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Original Research Article

Prognostic Significance of p53 Mutation in Oral Premalignant and Malignant Lesions- a Tertiary Care Center Study

Vaibhav Patel[1], Himani Patel[2], Meghavi Joshi[3]

[1]Assistant Professor, Department of Otorhinolaryngology, Nootan Medical College and Research Center, Visnagar, Mehsana

[2]Assistant Professor, Department of Pathology, Nootan Medical College and Research Center, Visnagar, Mehsana

[3]Associate Professor, Department of Pathology, Nootan Medical College and Research Center, Visnagar, Mehsana

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Corresponding Author: Dr. Himani Patel

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Abstract:

Introduction: Oral cancers accounts for up to 40% of all malignancies in developing countries including India. Early detection and treatment play pivotal roles in improving prognosis and overall survival rates. p53 immunomarker could be considered complementary to conventional prognostic evaluation.

Aim: The aim of present study is to analyse the expression of p53 in relation to histopathological diagnosis along with the utility of labelling Index of p53 protein in of oral premalignant and malignant lesions. Also, to evaluates clinicopathological profiles of patients with oral lesions in terms of age, gender and site.

Methods: The study was conducted over a period of 18 months at tertiary care center of north Gujarat. Total 39 cases of premalignant and malignant oral cavity lesions were examined for p53 immunomarker expression, staining pattern and staining intensity by immunohistochemistry study. Formalin fixed paraffin embedded tissue were used for both histopathology and immunohistochemical evaluation.

Results: Among 39 cases, 9 (23.08%) were dysplastic lesions and 30((76.92%) were malignant lesions with male predominance. Tongue (48.71%) is most common site.92.31% cases expressed p53 in over 5% of cells. Among them, 88.88% of dysplastic lesions expressed p53 in>5% of cells and only one dysplastic lesion (12.5%) showed p53 expression >25%. The mean p53 LI in dysplastic lesions, in the present study was found to be 13.33% and in carcinoma it was 71.83%. 93.33% of SCC cases were positive for p53. 26 out of 30 cases (86.66%) of SCC showed p53 LI >25%., mean LI for p53 in SCC was found to be 71.83%. In the present study, 5/9 cases (55.55%) of dysplastic lesions showed p53 expression only in basal layer, while 2/9 cases (22.22%) cases showed both basal and suprabasal staining pattern and only one case (11.11%) showed p53 expression in all layers while one case was negative. In cases of carcinoma, 2/30 cases (6.66%) showed basal and suprabasal staining pattern while 26/30 cases (86.66%) showed p53 expression in all layers. p53 expression was mild in mild dysplasia compared to moderate and severe dysplasia and staining intensity ranges from moderate to intense for carcinoma.

Conclusions: The significant correlation between progression of oral epithelium from dysplasia to neoplasia and increased expression of p53 suggest that it may be useful biomarker of malignant transformation in oral precancerous conditions and may serve as useful biomarker for cancer prevention programmes. P53 as prognostic markers may stand as useful supplements of histopathological assessment in the prognosis of potentially malignant oral lesions.

Keywords: p53, Oral Cancer, Premalignant Lesion, Squamous Cell Carcinoma.

Introduction

Oral cancers are indeed a significant health problem in many parts of developing countries including India where it accounts for up to 40% of all malignancies.[1] About 80- 90% of malignant oral lesions are oral squamous cell carcinoma.[2,3] With prompt intervention, 5 years survival rate for localized oral squamous cell carcinoma can ranges from 70-80%. Early detection and treatment play pivotal roles in improving prognosis and overall survival rates.[1] The etiology of oral squamous cell carcinoma is multifactorial and the important risk factors include personal habits of tobacco use, areca nut chewing and alcohol consumption as well as Human Papilloma virus (HPV) infection, poor

oral hygiene and micronutrient deficiencies. Tobacco usage can cause various genetic and molecular alterations in oral premalignant and malignant lesions.[4,5] Most of malignancies develop from oral premalignant lesion such as leukoplakia and oral submucous fibrosis (OSMF).

The clinical and histopathological changes in these lesions are preceded by changes at molecular level. The identification of these high risk premalignant lesions plays a significant role in reducing the morbidity, mortality and cost of treatment associated with oral squamous cell carcinoma.[1] Markers of proliferation, epithelial differentiation and genomic markers could potentially be good for improving the prognostic evaluation of precursors of oral cancers. p53 immunomarker could be considered complementary to conventional prognostic evaluation.[1]

In a normal cell, the p53 protein is kept at a low concentration by rapid degradation. In addition, p53 exists in a latent, inactive form. Several stressful situations including genotoxic DNA damage, hypoxia and deprivation of growth factors and loss of cell to cell contact can induce the formation of functional p53. Activation of p53 occurs by increasing the p53 protein concentration by enhanced translation or by the transformation of p53 protein from a latent to an active form or by the translocation of p53 protein from cytoplasm to the nucleus.[6] p53 is a proto-oncogen and mutated gene leads to carconogenesis. Approximately 50-60% of the cases of oral cavity cancer show p53 expression.[7,8,9] The Differential diagnosis should be established with other malignant lesions such as lymphoma, sarcoma and metastasis, which have rapid growth rates as opposed to the typical oral squamous cell carcinomas.[10]

The objective of our study was to study the labelling index (LI) of expression of p53 protein, pattern and intensity of staining along with to find out the utility of labelling index of p53 protein in oral premalignant and malignant lesion.

Materials and methods

A present study includes total 39 cases of premalignant and malignant lesions of oral cavity during period of 18 months at tertiary care center of North Gujarat. Oral mucosal biopsy specimens of all age groups were taken by ENT surgeon and histopathology reporting along with immunohistochemistry was done by pathologist. Informed consent was taken from all the patients. For histopathological examination, Hematoxylin and Eosin stained formalin fixed paraffin embedded tissue sections were examined. Among them, histologically confirmed dysplasic lesions and malignant lesions were selected for immunohistochemistry. Known positive immunostaining slides were used as controls. The prepared slides were examined for expression of p53 under Olympus microscope under 4x, 10x, 20x and 40x objective lens by pathologist. Clear brown colour staining of nucleus of epithelial cells was considered positive.. The pattern of staining was graded as Humayun S et al [1]: 1) Confined to basal layer only 2) Both basal and suprabasal layer 3)All layers of epithelium

Labelling index (LI) was calculated by counting the number of positive cells per 100 squamous cells and was recorded as percentage. The parameters used to analyze the expression of p53 antigen are: 1) Pattern of staining in epithelial layers 2) Intensity of staining in each slide 3) The percentage of positive cells or labelling index (LI).

Observation

Table 1: Cases according to age groups

Age (years)	Number of cases				Total	%
	Mild DP	Moderate DP	Severe DP	SCC		
21-30	0	0	0	3	3	7.7
31-40	0	0	0	6	6	15.4
41-50	2	1	0	8	11	28.2
51-60	1	1	3	9	14	35.9
61-70	0	1	0	3	4	10.3
71-80	0	0	0	1	1	2.7
Total	3	3	3	30	39	100

Table 2: Cases according to gender

Gender	Number of cases				Total	%
	Mild DP	Moderate DP	Severe DP	SCC		
Male	0	2	2	22	26	66.67
Female	3	1	1	8	13	33.33
Total	3	3	3	30	39	100

Table 3: Cases of oral lesions according to sites

Site	No of cases	%
Tongue	19	48.71
BM	8	20.51
BOT	6	15.38
Hard palate	3	7.69
Lip	1	2.56
FOM	1	2.56
Alveolus	1	2.56
Total	39	100.00

Table 4: Cases according to histopathological diagnosis

Premalignant lesions			SCC	Total
Mild DP	Moderate DP	Severe DP		
3	3	3	30	39
7.7%	7.7%	7.7%	76.9%	100%

Table-5: Histological grades of SCC

Histological grading of SCC	No of cases	%
Well differentiated	7	23.33
Moderate differentiated	19	63.33
Poorly differentiated	4	13.33
Total	30	100.00

Table 6: Cases according to groups and p53 LI

LI	Mild dysplasia	Moderate dysplasia	Severe dysplasia	SCC	%
0-5%	1	0	0	2	7.7
6-25%	2	3	2	2	23.08
26-60%	0	0	1	3	10.26
61-99%	0	0	0	23	58.96
Total	3	3	3	30	100.00

Table 7: Cases according to groups and p53 staining intensity

Staining intensity	Mild Dysplasia	Moderate Dysplasia	Severe Dysplasia	SCC	%
Negative	1	0	0	2	7.7
Mild	2	0	0	0	5.13
Moderate	0	3	2	4	23.08
Intense	0	0	1	24	64.10
Total	3	3	3	30	100.00

Table 8: Cases according to groups and p53 staining pattern

Pattern of staining	Mild Dysplasia	Moderate Dysplasia	Severe Dysplasia	SCC	%
Negative	1	0	0	2	7.7
Basal	2	2	1	0	12.82
B+SB	0	1	1	2	10.25
All layers	0	0	1	26	69.23
Total	3	3	3	30	100.00

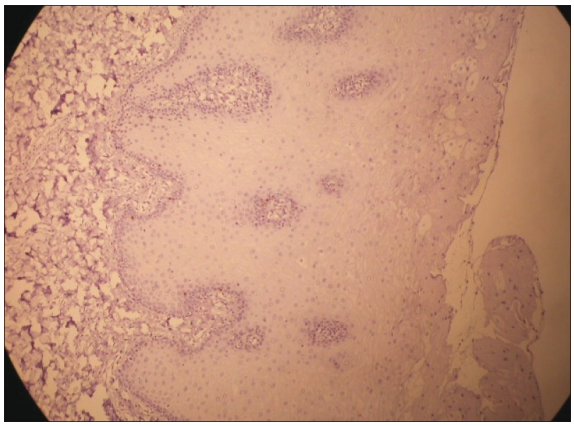

Figure-1: p53 expression in normal oral mucosa (40x view)

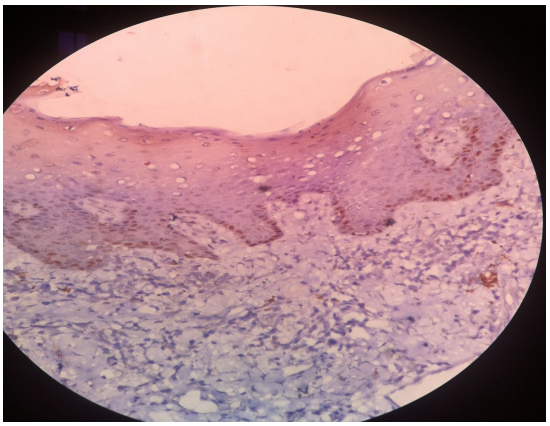

Figure-2: p53 expression in mild dysplasia (40x view) showing only basal layer staining

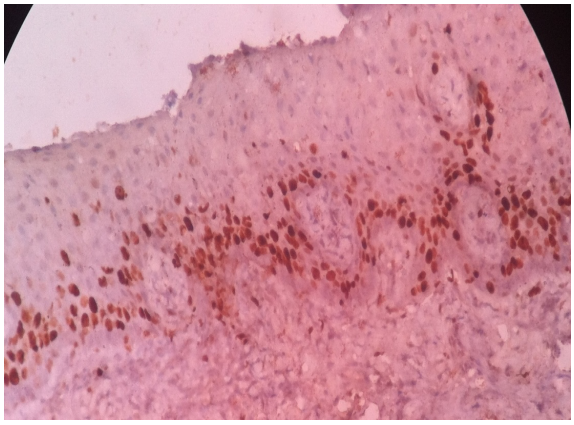

Figure-3: p53 expression in severe dysplasia (40x view) showing both basal and suprabasal layer staining

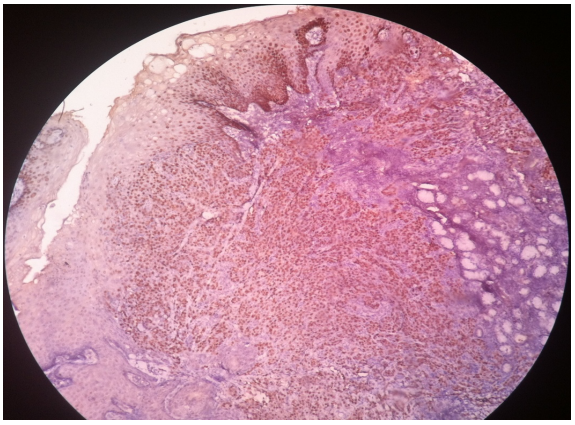

Figure-4: p53 expression in SCC (10x view) showing p53 immunostaining in all layers of epithelium and by invasive tumour cells

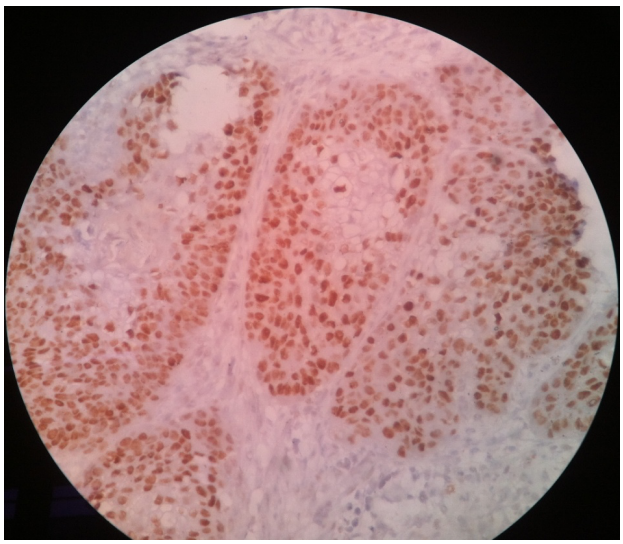

Figure 5: p53 expression in SCC (40x view) showing tumour invasion front

Discussion:

On the basis of above results and observations, in present study, maximum number of cases were found in the age group of 51-60 years(30%) followed by in 41-50 years (26.66%) which is similar to studies done by

Gopalakrishna et al[11], Thakur B.S et al and others. In our study, low incidence of oral lesions seen in 71-80 years (3.33%) followed by 21-30 years (10%) which are comparable with Thakur B.S et al[12]

Table 9: Comparison of studies according to age in cases of oral lesions

Age(years)	Sharma et al[13]	Gopalakrishna et al[11]	Thakur B.S et al[12]	Present study
21-30	1.60	2.1	4.20	10.0
31-40	32.80	16.0	12.50	20.0
41-50	33.70	34.0	28.50	26.66
51-60	26.70	35.1	32.00	30.0
61-70	5.70	6.2	19.00	10.0
71-80	0.0	6.2	2.30	3.33

Table 10: Comparison of studies according to gender in cases of Oral lesion

Studies	M:F
Sharma[13]	3.95:1
Mehrotra et al[14]	3.27:1
Dias et al[15]	4:1
Present study	2:1

From the above table, it can be observed that, male preponderance is seen in all studies, including the present study. This is because traditionally males are more likely to display oral habits such as tobacco smoking and betel quid chewing.[1] But there appears to be growing incidence in females.[16]

Table 11: Comparison according to histopathological diagnosis

Study	Malignant: premalignant
Mehrotra Ravi et al[14]	2.3 : 1
Maheshwari et al[17]	1.6 : 1
Present study	1.1 : 1

Among 39 total cases, 30 cases (76.9%) of malignancy and 09 cases of premalignant lesion (23.08%). Above table show, incidence of malignancy is increased in compare of non- malignant lesion in comparison of Mehrotra Ravi et al [14] and Maheshwari et al [17]

Table 12: Comparison of site wise distribution of oral lesions

Sites	Gopalakrishna et al[11]	Alvi et al[18]	Thakur B.S et al[12]	Present study
Tongue	24.7	30.1	30.3	48.71
Cheek(BM)	51.5	12	42.5	20.51
Lip	7.2	10.4	15	2.56
Palate	5.2	3.2	5.8	7.69
Alveolus	9.3	16.7	2.5	2.56
FOM	2.1	27.6	3.3	2.56

In our study tongue was the most common site of malignancy (48.71%) followed by buccal mucosa (20.51%). Mehrotra Ravi et al [14] also concluded tongue as the most common site of malignancy (42.57%) followed by buccal mucosa (19.14%). Alvi et al [18] also concluded that tongue was the most common site of oral lesions. The findings on oral cancer patients from India might be explained by the habit of keeping oral tobacco in the buccal pouch just before going to sleep-ensuring long contact between the buccal mucosa and the potential carcinogen.[19] In the present study, 92.31% cases expressed p53 in over 5% of cells which is comparable to the study of S Kannan et al[20] which observed that 70% cases expressed p53 in over 5% of cells.

In the present study 88.88% of dysplastic lesions expressed p53 in>5% of cells which is similar to the study of S Kannan et al[20] which observed it as 86%. Reddy V M et al [6] observed p53 expression in 70% of dysplastic lesions. Raju et al [19] observed p53 expression in 66% of premalignant lesions. In the present study, only one dysplastic lesion (12.5%) showed p53 expression >25% which was similar to the study of S Kannan et al [20], in which it was 13%.

The mean p53 LI in dysplastic lesions, in the present study was found to be 13.33% and in carcinoma it was 71.83%. This suggests p53 immunolocalization increases as normal mucosa becomes dysplastic and undergoes malignant transformation. This finding is similar with study of S. Humayun et al[1] and supported by other reports such as the study of S Kannan et al.[20] These findings suggest a strong correlation between p53 expression and degree of dysplasia, thus confirming that p53 may be involved in proliferative events as well as in neoplastic transformation.

In the present study, 93.33% of SCC cases were positive for p53 which is comparable with the studies of L.P. Dragomir et al (82.3%) [16], Raju et al (100%)[19] and S. Humayun et al(100%)[1]. In the present study, mean LI for p53 in SCC was found to be 71.83% which is comparable with the study of S.Humayun et al [1]. This concluded it to be 66.25%. In the present study, 26 out of 30 cases (86.66%) of SCC showed p53 LI >25%. S Kannan et al observed >25% p53 LI in 67% of carcinoma cases. [20]

In the present study, all the well differentiated and moderately differentiated carcinoma showed p53 LI>25% while the 2 cases of poorly differentiated carcinoma showed <25% p53 LI and other two cases of poorly differentiated carcinoma showed negative p53 staining. This might be attributed to the role of other oncogenes like H-ras, C-fos, jun family, C-myc, and tropical factors that participate in growth regulation and when inappropriately expressed, generate growth signals that may override the cellular control of p53.[6] Some mutations lead to transcription or translation errors in the p53 gene, which result in the arrest of p53 protein synthesis and negative staining on immunohistochemistry. A study from Sri Lanka found a low prevalence (11%) of p53 expression in oral carcinoma. Previously population differences in ras mutation have been reported in oral carcinoma for Indian and UK samples. Tumours with nonsense or frame shift mutations also result in the production of unstable, truncated proteins, which are also negative on immunohistochemistry.[20] In the present study, 5/9 cases (55.55%) of dysplastic lesions showed p53 expression only in basal layer, while 2/9 cases (22.22%) cases showed both basal and suprabasal staining pattern and only one case (11.11%) showed p53 expression in all layers while one case was negative. Expression of p53 in suprabasal cells in moderate and severe dysplasia states indicated that the superficial cells of the epithelium were mitotically active resulting in an abnormally proliferative state. This is in contrast to normal mucosa, where it is only the basal cells of the epithelium, which proliferate, differentiate and mature to form keratinised squames.[6]

In cases of carcinoma, 2/30 cases (6.66%) showed basal and suprabasal staining pattern while 26/30 cases (86.66%) showed p53 expression in all layers. Cruiz IB et al [21] in their study observed that 86% of premalignant lesions that showed p53 expression above the basal cell layer developed into SCC. They stated that p53 expression above the basal layer is an early event in oral carcinogenesis. Huang WX et al[22], in their study observed that there is a significant predilection for basal and suprabasal staining pattern with the progression of lesion towards malignancy compared to strictly basal layer staining in the normal mucosa. S. Humayun et al [1] in their study suggested that the expression pattern of p53 is significant in predicting the malignant transformation of oral premalignant lesions and conditions.

The above reports and the present study suggest that the expression of p53 above the basal layer could be an early event in oral carcinogenesis and an indicator of developing carcinoma. In the present study, for carcinoma staining intensity ranges from moderate (++) to intense (+++). 4 cases showed moderate (++) staining intensity and 24 cases showed intense (+++) staining. S. Humayun et al also observed moderate (++) to intense (+++) staining in carcinoma cases. In our study, comparison of p53 staining intensity in positive cases revealed that p53 expression was mild in mild dysplasia compared to moderate and severe dysplasia which showed intense staining intensity. This may be directly related to the activity of p53 which may be increased during the early stages of tumour progression.

Conclusion

Majority of patients with oral carcinoma and precancerous lesions are from age group of 41-60 years and predominantly a disease of males. Squamous cell carcinoma was most common malignant lesion of oral cavity. Tongue is the most common site in oral lesions. Oral carcinogenesis is a multistep process in which occurrence of a series of genetic events may lead to dysregulation of the cell cycle. Mutations in the p53 gene are the most common genetic changes observed in human carcinomas. Accumulation of p53 protein in the cell is generally considered to be the result of mutation of the p53 gene. These mutations lead to uncontrolled cell proliferation, resulting in further genetic abnormalities and finally in malignancy. P53 as prognostic markers may stand as useful supplements of histopathological assessment in the prognosis of potentially malignant oral lesions. The significant correlation between progression of oral epithelium from normal to neoplasia and increased expression of p53 suggest that they may be useful biomarker of malignant transformation occurs in oral precancerous lesions and conditions and also may useful for cancer prevention programmes.

References

1. S. Humayun, V. Ram Prasad. Expression of p53 protein and ki67 antigen in oral premalignant lesions and oral squamous cell carcinomas: An immunohistochemical study. National Journal of Maxillofacial Surgery. 2011 (May, Monday); 2(1):38-46.
2. Bray F, Sankila R, Ferlay J, Parkin DM. Estimates of cancer incidence and mortality in Eu-

rope in 1995: european journal of cancer (Oxford England: 1990)2002; 38:99-166.
3. 3.Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002.CA: A cancer journal for clinician.2005;55:74-108
4. Windsor LJ. Social And Behavioural Determinants Of Oral Cancer Dentistry.2013;4:11-3
5. Pitiyage G, Tilakaratne WM, Tavasoli M, et al. Molecular markers in oral epithelial dysplasia: Review. J Oral Pathology Med. 2009; 38:737-52
6. Reddy VM, Kamath A, Radhakrishnan RA. p53 immunoprofiling of potentially malignant oral disorders: A case series analysis. Indian Journal of Cancer. 2012 (Jan, Monday); 49(1):27-32.
7. Linden MD, Nathanson SD, Zarbo RJ. Evalution of anti-p53 antibody staining quality control and technical considerations. Appl Immuno- histochem. 1994; 2:218-24.8.
8. Tubiana M, Courdi A. Cell proliferation kinetics in human solid tumors:relation to probability of metastatic dissemination and long term survival. Radiother Oncol. 1989;15:1-18.9.
9. Forastiere A, Koch W, Trotti A, Sidransky D. Head and neck cancer. N Engl J Med. 2001; 345:1890-900.]
10. J Bagan, G sarrion, Y Jimenez ,oral cancer: clinical features oral oncology : 20104664147
11. Gopalakrishna, Saxena, Singh A.K.; Oral carcinoma; Ind J Surg; 1967.
12. Thakur B.S., J.H. Makannavar,; oral and oropharyngeal tumours clinicopathological study of 243 tumors(1991-1997); Karnataka; Karnataka university; 1998.
13. Sharma R.N.: oral carcinoma –A clinical study of 122 cases; J Ind Med Ass; 1964; 43: 263.
14. Mehtrotra, R., Singh, M., Kumar, D., Pandey, A. N., Gupta, R. K., Sinha, U. S., Age specific incidence rate and pathological spectrum floral cancer in Allahabad
15. Dias GS; A histological and clinical study on oral cancers, descriptive analysis of 365 cases .Med Oral Pathol Oral Cir Bucal.2007 Nov 1;12(7):E474-478.
16. L.P. Dragomir, Cristiana S., CL. Margaritescu, A.Stepan et al. P53, p16 and Ki67 immunoexpression in oral squamous carcinomas. Romanian Journal of Morphology & Embryology. 2012;53(1):89-93.
17. Maheshwari V, Sharma SC, Narula V, Verma S, Jain A, Alam K. Prognostic and Predictive Impact of Ki-67 in Premalignant and Malignant Squamous Cell Lesions of Oral Cavity. Int J Head Neck Surg 2013;4(2):61-65
18. Alvi Aijaz, Myers, E. N., Johnson, J. T. Cancer of the oral cavity in myers/ suen Editor, Cancer of the head and neck. W. B. Saunders, Company Phialdelophia, 1996; 3rd ed. P. 321-360.
19. Bina Raju, Ravi M., Gunnvor J., Ali K. et al. Expression of p53, Cyclin D1and Ki-67 in Premalignant and Malignant Oral Lesions: Association with Clinicopathological Parameters. Anticancer Research. 2005; 25:4699-4706.
20. S Kannan, G Jagadeesh, K Raveendran, B. Mathew et al. Expression of p53 in leukoplakia and squamous cell carcinoma of the oral mucosa: correlation with expression of Ki67. Journal of Clinical Pathology: Molecular Pathology. 1996; 49:170-175.
21. Cruz IB, Snijders PJ, Meijer CJ, Braakhuis BJ 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; 184:360-368.
22. Huang WY, Coltrera M, Schubert M, Morton T et al. Histopathological evaluation of PCNA and p53 in oral epithelial hyperplasias and oral pre-malignant lesions. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 1994; 78:748-754.