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Original Research Paper

Pathology

A STUDY OF ORAL PREMALIGNANT AND MALIGNANT LESIONS WITH IMMNUNOEXPRESSION OF P53 AND BCL2.

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ABSTRACT

CONTEXT- Histopathological examination of oral premaligant and malignant lesions, presenting the fundamental aspects of this cancer, focused on squamous cell carcinoma of the oral cavity (OSCC), moving from its definition and epidemiological aspects, addressing the oral carcinogenesis, oral potentially malignant disorders, epithelial precursor lesions and experimental methods for its study, therapies and future challenges. Also immunoexpression of genes p53 and bcl2 attributed in pathogenesis of development of oral cancer.

AIMS- Histopathological analysis of oral premalignant and malignant lesions and immunoexpression of Bcl 2 and p53 in these lesions.

MATERIAL AND METHODS- This study was done in department of pathology, GSVM medical college, Kanpur. This was prospective study done from 2019-2021. A total of 200 cases were studied. Specimen received were fixed in 10% formalin, processed and sections of 3-4 micron were prepared, stained with H and E. Histopathological examination was done. Anti p53, anti bcl2 antibody was used. Staining and evaluation of immunohistochemistry was done using Ab-5 (clone DO-7) ready to use as monoclonal primary antibody.

RESULTS- Maximum number of cases [24%] were in their 4th and 5th decades of life. Males predominated over females with male:female ratio of 3:1. In present study the most common site of oral mucosal lesions was buccal mucosa which accounted for 42% of total cases. . 88% cases had history of tobacco use, this data strongly suggest close association between tobacco and oral cancer. Most of the pre-neoplastic and neoplastic lesions of oral cavity & oropharynx occur after 20-30 years of exposure to tobacco (37%), followed by 27% each after 0-20 years and 30-40 years of exposure. Gutkha chewing (tobacco) was the commonest observed in almost 50% cases. 69.5%belong to rural areas whereas only 30.5% cases were belong to urban areas. . Among premalignant cases, majority were of leukoplakia (30%) cases.Majority of the malignant cases were well differentiated squamous cell carcinoma. [23%]. Expression of p53 was found in 772.8% of the pre-malignant cases and 90.7% of the malignant lesions of the oral cavity. . It was found that 54.34% of premalignant lesions and 66.6% of malignant lesions showed strong p53 positivity. Most of the premalignant lesion showed mild positivity [50%]. 35% cases showed moderate positivity while none of the premalignant cases showed strong positivity. 52% cases of malignant lesions showed weak positivity. 45% cases showed moderate positivity and 13% had strong positivity. Intensity of staining increases with increasing grade of dysplasia and cancer.

CONCLUSION- Pro apoptotic and anti apoptotic genes like p53 and bcl2 play an important role in early pathogenesis of oral cancer and their development from premalignant to malignant lesions. However, they are not the only genes involved as their tumorigenesis seems multifactorial. Targetted study towards these genes can still bring future prospects of gene therapy to light.

KEYWORDS :

INTRODUCTION

Oral cancer is a big health problem worldwide due to its morbidity and mortality. The prevalence of oral cancer presents variations around the world. These rates vary by as much as 20-fold among different countries, age groups, gender, races, and ethnic groups. In 2013, oral cancer incidence ranked eleventh among all sites of cancer. The Indian subcontinent accounts for one-third of the oral cancer burden in the world.

More than 95% of oral cancers are Squamous cell carcinomas. Etiology of oral cancers like most other malignancies is multifactorial involving both chemical carcinogens and genetic factors. The effect of chemical carcinogens on OSCC is quite overwhelming. In the western countries most are attributable to separate and combined habits of tobacco use and alcohol consumption. In the developing Asian countries chewing habits of betel quid, betel quid substitutes along with smoked and chewed tobacco are more prevalent. Genetics has a definite predisposing role in oral malignancies with genetic polymorphisms leading to carcinogenic potential and transformation.

Various studies have been conducted to determine the pathogenesis of development of malignancy from premalignant lesions. Apoptosis has been recognised as a physiologic process associated with cancer.Role of programmed cell death in the pathogenesis and treatment of cancer has therefore been an interest of study.

Wild type p53 protein is known to induce cell cycle arrest to repair the damaged DNA.

Bcl-2, Bcl-xL and Mcl-1 proteins appear to inhibit apoptosis, Bax, Bad, and Bcl-xS proteins apparently promote apoptosis.

Bcl-2 is thus an anti-apoptotic membrane-associated molecule residing in the nuclear and mitochondrial membranes. It is inversely related to p53 since its expression prevents apoptotic cell death and its anti-apoptotic function is modulating the mitochondrial release of cytochrome c.

Loss of TP53 tumor suppressor gene function due to mutation represents the most common genetic event known in human cancer. Wild-type p53 contributes to tumor suppression through at least two mechanisms in response to DNA damage, i.e., arrest of cell proliferation and induction of apoptosis. p53 may induce apoptotic cell death by down-regulating Bcl-2 and up-regulating Bax expression, which has been suggested to determine the survival or death of cells following an apoptotic

stimulus.

Overexpression of bcl-2 results in an alteration of programmed cell death with persistence of cells that fail to die.

An important consideration in the interactions of Bcl- 2 family members is the loss of the tumor suppressor p53. The role of wild type p53 protein in inducing cell cycle arrest to allow for repair of damaged DNA has been well characterized.

Oral cancer is one of the 10 most common cancers in the world, with a delayed clinical detection, poor prognosis, without specific biomarkers for the disease and expensive therapeutic alternatives. This review aims to present the fundamental aspects of this cancer, focused on squamous cell carcinoma of the oral cavity (OSCC), moving from its definition and epidemiological aspects, addressing the oral carcinogenesis, oral potentially malignant disorders, epithelial precursor lesions and experimental methods for its study, therapies and future challenges. Oral cancer is a preventable disease, risk factors and natural history is already being known, where biomedical sciences and dentistry in particular are likely to improve their poor clinical indicators

MATERIAL AND METHODS-

A total of 200 cases diagnosed in histopathology laboratory between year 2018-2019 were included as follows:

INCLUSION CRITERIA:

- Only histopathologically diagnosed cases were included.
- Patients who have undergone surgical therapy as a primary mode of treatment.

EXCLUSION CRITERIA:

- Patients with known primary other than oral cavity.
- Inadequate biopsies were excluded from the study.
- Patient who have undergone radiotherapy as a primary mode of treatment.
- Histopathologically ulcerative and necrotic areas will be excluded.

DISCUSSION-

Oral lesions according to Age group

Age Group in Years	No. of cases	percentage
0-10	0	0%
11-20	0	0%
21-30	20	10%
31-40	44	22%
41-50	48	24%
51-60	40	20%
61-70	22	11%
71-80	14	07%
81-90	12	06%
Total	200	100%

The study include 200 cases of oral lesion. Maximum number of cases [24%] were in their 4th and 5th decades of life, followed by 3rd and 4th decade [22%].

Sex-wise distribution of oral lesion

Sex	No. of cases	Percentage
Male	151	75.5%
Female	49	24.5%
Total	200	100%

Table 3 shows sex wise distribution of cases analysed. There were 151 males(75.5%) and 49 females(24.5%). Males predominated over females with male:female ratio of 3:1.

Site wise distribution of cases

Site	No. of cases	Percentage
Buccal mucosa	84	42%
Tongue	40	20%
Border of tongue	26	13%
Hard palate	16	08%
Lip	18	09%
Floor of mouth	12	06%
Alveolus	04	02%
Total	200	100%

In present study the most common site of oral mucosal lesions was buccal mucosa which accounted for 42% of total cases. Tongue is the next common site of oral lesions (20%), base of tongue & others were less common sites.

Incidence in relation to tobacco chewing

Addiction	No. of cases	Percentage
Tobacco users(in any form)	176	88
Non- tobacco users	24	12
Total	200	100

Table 5 shows incidence of oral lesions in relation to tobacco use. 88% cases had history of tobacco use, this data strongly suggest close association between tobacco and oral cancer.

Duration of tobacco use

Duration of tobacco use (yrs)	No. of cases	Percentage
1-10	7	3.5%
11-20	54	27%
21-30	74	37%
31-40	43	21.5%
>40	22	11%
Total	200	100

As evidenced from the table, majority of pre-neoplastic and neoplastic lesions of oral cavity & oropharynx occur after 20-30 years of exposure to tobacco (37%), followed by 27% each after 0-20 years and 30-40 years of exposure.

Habits As Observed In Different Patients

S. no.	Personal habits	Cases	Percentage
1.	Gutkha chewing	98	49%
2.	Tobacco chewing and smoking	70	35%
3.	Smoking alone	05	2.5%
4.	Pan masala	23	11.5%
5.	others	04	2%
	Total	200	100%

This table shows personal habits in relationship with oral lesions. Gutkha chewing (tobacco) was the commonest observed in 98 cases (49%), followed by 70 cases (35%) tobacco chewing & smoking, 23 cases (11.5%) of pan masala and 2.5% cases of smoking alone and 2% cases of others.

Distribution of oral lesion

Urban/rural	No. of cases	Percentage
Rural	139	69.5%
Urban	61	30.5%
Total	200	100%

As evident from the table, most of the cases (69.5%) belong to rural areas whereas only 30.5% cases were belong to urban areas.

Distribution of cases on the basis of histological type (n=200)

Premalignant cases(92)-

Epithelial lesions	Total no. of cases	Percentage
Leukoplakia	38	19%
-Mild dysplasia	16	08%
-Moderate dysplasia	20	10%
-Severe dysplasia	18	09%
Malignant cases(108)		
WDSCC	46	23%

MDSCC	37	18.5%
PDSCC	15	7.5%
Verrucous carcinoma	10	5%

On histopathology, 92 (46%) cases were premalignant and 108 (54%) malignant. Premalignant including majority of leukoplakia 60(30%) cases.Majority of the cases were well differentiated squamous cell carcinoma in malignant lesion [23%]

In our series, Malignant lesions had higher incidence in comparision to benign lesions. Well differentiated squamous cell carcinoma is most common entity among the malignant lesions.

Immunohistochemistry studies of premalignant and malignant lesions

Presence of p53 in epithelium of different groups

S.No	Result	Premalignant		Malignant	
		N	%	N	%
1.	Absent	25	27.1%	10	9.2%
2.	Present	67	72.8%	98	90.7%

Expression of p53 was found in 772.8% [67/92] of the pre-malignant cases and 90.7%(98/108) of the malignant lesions of the oral cavity.

Intensity of staining in oral dysplasia with p53 immunoreaction in relation to histological grading

	Mild dysplasia [n=15]	Moderate dysplasia [25]	Severe dyplasia [27]
Mild +	9 [60%]	8 [32%]	1 [3.7%]
Moderate ++	6 [40%]	10 [40%]	18 [66.66%]
Strong +++	0 [0%]	7 [28%]	8 [29.6%]

Intensity of staining in oral squamous cell carcinoma with p53 immunoreaction in relation to histological grading

	WDSCC [40]	MDSCC [35]	PDSCC [15]	Verrucous carcinoma [8]
Mild ++	2 [5%]	2 [6%]	0 [0%]	3 [37%]
Moderate ++	15 [37.5%]	13 [37%]	5 [33%]	4 [50%]
Strong +++	23 [57.5%]	20 [57%]	10 [677%]	1 [13%]

Brown nuclear staining is taken as positive. It was found that 54.34% of premalignant lesions and 66.6% of malignant lesions showed strong p53 positivity. Intensity of p53 expression increases with degree of dysplasia and grade of carcinoma.

Bcl2 staining in oral premalignant and malignant lesions

S.No	Result	Premalignant		Malignant	
		N	%	N	%
1.	Absent	63	70%	70	66%
2.	Present	29	30%	38	34%

30% cases of premalignant lesions showed bcl2 positivity while 38% malignant lesions were positive for bcl2.

Intensity of bcl2 staining in oral premalignant lesions

	Mild dysplasia [%]	Moderate dysplasia[%]	Severe dysplasia[%]
Negative staining	**75**	**82**	**58**
Mild positive +	**25**	**8**	**17**
Moderate positive ++	**0**	**10**	**25**
Strong positive +++	**0**	**0**	**0**

Most of the premalignant lesion showed mild positivity [50%]. 35% cases showed moderate positivity while none of the premalignant cases showed strong positivity.

Intensity of bcl2 staining in oral premalignant lesions

	WDSCC[%]	MDSCC[%]	PDSCC[%]
Negative staining	**70**	**68**	**52**
Weak positive +	**15**	**14**	**23**
Moderate positive ++	**15**	**18**	**12**
Strong positive +++	**0**	**0**	**13**

52% cases of malignant lesions showed weak positivity. 45% cases showed moderate positivity and 13% had strong positivity Brown granular cytoplasmic staining and nuclear staining taken **positive.**

Intensity of staining increases with increasing grade of dysplasia and cancer.

CONCLUSIONS-

1. In present study it was found that the peak incidence of oral carcinoma was in the fourth and fifth decade of life. This was in accordance with findings of **Kittipong et al** [2018] [1] who stated that the mean age of the Asian patients ± SD was 56.37 ± 14.98 years.
   KenRussell Coelho et al [2]cited most of the oral cancer cases occur between the age of 50 to 70 years, but it could also affect children as early as 10 years. Incidence of oral cancer increases by age[16].
   SS Rahman et al [3] stated the commonest age is the fifth decade of life[17].
2. Male preponderance was noted with male female ratio of 3:1, which is in accordance with **Oliver et al, (1996) [4] and Agarwal et al, (2001) [5].** This is may be primarily due to their addiction to tobacco chewing and smoking, which is the most important risk factor for oral cancer and presence resultant precancerous lesions, secondarily low incidence in females . This was also in accordance with findings of Kittipong Dhanuthai **et al** [2018] et al who cited the male-to-female ratio was 2.22:1.
3. Tobacco addiction in any form has been widely impied in causative agent of oral premalignant and malignant lesions. In present study 88% cases were tobacco users, which strongly suggest close relation between tobacco use & oral cancer. Similar findings were reported by **P.N.Wahi 1968 [6]** .
   KenRussell Coelho et al estimates indicate 57% of men and 11% of women between 15- 49 years of age use some form of tobacco[16].
   Our findings were also in line with findings of **Sree Vidya Krishna Raol [2012] [7] .** More than 90% of OC cases report using tobacco products[8].
4. Majority of cases(96%) had a history of more than 10 years of tobacco use, which indicates that the risk of developing carcinoma increases with the duration of tobacco use. The findings were consistent with **Hirayama 1966 and P.N. Wahi 1968,** who suggested that increase in frequency and doses of tobacco use increases risk of developing carcinoma of oral cavity.
5. Most common site of oral lesion was buccal mucosa which accounted for 42% of cases with tongue being the second most common site [20%]. Our findings were in line with **V Singhania et al** [8] who cited that Carcinoma of the buccal mucosa is the most common cancer of the oral cavity in India and **Sampurna Ghosh et al. Ear Nose Throat J.[2017] [9]** stating that buccal mucosa was the single most common site of white lesions.
   Ravi et al,[2008] [10] stated that buccal mucosa was the most frequently involved site in benign and premalignant lesions, however in malignant lesions, the tongue was most common site.
6. Well differentiated SCC & moderately differentiated SCC were found in higher number in our study, which is in accordance with **Zedan et al 2015[11]** who reported Well differentiated SCC as the most common histological type.

Our study is in contrast to some stodies that found that poorly differentiated squamous cell carcinoma was most prevalent histological variant.

7. Our attempt to analyse p53 and bcl2 expression showed uniform pattern and intensity of expression in all batches compared at premalignant and malignant lesions suggesting that immunohistochemical procedure utilised was standardised. **Nylander K, Nilsson P, Mehle C, [1995] [12]** stated mutation of p53 gene is one of the most common events in carcinogensis including squamous cell cancers of the head and neck [5].
8. Expression of p53 was found in 72.8% [67/92] of the pre-malignant cases and 90.7%(98/108) of the malignant lesions of the oral cavity.
   Sharmistha M Patel, et al [2014] [13] In their study showed 8 (88.89%) dysplastic lesions and 28 (93.33%) SCC cases showed p53 overexpression. Similar findings were observed by **Panjwani et al** [14] in their study that showed 75% positive cases.
9. Intensity of p53 staining increased with increasing grade of dysplasia with mild dysplasia showing weak positive [+][60%] cases to strong positive in 29% cases of severe dysplasia.
   Intensity of p53 staining increased with increasing grade of carcinoma although all grades showed mostly moderate or strong positivity upto 67%.
   Shin DM, Kim J, *et al*.[1994][15] , **Lavieille JP, [1995] [16] and Regezi JA, Zarbo RJ et al [1995] [17]** also found that the number of cases that expressed p53 increased as the epithelial disorder progressed from hyperplasia to epithelial dysplasia.
10. **Juneja S, Chaitanya NB et al** [18] concluded that the altered expression of Bcl-2 may be an early molecular event, which leads to prolonged cell survival, an increased chance of accumulation of genetic alterations, and subsequent increase in malignant transformation potential.
    We observed positive expression of bcl2 in 30% of premalignant cases and 34% of malignant cases. This was in line with findings of **Vandana Arya a, Subash Singh b et al [2016] [19]** who stated positive Bcl-2 expression was present in 36.66% (11/30) oral cancer cases.
11. Yellowish-Brown granular cytoplasmic staining in central and peripheral areas of tumor was taken as positive.
    Patielli et al [20] also observed the same pattern of Bcl-2 immunostaining in dysplastic and oral cancer cases.
    Bcl2 staining was seen more intense in poorly differentiated cases while relatively less intense in well and moderately differentiated carcinomas.
    This was in line with findings of **Rahmani et al [2012]** [21] who concluded that the expression of bcl2 was found to be restricted to the tumor cells in well- and moderately differentiated tumors. The intense expression of Bcl2 was observed throughout the tumor cell in poorly differentiated tumors.

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