Original Research Paper

Volume-9 | Issue-1 | January-2019 | PRINT ISSN - 2249-555X

Pathology

STUDY OF P53 IMMUNOHISTOCHEMISTRY IN ORAL MALIGNANT LESION AND ITS COMPARISON WITH PREMALIGNANT AND BENIGN ORAL LESION

Dr. Sumanlata Verma Professor, G.S.V.M. Medical College, Kanpur.

Dr. Mehnaz Bi* P.G. Student, G.S.V.M. Medical College, Kanpur. *Corresponding Author

Dr Soni Verma Assistant professor, G.S.V.M. Medical College, Kanpur.

ABSTRACT Expression of p53 tumor suppressor gene is one of the common findings in human cancers including the oral cancer. The p53 protein is known to be expressed in benign, premaignant and oral squamous cell carcinoma.

Oral carcinogenesis is a multistep process. The premalignant oral lesions develop into the malignant lesions tumors due to chronic exposure to various carcinogens such as tobacco, alcohol, beetle chewing and HPV in a genetically predisposed person.

Objective :

- To study the expression of p53 by immunohistochemistry in oral malignant lesions & compared it with premalignant & benign oral lesions.
- To correlate the immunoexpression of p53 with grading & staging of oral premalignant & malignant lesions

Materials & Methods : In this study, histopathological examination and immunohistochemical staining (Dako cytomation) was performed on 200 cases of oral biopsies to see the p53 expression in these oral epithelial lesions.

Results : Immunohistochemical expression of p53 observed in 200 cases of oral epithelial lesions, consisting of 34 benign, 60 pre-malignant and 106 malignant lesions. Out of all, 170 cases were showed p53 positivity. All benign lesions were negative or showed only basal positivity. P53 was expressed in 83.33% of dysplastic lesions & 94.27% malignant lesions. The staining intensity increased from basal to suprabasal region with increase in grade of dysplasias.

Conclusion- Combined histological analysis with p53 immunoexpression could be a useful and simple molecular marker to detect the possibility of transformation from a premalignant to malignant lesion of oral epithelium.

KEYWORDS : Oral squamous cell carcinoma, p53, immunohistochemistry.

INTRODUCTION-

Oral cancer constitutes a major health problem in developing countries and represents leading cause of death. Squamous carcinomas represent about 3% of human cancers and over 90% of malignant tumors at oral location, being diagnosed worldwide each year in over 350,000 new cases.[1]

The etiology of oral squamous cell carcinoma (SCC) is multifactorial and the most important risk factors include personal habits of tobacco use and alcohol consumption. Tobacco is considered the most important risk factor in the development of oral dysplasia and oral SCC. Various genetic and molecular alterations are observed in oral premalignant and malignant lesions due to consumption of tobacco. Carcinogens in tobacco affect the normal oral epithelium by increasing the number of aneuploid nuclei. Most of the oral SCC develops from precursor lesions, such as leukoplakia and oral submucous fibrosis.[2,3]

The p53 tumor suppressor gene was first described in 1979 as a protein that binds SV40 virus large T antigen. The gene exists on chromosome 17p and behaves as a tumor suppressor. However, mutation of the gene can inactivate this tumor suppressor activity.[4,5] Normal or wild– type p53 gene is a negative regulator of cell proliferation, whereas mutations in the gene are main culprit for malignant transformation.[6] Thus, p53 has a predictive as well as a prognostic significance in oral malignancy.

MATERIALS AND METHODS Specimen received in 10% formalin. Put the specimen in 10% formalin after loafing for 6-8 hr. Perform grossing. Paraffin sections of 5µm thickness will be stained by haematoxylin and eosin (H & E) for histopathological study.

- For immunohistochemistry- Sections of 3µm taken on charged/coated slides with poly -L-lysine. Slides are put on the hot plate for dewaxing for 1 hr. As dewaxing is completed dip the slides into xylene directly from hot plate. Keep the slides in the xylene for 10 min. Transfer the slides into second xylene solution for 10 min. After it pass the slides into downgrading concentration of alcohol in order of 100%, 70%, and 50% for 5 min in each. Wash the slides with distilled water for 5-10 min. Next step is antigen retrieval. . Next step is antigen retrieval. For p53 retrieval done by the citrate buffer (pH6) at high temperature and high pressure When retrieval is complete put the slides in moist chamber and allow it to cool down to room temperature for app. 30 min. Wash the slides with PBS (pH-7.5) (2-3 times). Add the peroxidase blocker to each section for 15 min. 3 washing with PBS buffer. and incubate the slides with primary antibody for 1 hr. 3 washing with PBS. Incubate the slides with secondary antibody for 30 min each. 3 washing with PBS buffer. Mix with DAB buffer in the ratio of 1:50 and apply it for 30 mins. Give 3 washing of distilled water. Counterstain the slides with hematoxylin for appropriate time (3-5min).

OBSERVATION

The performed study include 200 cases of oral lesions. Most of the benign lesions were present in between 20-40 years of age, whereas premalignant lesions were being diagnosed in between 40-60 years of age and malignant lesions more common in 4-5th decade.

Table-1:Oral lesions according to Age group

Age Group in Years	Benign	Premalignant	Malignant	Total
20-30	15	04	04	23
30-40	12	18	12	42
40-50	04	16	34	54
50-60	03	12	28	43
60-70	00	06	20	26
70-80	00	04	08	12
Total	34	60	106	200

Table-2: Incidence in relation to tobacco chewing

Addiction	Male	Female	Total	Percentage
Tobacco users (in any form)	136	40	176	88
Non- tobacco users	15	9	24	12
Total	151	49	200	100

Table shows incidence of oral lesions in relation to tobacco use. Most of the oral lesions (88%) due to tobacco use. Oral lesions were more common in males who take tobacco in any form with male : female of 3:1.

Table-3: Histopathological Distribution of Oral Epithelial Lesions (n=200)

Epithelial lesions	Male	Female	Total	Percentage
Benign Lesions				
Hyperplasia without dysplasia	23	05	28	9.52
Squamous Cell Papilloma	05	01	06	2.04

Dysplasia				
Mild	06	04	10	06
Moderate	10	05	15	09
Severe	14	06	20	12
Carcinoma in situ	12	03	15	09
Malignant lesions	81	25	106	52.44

In this study, there were 106 (52.44%) cases of malignant oral lesions followed by (36%) cases of premalignant lesions and (11.56%) cases of benign lesions. Malignant lesions are common in males then females.

Table-4: Presence of P53 in epithelium of different groups

S.No	Result	Benign		Premalignant		Malignant		p-value
		N	%	N	%	N	%	
1.	Absent	14	41.17%	10	16.66%	6	5.6%	<0.00001
2.	Present	20	58.8%	50	83.33%	100	94.3%	<0.00001

Expression of p53 was found in 58.8% of benign lesions , 83.33% of premalignant lesions and 94.3% of malignant lesions of oral cavity.

Table-5 Distribution of p53 expressing cells

Oral lesion	Negative	Basal cells	Supra basal cells	Total	p- value
Benign	14(41.17%)	10(29.4%)	10(29.4%)	34	<0.00001
Premalignant	10(16.6)	20(33.3%)	30(50%)	60	<0.00001
Malignant	6 (5.6%)	20(18.8%)	80(75.47%)	106	<0.00001

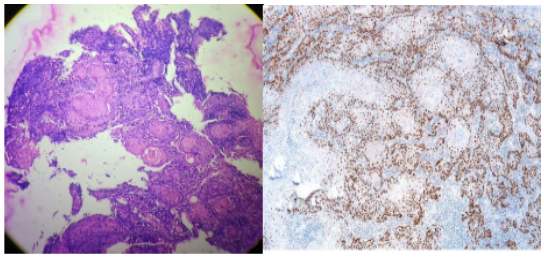
Expression of p53 was limited to basal and supra basal layer of benign oral lesions. And the expression of p53 increases from benign to premalignant to malignant oral lesions. In all cases of OSCC showed staining in basal and supra basal layers upto 58.7% except 6 cases were negative.

Table-6: p53 Staining Intensity in oral lesions

Epithelial Lesions	Intensity			Total
	Weak	Moderate	Strong	
Benign	10 (50%)	5 (25%)	5 (25%)	20
Premalignant	10(20%)	20 (40%)	20(40%)	50
Malignant	20(20%)	40 (40%)	40 (40%)	100

1.weak staining + 6-25% cells take staining.
2.moderate staining ++ 26-50% cells take staining.
3.strong staining +++ 51-100% cells take staining.

It was found that 40% malignant and 40% premalignant lesions showed strong p53 positivity including 25% of benign oral lesions. . Intensity of p53 increases with the degree of dysplasia and grade of carcinoma.



DISCUSSION-

In the present study, relative incidence of oral lesion was 57.14%, (n=200) and most common oral epithelial lesion found was OSCC 53%. Malignant lesions were seen in 4-5th decade of life, whereas benign lesions were common in 20-40 years of age. The average age was 50 years that was lower than authors reported by Gervasio et al[7] i.e. 58.6 years and Mirza et al i.e. 54.3%[8]. The male and female distribution in our study was 3.:1. In accordance by Pinholt et al[9] the male and female ratio is 1.19 : 1. However, in studies of Greek and Brazilian population show quiet a higher ratio of 9.2 : 1 to 4.8 : 1 respectively.[10,11]

Epidemiological studies have shown that the site of occurrence of oral S.C.C. differs widely. In the present study, most common location of oral lesions was tongue i.e. 37.5%, of which S.C.C. comprised of 56%. This was compatible as compared to most other studies.[12,13,14]

Well differentiated (25%) and moderately differentiated S.C.C (20%) were found in high number in our study, which is in contrast to Haq ME et al, who found that poorly differentiated S.C.C. was most prevalent histological variant. Zedan et al reported WDSCC as the most common histological type.[15]

In present study out of 200 oral cases, 85% cases were p53 positive and 15% cases were p53 negative these findings were similar with the findings of **Jang-Jaer et al. 2005**[16]**.** P53 immunostain were expressed at a nuclear level in 58.8% of benign lesions, 83.3% of premalignant lesions and 94.3% of malignant lesions of oral cavity. Benign lesions showed only basal positivity or p53 negative. The positive p53 staining in the hyperplastic tissue was likely to be due to in part to the microwave antigen retrieval technique which has been shown to reduce p53 detection thresholds[17] due to detection of wild type p53[18]. Another possible explanation for the number of positive cases of hyperplasia in present study may be due to proliferation activity in these tissue as there is a positive relation between p53 expression and cellular proliferation[19,20,21].

In premalignant cases, p53 positivity found in 83.3% cases, with an increased in suprabasal positivity as the grade of dysplasia increased. Such pattern of staining was also observed by Cruz et al[22,23], Kerdpon et al[24], Vered et al[25]and Nasser et al[26]. These investigators also found that the p53 expression pattern was significantly related to the development of carcinoma. While 94.3% malignant lesions showed basal and suprabasal positivity, suprabasal positivity increases as the grade of carcinoma increases. Our results were similar as reported by Kaur et al[27]. An interesting observation noted in this study was that, well differentiated tumours had a high p53 immunostaining, while poorly differentiated SCC showed significant weaker p53 expression. These results were consistent with other studies, who have demonstrated statistically significant correlation between histological grade and p53 expression[28,29].

P53 intensity increases from benign to malignant lesions. 40% of malignant & 40% of premalignant lesions showed strong p53 intensity while only 25% of benign lesion shows strong intensity. These findings were similar with other studies.

CONCLUSION

The most common oral epithelial lesion found in our study is SCC. p53 immunoreactivity is seen in 58.8% of benign, 83.3% of premalignant and 94.3% of squamous cell carcinoma. p53 immunoreactivity has no relation with age, sex and site of lesion. The immunoexpression of p53 increased with increasing grades of dysplasia and grades of carcinoma increases indicating that they may be used as predictive markers in oral cancer development. Based on these findings, combined histological analysis with p53 immunoexpression, evaluation of premalignant lesions could be improved.

REFERENCES

1. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008, Int J Cancer, 2010; 127: 2893-917.
2. R. Sankaranarayanan, K. Ramadas, G. Thomas et al., "Effect of screening on oral cancer mortality in Kerala, India: a cluster-randomised controlled trial," The Lancet, vol. 365, no. 9475, pp. 1927-1933, 2005.
3. Choi S, Myers JN. Molecular pathogenesis of oral squamous cell carcinoma: Implications for therapy. J Dent Res 2008;87:14-32.
4. Prives C, Hall PA. The p53 pathway. J Pathol 1999;187:112-26.
5. Abbas NF, Labib El-Sharkawy S, Abbas EA, Abdel Monem El-Shaer M. Immunohistochemical study of p53 and angiogenesis in benign and preneoplastic oral lesions and oral squamous cell carcinoma. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2007;103:385-9
6. CHENG an, Jiang SS, Fan CC, Lo YK, Kuo CY, Chen CH, Liu YL, Lee CC, Chen WS, Huang TS, wang TY, Lee AY (2013).Inceased Cdc7 expression in a marker of oral squamous cell carcinoma and overexpression of Cdc7 contributes to the resistance to DNA-damaging agents. Cncer Lett 337(2): 218-22
7. Kashiwazaki h et al high frequency of p53 mutations in human oral y epithelial dysplasia & primary SCC detected by yeast functional assay, oncogene 1997;15(22):2607-74.
8. Gervasio OLAS, Dutra RA, Tartaglia SMA, Vasconcellos WA, Barbosa AA, Aguiar MCF. Oral squamous cell carcinomas: A retrospective study of 740 cases in Brazilian population. Braz Dent J 2001;12(1):57-61.
9. Mirza T, Alam SM, Pringle IL, Zaidi SH. Molecular analysis of human papillomavirus and oncosuppressor genes in tobacco related oral cancer. Pak J Otolaryngol 1998;14:27-33.
10. Pinholt EM, Rindum J, Pindborg JJ. Oral cancer: a retrospective study of 100 Danish cases. Br J Oral Maxillofac Surg 1997;35:77-80.
11. Antoniades DZ, Stynidis K, Papanatou P, Trigonidis G. Squamous cell carcinoma of the lips in a northern Greek population. Evaluation of prognostic factors on 5-year survival rate-I. Oral Oncol Eur J Cancer B 1995;31B:333-9.
12. Walid Zedan et al. Cytogenetic significance of chromosome 17 abberation anf p53 gene mutation as prognostic marker in oral SCC.
13. Haq MEV, Abid I, Hanit MK, Warraich RA, Mohamood HS, Saddique K. Frequency and pattern of oral and Maxillo-facial carcinoma. J. Orofacial Res Ann.2009; 15(4): 171 -5.
14. Rich AM, et al. p53 expression in oral precancer & cancer Aust,Dent. J. 1999;44:103-5.
15. Dowell SP, Ogden GR. The use of antigen retrieval for immuno-histochemical detection of p53 over-expression in malignant and benign oral mucosa: a cautionary note. J Oral Pathol Med1996;25:60-64.

16. McKee PH, Hobbs C, Hall PA. Antigen retrieval by microwave irradiation lowers immunohistochemical detection thresholds. Histopathology 1993;23:377-379.
17. Lippman SM, Shin DM, Lee JJ, et al. p53 and retinoid chemo-prevention of oral carcinogenesis. Cancer Res 1995;55:16-19.
18. Warnakulasuriya KAAS, Johnson NW. Association of over-expression of p53 oncoprotein with the state of cell proliferation in oral carcinoma. J Oral Pathol Med 1994;23:246-250.
19. Nylander K, Stening R, Gustafsson H, et al. p53 expression and cell proliferation in squamous cell carcinomas of the head and neck. Cancer 1995;75:87-93.
20. Cruz I, Napier SS, van der Waal I, Snijders PJF, Walboomers JMM, Lamey PJ, et al. Suprabasal p53 immunoexpression is strongly associated with high grade dysplasia and risk for malignant transformation in potentially malignant oral lesions from Northern Ireland. J Clin Pathol. 2002 Feb;55(2):98-104.
21. Cruz IB, Snijders PF, Meijer CJ, Braakhuis BJ, Snow BG, Walbloomers JM, et al. p53 expression above the basal cell layer in oral mucosa is an early event of malignant transformation and has predictive value for developing oral squamous cell carcinoma. J Pathol. 1998 Apr;184(4):360-8.
22. Kerdpon D, Rich AM, Reade PC. Expression of p53 in oral mucosal hyperplasia, dysplasia and squamous cell carcinoma. Oral Dis. 1997 Mar;3(2):86-92.
23. Vered M, Allon I, Dayan D. Maspin, p53, and Ki- 67 in epithelial lesions of the tongue: from hyperplasia to dysplasia to carcinoma. J Oral Pathol Med. 2009 Mar;38(3):314-20.
24. Nasser W, Flechtenmacher C, Holzinger D, Hofele C, Bosch FX.Aberrant expression of p53, p16INK4a and Ki-67 as basic biomarker for malignant progression of oral leukoplakias. J Oral Pathol Med. 2011 Sep;40(8):629-35.
25. Kaur J,et a 1. Prognostic significance of p53 protein over expression in betel & tobacco related oral oncogenesis. Int. J. Cancer 1990;79:370-5.
26. Mirza T et al. Molecular analysis of HPV and oncosuppression gene in tobacco related oral cancer. Pak. J. Oto laryng.1998;14:27-33.
27. Yan JJ, Tzeng CC, Jin YT. Overexpression of p53 protein in squamous cell carcinoma of buckle mucosa and tongue in Taiwan: An immunohistochemical and clinicopathology study. J. Oral Pathol Med 1996;25:55:9.
28. Mullel PAJ, et al. p53 mutation in cancer. Nat Cell Biol 2013;15:2-8. (3^)
29. Vousden KH, et aL The cells response to p53. Nat Rev Cancer 2002;2:594-60