

Diagnostic And Prognostic Significance Of p53 Protein Expression In Squamous Cell Lesions Of The Oral Cavity

A Jain, V Maheshwari, G Mehdi, K Alam, S Sharma

Citation

A Jain, V Maheshwari, G Mehdi, K Alam, S Sharma. *Diagnostic And Prognostic Significance Of p53 Protein Expression In Squamous Cell Lesions Of The Oral Cavity*. The Internet Journal of Otorhinolaryngology. 2007 Volume 7 Number 2.

Abstract

Context: Oral cancers are a major health problem in India. Recently, parameters of cell proliferation and cell death have emerged as important diagnostic and prognostic tools.

Aims: The present study evaluated the role of tumour suppressor gene protein p53 in squamous cell lesions of the oral cavity.

Settings and Design: A single institutional prospective study.

Methods and Material: The evaluation of p53 (by IHC using Clone DO7 monoclonal antibody, DAKO, USA) was done on 50 cases of squamous cell lesions of oral cavity.

Statistical analysis used: Student's't' test was performed.

Results: The p53 expression increased with increasing degree of dysplasia and grade of SCC. There was a statistically significant difference of p53 positivity between dysplasia & SCC ($P < 0.001$), WD-SCC & MD-SCC ($P < 0.05$), and WD-SCC & PD-SCC ($P < 0.001$). Significantly high p53 positivity ($P < 0.001$) was noted in cases presenting with lymph node metastasis.

Conclusions: Alterations of p53 gene seem to be an early event in multistage oral carcinogenesis. Role of p53 gene even at later stages when metastasis has occurred has not been properly dealt with; taking the research a step further, our study has found a statistically significant increase in p53 expression in cases presenting with nodal metastasis thus suggesting that the mutant p53 gene continues to be important for progression and metastasis of SCC, and a higher p53 expression indicates poorer prognosis.

INTRODUCTION

Oral cavity cancer is the sixth most common cancer worldwide, with diagnosis of over a quarter million new cases world-wide in the year 2000 out of which 80000 (30%) new cases and 47000 cancer related deaths were in India [1].

In India, oral cavity cancers are the most common cancers in males and third most common in females [2].

Most of the oral cancers are squamous cell carcinomas and majority is unequivocally associated with tobacco chewing and usually preceded by premalignant lesions; most often a persistent leukoplakia and oral submucous fibrosis.

Researchers agree that an early diagnosis greatly increases the probability of cure with minimum impairment and

deformity. In the last decade, histological methods which reveal parameters of cell proliferation and cell death have become important; and might facilitate identification of individuals who are at high risk of developing carcinoma besides having prognostic significance.

The immunohistochemical (IHC) detection of p53 in biopsy tissue specimens is of immense interest to oncopathologists as a potential tumour marker; since it is the most commonly identified and mutated gene in diverse types of human cancers. The wild type p53 protein has a half life of 6-20 minutes[3], while the mutant form has a half life of several hours and can be detected immunohistochemically.

The present study was undertaken with the purpose of evaluating the role of tumour suppressor gene protein p53 in

oral carcinogenesis, assessing its significance as a tumor marker, and a possible prognostic marker in premalignant and malignant squamous cell lesions of the oral cavity.

SUBJECTS AND METHODS

The study included 50 excisional/ incisional oral biopsy specimens. These included 5 cases each, of mild, moderate and severe dysplasia; 18 of well differentiated squamous cell carcinoma, 12 of moderately differentiated and 5 of poorly differentiated squamous cell carcinoma. For p53 immunohistochemical staining monoclonal Clone DO7 antibody (LSAB kit, DAKO cytomation, USA) was used. The antibody is an isotype IgG_{2b}? monoclonal mouse, antihuman P53 antibody whose immunogen is recombinant human wild type p53 protein expressed in E. coli. It recognizes an epitope in the N-terminus of the human p53 protein, which is known to reside between amino acid 19 to 26; and reacts with wild type as well as mutant type of the p53 protein [4].

Breast carcinoma cases with known positivity for p53 were taken as positive controls.

As p53 is a nuclear protein, epithelial cells showing only nuclear positivity (strong brown staining) were considered positive, and were assessed quantitatively. Cells showing cytoplasmic staining were not counted.

The quantification of p53 positivity was done according to the method adopted by Chiang et al [3]. Only the percentage of cells showing nuclear expression of p53 irrespective of intensity of staining was quantified. The percentage of positively stained cells in the full thickness of the epithelium was determined by scanning the entire section.

The scores obtained were expressed as follows:

Positive Cases (%): The percentage of cases showing positive staining for p53.

p53 Positivity (%): The percentage of cells showing a positive staining for p53 in each case.

RESULTS

Nuclear positivity for p53 was found in 60% cases of dysplasia (Fig.1) with a mean p53 positivity of $4.7\% \pm 6.4$, and 74.3% cases of SCC(Fig. 2,3) with a significantly high value of $30.8\% \pm 23.4$ ($P < 0.001$). Both the percentage of positive cases and p53 positivity showed a corresponding increase in values with increasing degree of dysplasia and

grade of squamous cell carcinoma. A significant difference in p53 positivity was noted between cases of well differentiated (WD-SCC) and moderately differentiated SCC (MD-SCC) ($P < 0.05$); and between well differentiated SCC and poorly differentiated SCC (PD-SCC) ($P < 0.001$). The p53 positive cells were seen to be located mostly in the suprabasal as well as superficial layers in dysplasia, whereas in SCC they were diffusely distributed.

Figure 1

Figure 1: (a) mild dysplasia showing scattered brown nuclear staining conferred to basal and parabasal layer (p53 positivity= 2%), (b) moderate dysplasia with 3% p53 positivity. [p53 IHC, Clone DO7, x400]

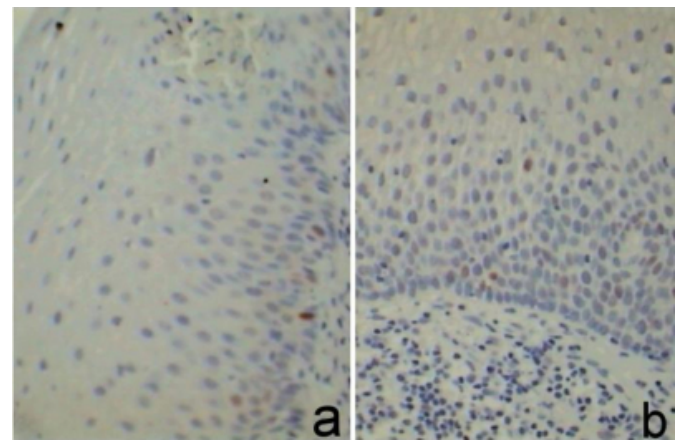


Figure 2

Figure 2: (a) WD-SCC [H & E, x 400], (b) same section with 44% p53 positivity [p53 IHC, Clone DO7, x400], (c) PD-SCC [H & E, x 400], (d) same section showing diffuse brown nuclear staining of tumour cells (p53 positivity= 75%) [p53 IHC, Clone DO7, x400].

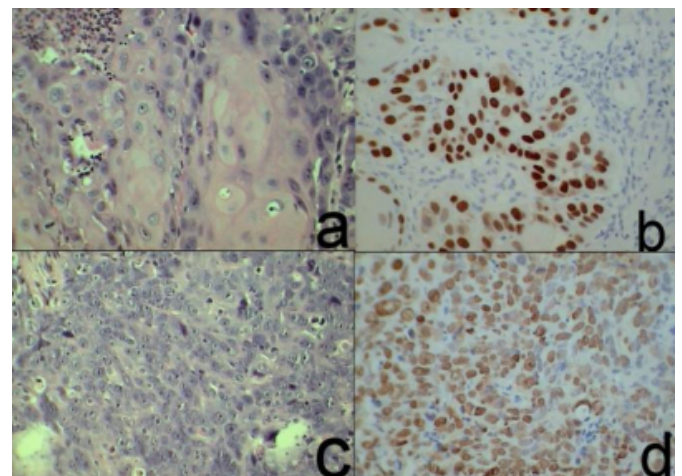
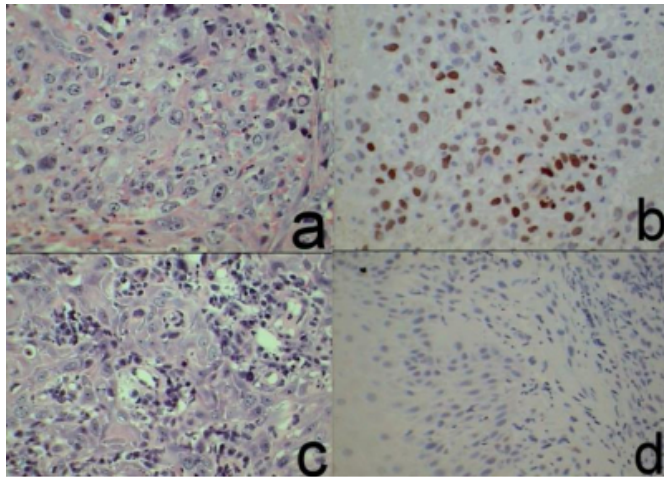


Figure 3

Figure 3: (a) MD-SCC [H & E, x 400], (b) same section showing diffuse and strong p53 expression (p53 positivity= 56%) [p53 IHC, Clone DO7, x400], (c) another case of MD-SCC [H & E, x 400], (d) same section showing complete absence of p53 expression [p53 IHC, Clone DO7, x400].



All cases of recurrent SCC were positive with a remarkably higher p53 positivity ($60\% \pm 10$) compared to values obtained in primary SCC ($28.1\% \pm 22.4$).

Cases presenting with lymph node (LN) metastasis (12 cases) were 100% positive with a mean p53 positivity of $51.6\% \pm 13.2$, in contrast to those without metastasis (60.9% cases positive, with $20\% \pm 19.5$ positivity). The difference between p53 positivity was found to be statistically significant ($P < 0.001$). (Table 1)

Few cases (7 cases of well differentiated and 1 each of moderate and poorly differentiated SCC) were negative for p53 expression (Fig.3c, d).

DISCUSSION

TP 53 gene is located on human chromosome 17p 13.1 and encodes a 53 KDa nuclear phosphoprotein which plays an important role in regulation of normal cell proliferation^[5], and may induce growth arrest or cell death (apoptosis)⁶. In the recent studies 90% of mutations at p53 locus were found to be mis- sense mutations with in the region of exons 5 to 8^[6].

It has been suggested that mutations in the p53 gene are intimately involved in the genesis of oral SCCs. Immunohistochemical studies of p53 expression in SCCs of oral mucosa have shown over expression of p53 protein^[3,7,8].

We observed an increased p53 expression as the degree of dysplasia and the grade of SCC advanced. Sauter et al ^[9] have reported increasing number of positive cases (from 28% in mild dysplasia, to 45% in moderate dysplasia, to 54% in severe dysplasia and 50% in carcinoma in-situ); which is very similar to our observations in finding 40% positive cases in mild dysplasia, 60% in moderate and 80% in severe dysplasia. Moreover, the p53 positivity also increased with advancing dysplasia. Similar results were expressed by Saito et al^[10].

More importantly, we found significantly higher mean p53 positivity ($P < 0.001$) in SCC in contrast to dysplasia; similar results have been observed in studies done by Chiang et al ^[3] and Raju et al ^[11].

We also observed that if p53 over-expression was present, it was limited to basal layer in normal epithelium adjacent to tumour; it expanded to parabasal and superficial layers in dysplasia, and was diffusely distributed in tumour cells of SCC, a finding that has been recorded by Chiang et al ^[3] and Hafian et al ^[10] as well. Thus, suprabasal expression of p53 here would indicate that a larger part of epithelium than normal is dividing ^[12].

All of these observations suggest that alterations of p53 may be an early event in multistage carcinogenesis of oral epithelium.

Some contradictory observations have been made by Girod et al ^[7] and Tsuji et al ^[13]. The variable results found among studies may be due to subjectivity in assessment of dysplasia, differences in the populations studied, sampling method, antibodies used, or to variations in fixation and IHC procedures ^[5].

A significantly increased positivity with increasing tumour grade was noticed by us, suggesting that a higher p53 expression indicates poorer prognosis. The relationship between p53 mutations and tumour grade has been evaluated in many studies ^[9,13,14,15,16]. Zariwala et al ^[13] found a tendency toward a higher incidence of p53 positivity in highly malignant, poorly differentiated carcinomas. Over expression of p53 has been correlated with increased dedifferentiation ^[14] and with cellular atypia ^[15]. Contrarily, few other studies have shown no positive relationship ^[9,16].

Major interest exists on the role played by p53 mutations in progression and prognosis of oral SCCs. We observed that the prevalence of p53 expression was higher in patients with

lymph node involvement than in those without lymph node metastases. Wang et al [17] have correlated mutated p53 with the invasiveness and metastasizing ability of cancer cells. However, some authors either found a discordant staining [15]; or no significant difference [11] between lymph node metastases and primary tumours.

Some studies have also evaluated and scored the p53 staining intensity; however, according to Nylander et al [12], evaluation of staining intensity is a highly difficult task since it depends on many variables, such as pre-treatment, antibody concentration and more general staining conditions, such as ambient temperature. In a strict sense, intensity can only be evaluated when staining has been performed under reproducible conditions, for example, by using a programmable staining machine. So we did not perform intensity scoring in our study.

A noticeable observation in the present study was the complete absence of p53 positivity in some SCCs. This has been aptly explained by Nylander et al [12], as, the tumours completely lacking detectable p53, could have a mutation in TP53 gene resulting in production of a truncated, non-functional and non-detectable protein (if there are mutations involving the antibody recognizing epitopes).

We also found higher p53 positivity in cases of recurrent SCC, but due to a small sample size, statistical analysis was not possible. Girod et al [10] also observed similar results. Neither the recurrence rate, nor the time taken to recur was found to be dependent on p53 positivity or negativity [18].

Thus a higher degree of p53 positivity in recurrent SCC and those with lymph node metastasis may suggest that mutant p53 gene continues to be important for squamous cell carcinoma progression and metastasis.

We conclude our study by emphasizing that perhaps now p53 should be included as a prognostic marker for oral cancers, thus helping in predicting cancer behaviour at not only the stage of dysplasia, but also invasive and metastatic carcinoma. The importance for targeted therapy towards p53 has increased further with the results of our study indicating the role of p53 even till late stages, since it can not only help in prevention but also treatment of progressive disease.

References

1. Saranath D. Molecular biomarkers in oral cancers. *Indian J Med Res (Suppl)* 2005; 1-3.
2. Park K. *Park's Textbook of Preventive and Social Medicine*. 18th ed. Jabalpur: M/s Banarsidas Bhaot; 2005.

3. Chiang CP, Lang MJ, Lin BY, Wang JT, Len JS, Hahn LJ, et al. Expression of p53 protein in oral submucous fibrosis, oral epithelial hyperkeratosis and oral epithelial dysplasia. *J Formos Med Assoc* 2000; 99: 229-34.
4. Dako M7001, Dako Cytomation. Available from: URL: <http://www.alzforum.org/res/com/ant/p53redo/dakM7001.asp?CompanyID=148>
5. Raybaud-Diog'ine, Te'tu B, Morency R, Fortin A, Monteil RA. p53 Overexpression in head and neck squamous cell carcinoma: Review of the Literature. *Eur J Cancer B Oral Oncol* 1996; 32B: 143-9.
6. Lain S, Midgley C, Sparks A, Lane EB, Lane DP. An inhibitor of nuclear export activates the p53 response and induces the localization of mdm2 and p53 to U1A-positive nuclear bodies associated with the PODS. *Exp Cell Res* 1999; 248: 457-72.
7. Girod SC, Pape HD and Krueger GRF. p53 and PCNA expression in carcinogenesis of the oropharyngeal mucosa. *Eur J Cancer B Oral Oncol* 1994; 30B: 419-23.
8. Raju B, Mehrotra R, Oijordsbakken G, Al-Sharabi AK, Vasstrand EN, Ibrahim SO. Expression of p53, cyclin D, and Ki-67 in premalignant and malignant oral lesions: association with clinicopathological parameters. *Anticancer Res* 2005; 25: 4699-706.
9. Sauter ER, Cleveland D, Trock B, Ridge JA, Klein-Szanto AJP. p53 is over expressed in fifty percent of pre-invasive lesions of head and neck epithelium. *Carcinogenesis* 1994; 15: 2269-74.
10. Saito T, Nakajima T, Mogi K. Immunohistochemical analysis of cell cycle-associated proteins p16, pRb, p53, p27 and Ki-67 in oral cancer and precancer with special reference to various carcinomas. *J Oral Pathol Med* 1999; 28: 226-32.
11. Hafian H, Venteo L, Sukhanova A, Nabier I, Lefevre B, Pluot M. Immunohistochemical study of DNA Topoisomerase I, DNA Topoisomerase II , p53, and Ki-67 in oral preneoplastic lesions and oral squamous cell carcinomas. *Human Pathol* 2004; 35: 745-51.
12. Nylander K, Dabelsteen E, Hail PA. The p53 molecule and its prognostic role in squamous cell carcinomas of the head and neck; *J Oral Pathol Med* 2000; 29: 413-25.
13. Zariwala M, Schmid S, Pfaltz M, Ohgaki H, Kleihues P, Schafer R. p53 gene mutations in oropharyngeal carcinomas: a comparison of solitary and multiple primary tumours and lymph node metastases. *Int J Cancer* 1994; 56: 807-11
14. Nees M, Homan N, Discher H, Andl T, Enders C, Herold- Mende C, et al. Expression of mutated p53 occurs in tumor-distant epithelia of head and neck cancer patients: a possible molecular basis for the development of multiple tumors. *Cancer Res* 1993; 53: 4189-96.
15. Slootweg PJ, Koole R, Hordijk GJ. The presence of p53 protein in relation to Ki-67 as cellular proliferation marker in head and neck squamous cell carcinoma and adjacent dysplastic mucosa. *Eur J Cancer B Oral Oncol* 1994; 30B: 138-41.
16. Tsuji T, Mumura Y, Wen S, Li X, Kanekawa A, Shinozaki F. The significance of PCNA and p53 protein in some oral tumors. *Int J Oral Maxillofac Surg* 1995; 24: 221-5.
17. Wang DY, Xiang YY, Tanaka M, Li XR, Li JL, Shen Q, et al. High prevalence of p53 over expression in patients with esophageal cancer in Linxian, China and its relationship to progression and prognosis. *Cancer* 1994; 74: 3089-96.
18. Hogmo A, Munck-Wikland E, Kuylestierna R, Lindholm J, Aner G. Nuclear-DNA content and p53 immunostaining in oral squamous-cell carcinoma - an analysis of a consecutive 10-year material. *Int J Oncol* 1994; 5: 915-20.

Author Information

Anshu Jain, M.D. Pathology

Senior Resident, Department of Pathology, JNMC

Veena Maheshwari, M.D. Pathology

Professor, Department of Pathology, JNMC

Ghazala Mehdi, M.D. Pathology

Reader, Department of Pathology, JNMC

Kiran Alam, M.D. Pathology

Lecturer, Department of Pathology, JNMC

S.C. Sharma, M.S. ENT

Professor, Department of ENT, JNMC