patients. *Cancer* 1998, 83:2201-2207.
10. Anneroth G, Hansen LS, Silverman S Jr. Malignancy grading in squamous cell carcinoma. Squamous cell carcinoma of the tongue and floor of mouth. Histologic grading in the clinical evaluation. *J oral Pathol* 1986;15:162-168.

11. Bryne M, Nielsen, Koppang HS, Dabelsteen E. Reproducibility of two malignancy grading systems with reportedly prognostic value for oral cancer patients *Oral Pathol Med* 1991;20:369-372.
12. Crissman JD, Gluckman J, Whiteley J, Quenelle D. Squamous-cell carcinoma of the floor of the mouth. *Head Neck Surg* 1980;3:2-7.
13. Frieson HF Jr, Cooper PH. Prognostic factors in squamous cell carcinoma of the lower lip. *Human Pathol* 1986;17:346-354.
14. Stein AL, Tahan SR. Histologic correlates of metastasis in primary invasive squamous cell carcinoma of the lip *Cutan Pathol* 1994;21:16-21.
15. Moore L, MB, Greenberg RA. Evaluation of size in prognosis of oral cancer. *Cancer* 1986;58:158-162.
16. Dorta RG, Landman G, Kowalski LP, Lauris JRP, Latorre MRDO, Oliveira DT. Tumor-associated tissue eosinophilia as a prognostic factor in oral squamous cell carcinomas. *Histopathology* 2002;41:152-157.
17. Grandi C, Allosio M, Moglia D, Podrecca S, Sala L, Salvatore P, Molinari R. Prognostic significance of lymphatic spread in head and neck carcinomas. Therapeutic implications. *Head Neck Surg* 1985;8:67-73.
18. Platz H, Fries R, Hudec M (1985) Retrospective DOSAK Study on carcinomas of the oral cavity: results and consequences. *J Maxillofac Surg* 13:147-153.
19. Kearsley JH, Thomas S (1993). Prognostic markers in cancers of the head and neck region. *Anticancer Drugs* 4:419-429.
20. Roland NJ, Caslin AW, Nash J, Stell PM (1992). Value of grading squamous cell carcinoma of the head and neck. *Head Neck* 14:224-229.
21. Kerdpan D, Rich AM, Reade PC. Expression of p53 in oral mucosal hyperplasia. Dysplasia and squamous cell carcinoma. *Oral disease* 1997;3(6):86-92.
22. Po Wing Yuen et al, The clinicopathological significance of p53 and p21 Expression in the Surgical Management of lingual Squamous Cell Carcinoma. *Am J Clin Pathol* 2001;116:240-245.
23. Nighat Ara et al, Frequency of p53 Gene Mutation and protein Expression in Oral Squamous Cell Carcinoma. *Journal of the college of Physicians and Surgeons Pakistan* 2014, Vol, 24(10):749-753
24. Jaun C Cuevas et al, p53 and p16 in oral epithelial dysplasia and oral squamous cell carcinoma: A study of 208 cases. *Indian Journal of pathology and microbiology*. 2016;59;153-8.
25. Raefael Da Ros Motta et al, Ki67 and p53 correlation prognostic value in squamous cell carcinomas of the oral cavity and tongue. *Braz J Otorhinolaryngol*. 2009;75(4):544-49.
26. Faris Foco et al, Pertumoral p53 Expression in oral carcinoma. *Coll Antropol* (2011) 2:129-132.
27. Shinohara S et al, Prognostic impact of p16 and p53 expression in oropharyngeal squamous cell carcinomas. PubMed –NCBI.
28. A Jain et al, Diagnostic and prognostic significance of p53 protein expression in squamous cell lesions of the oral cavity. *The internet journal of Otolaryngology* vol.-7, number 2.
29. Ghanghoria S et al, p53 Expression in Oral cancer: A study of 50 cases. *Journal of pathology of Nepal* (2015) Vol. 5, 747-751.
30. Umaswaminathan et al, Expression of p53 and Cyclin D1 in oral squamous cell carcinoma and normal mucosa: An Immunohistochemical study. *J Oral Maxillofac Pathol*. 2012 May-Aug;16(2):172-177.
31. Nishant Sultana et al, P53 Expression in Oral Submucous Fibrosis and Oral Squamous Cell Carcinoma. *International Journal of oral and Maxillofacial Pathology*. 2011;2(1):9-14.
32. Efrain Alvarez Martinez et al, Immunoexpression of p53 in Oral Squamous Cell Carcinoma and Oral Dysplastic Lesions in Patients with the habit of reverse smoke. *Int. J. Odontostomat*;7(2):185-191, 2013.

How to cite this article: Khandelwal R, Sharma R, Agarwal D, Baijal A. Study of p53 gene over expression as a prognostic marker in squamous cell carcinoma of the oral cavity. *Ind J Pathol Oncol*, 2018;5(3):441-445.