Section: Pathology

Original Research Article

EXPRESSION OF p53 PROTEIN AND ki-67 ANTIGEN IN ORAL PREMALIGNANT AND ORAL SQUAMOUS CELL CARCINOMAS: AN IMMUNOHISTOCHEMICAL STUDY

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ABSTRACT

Background: Oral squamous cell carcinoma (OSCC) constitutes the 6th most common cancer worldwide. They are a major health problem in most of the developing countries. They are usually preceded by premalignant conditions of the oral cavity. Hence, early diagnosis of these premalignant lesions and understanding the degree of differentiation in Oral squamous cell carcinoma by histopathology aided by immunohistochemistry can prevent morbidity. The aim is to evaluate the Immunohistochemical expression of p53 and ki67 in premalignant lesions and invasive squamous cell carcinoma of the oral cavity, as potential biomarkers for histological grading and differentiation. **Materials and Methods:** This study consists of 55 histopathological specimens of Oral Premalignant lesions and Invasive squamous cell carcinoma of the oral cavity received at the Department of pathology, DR B.R Ambedkar Medical College and hospital between August 2019 to June 2021. After processing of representative tissue blocks, H&E and IHC stains for Ki-67 and p53 immunomarkers were carried out. The respective cases were graded with histopathology as the gold standard and analyzed for the expression of p53 and Ki-67. **Results:** A total of 55 cases were included in the present study, out of which 37 cases were of malignant OSCC and 18 cases were of oral premalignant cases with the mean age of presentation of 55.67 years and there was male preponderance. Tongue was the commonest site to be involved. Out of the total 55 cases under study, 18 cases (32.7%) belonged to Premalignant lesions, 22 cases (40%) were Well differentiated oral squamous cell carcinoma, 12 cases (21.8%) were Moderately differentiated oral squamous cell carcinoma and 3 cases (5.5 %) were belonging to Poorly differentiated oral squamous cell carcinoma. Out of the 55 cases, 44(80%) cases showed positivity for p53 and 52 (94.55%) cases showed positivity for ki-67. Statistical significance was present with respect to comparison between p53 and ki-67 LI, intensity and pattern of staining and histopathological diagnosis. A positive correlation was shown between the p53 and Ki-67 expression and this was established by the Fisher Exact Test and the Pearson correlation. **Conclusion:** The increased expression of p53 and ki-67 with an increase in the grade of dysplasia seen in the current study implies that their co-expression could be used to identify high-risk lesions and help with accurate OSCC histological grading. As a result, p53 protein and ki-67 antigen have the potential to be used as biomarkers of malignant transformation and carcinogenesis in oral dysplastic lesions and OSCC.

INTRODUCTION

"Oral squamous cell carcinoma (OSCC) is a carcinoma with squamous differentiation arising from the mucosal epithelium". It is an epithelial neoplasm generally beginning as focal overgrowth of altered stem cells near the basement membrane, moving upward and laterally, replacing the normal

epithelium. Oral squamous cell carcinoma (OSCC) constitutes the 6th most common cancer worldwide. More than 90% of cancers in the oral cavity are Oral squamous cell carcinomas.[1]

The GLOBOCAN project estimated 377,713 new cases in 2020, with a global age standardized incidence rate of 4.1 cases per 100,000 population per year and a global mortality rate of 1.9 deaths per 100,000 population per year.[2]

In India, oral cancer is the most frequent type of cancer accounting for up to 40% of all cancers. This can be attributed to the fact that most of the cases are diagnosed at advanced stages. Of all Oral Cancer cases, Oral Squamous Cell Carcinoma accounts for the majority of the cases with incidence rates of 12.8 and 7.5/ 100,000 in men and women, respectively and majority of the patients fall within the age of 40 – 70 years.[3]

According to the 'Three Year Report of the Population Based Cancer Registries' (2012-2014), the AAR (Age adjusted incidence Rates) per 100,000 population for the sites of Tongue and Mouth cancer in males in Bangalore was 4.2 and 3.9 respectively and in females with Mouth cancer was 5.[4,5]

It is the most common cancer among male and the third most common among female population, which is related to the deleterious oral habits such as tobacco chewing, betel quid chewing, tobacco smoking, reverse smoking, as well as other factors such as alcohol consumption, low socioeconomic status, poor hygiene, poor diet, viral infections, chronic irritation from ill fitting dentures, rough, or fractured teeth. OSCC can affect any area of the oral mucosa. The most common sites in many populations are the tongue, floor of the mouth, and gingival and among the Asian populations, OSCC commonly affects the buccal mucosa, due to tobacco chewing and betel quid chewing.[1]

The concept of a sequential development of carcinoma in the oral mucosa, in which a precursor lesion appears first and then develops into cancer, is well known. Mouth precancerous lesions were found to be present in roughly 80% of cases of oral cancer, according to studies. Leukoplakia, Oral Submucous Fibrosis, and Oral Epithelial Dysplasias are the most common in India.[6]

Oral leukoplakia is a white patch on the mucosa, which cannot be scraped off and cannot be attributed to any other disease process. 'Leuko' means white and 'plakia' means patch.[7]

The percentage of leukoplakias that progress to invasive SCC is accepted to be directly related to the severity of the dysplastic changes and it ranges from 5 to 43%.[6]

Oral Submucous Fibrosis (0SMF) is a high-risk precancerous condition marked by alterations in the connective tissue fibers of the lamina propria and deeper portions of the mouth, resulting in mucosal stiffness and mouth opening restriction. Almost all cases of OSMF have been documented in Indians and Asians. Over a 10-year period, malignant transformation rates as high as 7.6% have been documented.

The word dysplasia is to describe abnormalities in development and proliferation in the epithelium. Dysplasia refers to cellular and structural changes in the epithelium. The presence of dysplasia is essential in predicting malignant transformation, which occurs in 36.4 percent of cases.[8]

In OSCC, important biomarkers include p53, cyclin D, ki67, p16 and bcl¬2. Mutations in the p53 gene are the most common genetic changes observed in OSCCs. These mutations lead to uncontrolled cell proliferation, resulting in further genetic abnormalities and finally in malignancy. P53 is a tumor suppressor gene that encodes a 53-kDa nuclear phosphoprotein and is found on chromosome 17p13.1 in the human genome, earning it the nickname "guardian of the genome." It's called a tumor suppressor gene since its primary role is to prevent tumor growth by identifying genetic errors in G1 cells, which result in cell cycle arrest or cell death (programmed cell death). The tumor suppressor function of the mutant p53 protein is lost since the protein is usually active. A point mutation in exons 5 to 8 of the p53 gene is the most frequent p53 mutation. p53 mutations impede cells' ability to repopulate. Ki-67 is a cell cycle-related human nuclear protein that is found in the peri chromosomal area and whose expression is tightly linked to cell proliferation.[9]

Therefore, the nature of the p53 gene and the proliferative status of a cell are closely linked and the loss of this linkage is one of the main causes of tumor formation and is considered to be an early event in this process. The marker ki67 recognizes a proliferation related nuclear antigen present at G1, G2 , S and M phases of cell cycle.[10]

Histopathology is the gold standard for diagnosing Oral squamous intraepithelial lesions, yet there can be discrepancies due to interobserver agreement or differences. Oral epithelial dysplasia (OED) has been classified in a variety of ways, including by the World Health Organization (WHO) in 2005, which divided OED into mild, moderate, severe, and Carcinoma in Situ. Smith and Pindberg described the first dysplasia categorization system for oral mucosa, which Katz et al. eventually adopted. The World Health Organization (WHO) recently classified OED as low and high degree dysplasia, which was used in this study.

The study has been undertaken with the aim to evaluate the expression of p53 and ki67 markers as potential biomarkers for the histological grading and differentiation in oral premalignant lesions and OSCC by Immunohistochemistry. Hence, this study emphasizes the potential use of these cell cycle regulatory proteins as markers of malignant transformation in Oral Premalignant lesions and may serve as prognostic tools in early detection of Oral Squamous Cell Carcinoma in Cancer Prevention Programs.

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MATERIALS AND METHODS

All Histopathological specimens of Oral Premalignant lesions and Invasive squamous cell carcinoma of the oral cavity received at the Department of pathology, Dr B.R Ambedkar Medical College and hospital between August 2019 to June 2021 were studied prospectively. A total of 55 cases were received and analysed during this study period including oral premalignant lesions and Oral Squamous Cell Carcinoma cases

Inclusion Criteria

All oral biopsy specimens of oral premalignant lesions and radical resection specimens from patients with oral squamous carcinoma of age >18 were included.

Exclusion Criteria

1. Primary malignancies of the oral cavity other than Oral squamous cell carcinoma.
2. Recurrence and metastatic tumors of the oral cavity.
3. Cases with extensive tumor necrosis without sufficient viable tissue for accurate evaluation of Immunohistochemistry results.

After taking an informed consent from the patient in his /her own language, detailed clinical history and results of relevant investigations were collected from patient's case files.

Oral biopsy Specimens were received in Pathology Department in 10% formalin. In every case the standard protocol for surgical grossing of resection specimens was followed.

Specimen were kept for fixation for 24 hours. After a detailed specimen description, multiple sections were taken as per the standard protocol from the viable sections of the tumor including surgical margins. After conventional processing, the specimen were embedded in paraffin wax, sections of 4-5µm thickness were coated cut using Leica RM 2125 and stained using haematoxylin and eosin for histopathological study. In addition, 4µm sections were cut from paraffin blocks of tissue and taken on 2 Poly-L-Lysine glass slides for further Immunohistochemical analysis to detect P53 and Ki-67 expression. The percentage, intensity and pattern of expression was evaluated in each marker

Evaluation of Staining

The strength of immunohistochemical staining was evaluated as negative (-, no color), mild (+, light brown color), moderate (++, dark brown color), or intense (+++, very dark brown color) based on subjective evaluation of color exhibited (brown color) by antigen, antibody, and chromogen complex.

The staining was classified as only confined to the basal layer, both basal and supra basal layers, and all epithelial layers. Only nuclear staining of epithelial cells was detected, and nuclei with a distinct brown color were considered positive, independent of staining intensity. The pattern of expression was also analyzed semi quantitatively by counting the number of positive cells per 100 basal or parabasal cells and was recorded as percentage.

All the samples histopathologically and immunohistochemically studied were categorized into two groups such as oral premalignant group and OSCC group. The parameters used to analyze the expression of both p53 protein and ki-67 antigen are: 1) pattern or distribution of expression in the epithelial layers; 2) intensity of staining in each slide; 3) the percentage of positive cells or labeling index (LI).

Statistical Analysis: Descriptive and inferential statistical analysis has been carried out in the present study. Results on continuous measurements are presented on Mean SD (Min-Max) and results on categorical measurements are presented in Number (%). Significance is assessed at 5 % level of significance.

Chi-square/ Fisher Exact test has been used to find the significance of study parameters on categorical scale between two or more groups, non-parametric setting for Qualitative data analysis.

The calculated data was entered with excel sheets as study variables and were qualitative in nature. All data were analyzed with SPSS (Statistical Package for the Social Sciences) software.

RESULTS

This prospective study was conducted in the Department of Pathology, Dr B R Ambedkar Medical College and Hospital, Bangalore during August 2019 to June 2021. A total of 55 cases were received and analysed during this study period including oral premalignant lesions and Oral Squamous Cell Carcinoma cases.

Descriptive and inferential statistical analysis has been carried out in the present study. Results on continuous measurements are presented on Mean SD (Min-Max) and results on categorical measurements are presented in Number (%). Significance is assessed at 5 % level of significance.

Chi-square/ Fisher Exact test has been used to find the significance of study parameters on categorical scale between two or more groups, non-parametric setting for Qualitative data analysis.

Table 1: Age distribution of patients studied

Age in years	No. of patients	%
30-40	7	12.7
41-50	13	23.6
51-60	21	38.2
61-70	10	18.2
>70	4	7.3
Total	55	100.0

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Mean ± SD: 55.67±12.32

In the present study, majority of the cases were between the 51-60 years of age group with a mean age of 55.67±12.32.

Table 2: Gender distribution of patients studied

Gender	No. of patients	%
Female	22	40.0
Male	33	60.0
Total	55	100.0

Table 3: Distribution of sites effected in the oral cavity in relation to gender

Site	Gender		Total
	Female	Male	
TONGUE	11(50%)	19(57.6%)	30(54.5%)
BUCCAL	10(45.5%)	9(27.3%)	19(34.5%)
LIP	1(4.5%)	3(9.1%)	4(7.3%)
MAXILLARY ALVEOLUS	0(0%)	2(6.1%)	2(3.6%)
Total	22(100%)	33(100%)	55(100%)

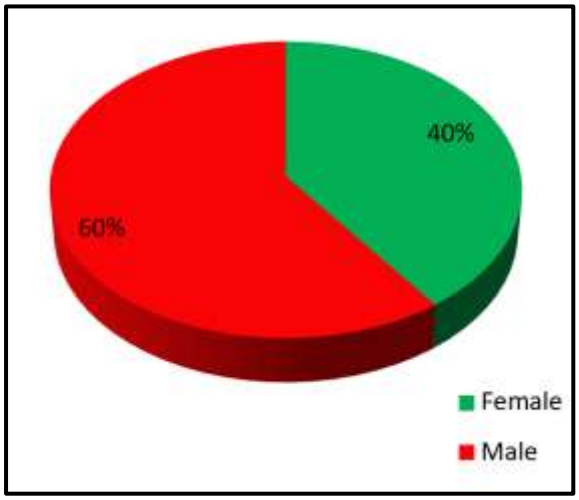

Figure 1: Gender distribution of patients studied

In the present study, the majority of the cases belonged to Male i.e 60 % and Female were 40% with a male: female ratio of 1.5:1.

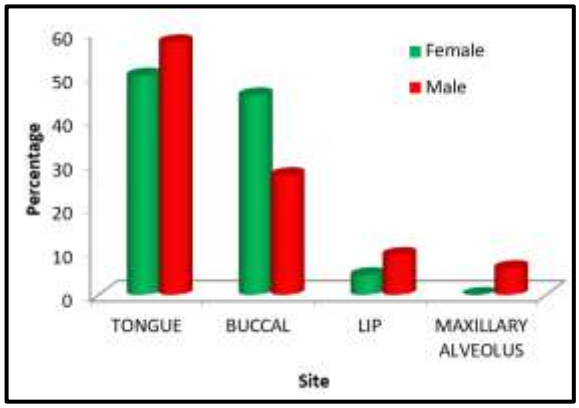

Figure 2: Distribution of sites effected in the oral cavity in relation to gender

In the present study, the most common site of lesion was Tongue seen in 30 cases (54.5%) and the next most common location was found to be buccal mucosa seen in 19 cases (34.5%). In males (57.6%) and females (50%) the most common location was Tongue.

Table 4: Frequency of premalignant and malignant lesions of oral cavity in the present study population

Histopathological Diagnosis	No. of patients	%
ORAL SUBMUCOUS FIBROSIS	2	3.6%
LEUKOPLAKIA	4	7.3%
LEUKOPLAKIA WITH ATYPIA	2	3.6%
LOW GRADE DYSPLASIA	6	10.9%
HIGH GRADE DYSPLASIA	4	7.3%
WDOSCC	22	40.0
MDOSCC	12	21.8
PDOSCC	3	5.5
Total	55	100.0

Table 5: Site involved in relation to Histopathological Diagnosis of patients studied.

Site	Histopathological Diagnosis				Total
	Premalignant	WDOSCC	MDOSCC	PDOSCC	
Tongue	8(44.4%)	9(40.9%)	11(91.7%)	2(66.7%)	30(54.5%)
Buccal mucosa	7(38.9%)	10(45.5%)	1(8.3%)	1(33.3%)	19(34.5%)
Lip	3(16.7%)	1(4.5%)	0(0%)	0(0%)	4(7.3%)
Alveolus maxilla	0(0%)	2(9.1%)	0(0%)	0(0%)	2(3.6%)
Total	18(100%)	22(100%)	12(100%)	3(100%)	55(100%)

Out of the total 55 cases in the present study, 18 cases (32.7%) belonged to Premalignant lesions, 22 cases (40%) were Well differentiated oral squamous cell carcinoma, 12 cases (21.8%) were Moderately

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differentiated oral squamous cell carcinoma and 3 cases (5.5 %) were belonging to Poorly differentiated oral squamous cell carcinoma.

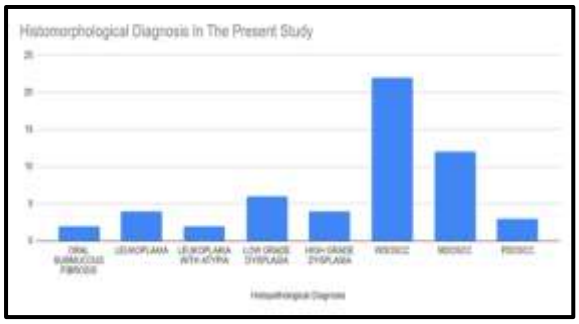

Figure 3: Frequency of premalignant and malignant lesions of oral cavity in the present study population

In the present study, the most common location in Premalignant lesions i.e 8 cases (44.4%) was Tongue. The most common location in Well differentiated squamous cell carcinoma was Buccal mucosa seen in 10(45.5%) cases. In moderately differentiated squamous cell carcinoma and poorly differentiated carcinoma the most common location of the lesion was found to be tongue 11(91.7%) cases and 2 (66.7%) cases respectively.

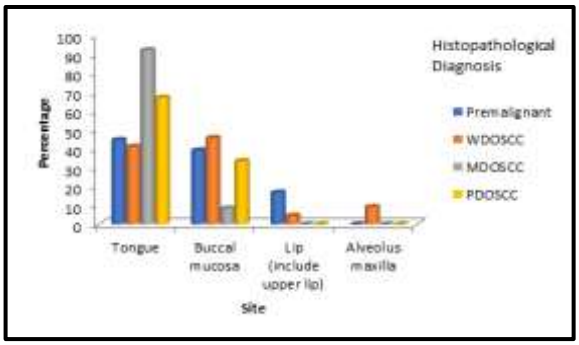

Figure 4: Site involved in relation to Histopathological Diagnosis of patients studied

Table 6: Distribution of histomorphological diagnosis in relation to gender

Histomorphological Diagnosis	Gender		Total
	Female	Male	
ORAL SUBMUCOUS FIBROSIS	1(4.5%)	1(3%)	2(3.6%)
LEUKOPLAKIA	3(13.6%)	1(3%)	4(7.3%)
LEUKOPLAKIA WITH ATYPIA	0(0%)	2(6.1%)	2(3.6%)
LOW GRADE DYSPLASIA	1(4.5%)	5(15.2%)	6(10.9%)
HIGH GRADE DYSPLASIA	1(4.5%)	3(9.1%)	4(7.3%)
WDOSCC	10(45.5%)	12(36.4%)	22(40%)
MDOSCC	5(22.7%)	7(21.2%)	12(21.8%)
POOR OSCC	1(4.5%)	2(6.1%)	3(5.5%)
Total	22(100%)	33(100%)	55(100%)

In the present study, low grade dysplasia was the most common oral premalignant lesion in males 5(15.2%) and Leukoplakia was the most common lesion in females 3(13.6%). The most common malignant lesion of the oral cavity in males and females was Well differentiated OSCC i.e 12(36.4%) and 10(45.5%) respectively.

Statistical analysis of immunohistochemical expression of P53 and KI67 in oral premalinant and malignant lesions.

Table 7: P53 %, intensity and pattern of staining in relation to Histopathological Diagnosis of patients studied

P53 details	Histopathological Diagnosis				Total (n=55)	P value
	Premalignant (n=18)	WDOSCC (n=22)	MDOSCC (n=12)	PDOSCC (n=3)		
P53%						
0-25%	13(72.2%)	4(18.2%)	1(8.3%)	0(0%)	18(32.7%)	0.006**
26-60%	5(27.8%)	18(81.8%)	11(91.7%)	0(0%)	34(61.8%)	
61-100%	0(0%)	0(0%)	0(0%)	3(100%)	3(5.5%)	
P53 Intensity						
0	8(44.4%)	2(9.1%)	1(8.3%)	0(0%)	11(20%)	0.065+
2+	2(11.1%)	2(9.1%)	3(25%)	0(0%)	7(12.7%)	
3+	8(44.4%)	18(81.8%)	8(66.7%)	3(100%)	37(67.3%)	
P53 Pattern						
0	8(44.4%)	2(9.1%)	1(8.3%)	0(0%)	11(20%)	<0.001**
Periphery	0(0%)	19(86.4%)	11(91.7%)	0(0%)	30(54.5%)	
Basal+suprabasal	7(38.9%)	0(0%)	0(0%)	0(0%)	7(12.7%)	
Diffuse	0(0%)	1(4.5%)	0(0%)	3(100%)	4(7.3%)	
Upper 2/3rd	3(16.7%)	0(0%)	0(0%)	0(0%)	3(5.5%)	

Chi-Square/Fisher Exact Test

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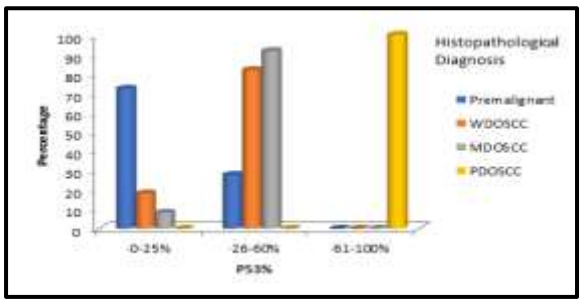

Figure 5: P53 % of staining in relation to Histopathological Diagnosis of patients studied

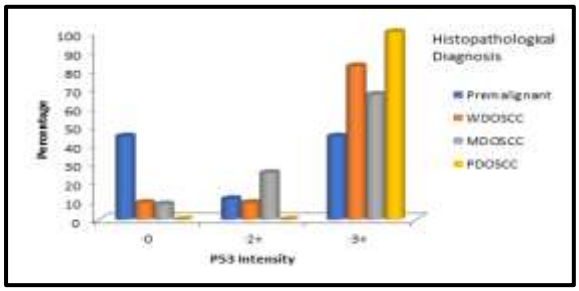

Figure 6: P53 intensity of staining in relation to Histopathological Diagnosis of patients studied

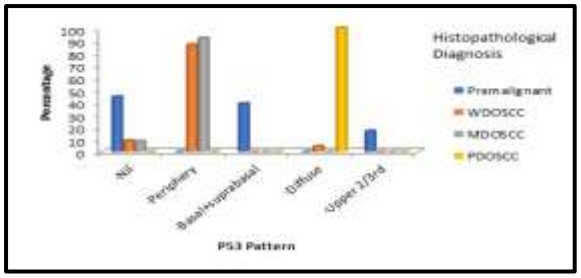

Figure 7: P53 pattern of staining in relation to Histopathological Diagnosis of patients studied

The highest expression of P53 % was seen in PDOSCC with all the three cases showing 100% expression followed by MDOSCC (91.7%) and then WDOSCC (81.8%). Two cases of WDOSCC and one case of MDOSCC did not show any staining for P53. A gradual increase in the p53% was observed from premalignant lesions to OSCC with 0-25% LI observed in 13 cases (72.2%) of premalignant lesions and within the different histological grades of OSCC the p53% was found to have increased from WDOSCC to PDOSCC. 26-60% of cells expressing p53 were observed in 18(81.8%) cases of WDOSCC, 11 (91.7%) cases of MDOSCC and few of the premalignant lesions i.e 5 (27.8%) cases which mainly involved the dysplastic lesions and this was statistically significant with a p value = 0.006.

The highest intensity of p53 expression i.e 3+ was observed in 37 cases (67.3 %) followed by 2+ observed in 7(12.7%) cases and no expression was observed in 11(20%) of the cases. The intensity of p53 expression was observed to have increased from premalignant lesions to OSCC and this showed moderate statistical significance.

The pattern of staining of P53 was seen in suprabasal and basal layers in majority of the premalignant lesions i.e 7 (38.9%) cases while 3 (16.7%) cases showed upper two-thirds pattern of staining mainly belonging to high grade dysplasia cases. 19 (86.4%) cases of WDOSCC and 11(91.7%) cases of MDOSCC showed predominantly peripheral pattern of staining in the islands of malignant cells. Two cases of WDOSCC and one case of MDOSCC did not show any staining with P53 and hence no pattern was observed. All the 3 cases (100%) of PDOSCC showed diffuse pattern of staining with P53.

Table 8: KI 67%, intensity and pattern of staining in relation to Histopathological Diagnosis of patients studied

KI67 details	Histopathological Diagnosis				Total (n=55)	P value
	Premalignant (n=18)	WDOSCC (n=22)	MDOSCC (n=12)	PDOSCC (n=3)		
KI67%						
0-25%	10(55.6%)	6(27.3%)	1(8.3%)	0(0%)	17(30.9%)	0.007**
26-60%	8(44.4%)	16(72.7%)	11(91.7%)	2(66.7%)	37(67.3%)	
61-100%	0(0%)	0(0%)	0(0%)	1(33.3%)	1(1.8%)	
KI67 Intensity						
0	2(11.1%)	1(4.5%)	1(8.3%)	0(0%)	4(7.3%)	0.863
2+	4(22.2%)	3(13.6%)	3(25%)	0(0%)	10(18.2%)	
3+	12(66.7%)	18(81.8%)	8(66.7%)	3(100%)	41(74.5%)	
KI67 Pattern						
0	2(11.1%)	1(4.5%)	0(0%)	0(0%)	3(5.5%)	<0.001**
PERIPHERY	0(0%)	21(95.5%)	11(91.7%)	0(0%)	32(58.2%)	
BASAL	6(33.3%)	0(0%)	1(8.3%)	0(0%)	7(12.7%)	
BASAL+SUPRABASAL	7(38.9%)	0(0%)	0(0%)	0(0%)	7(12.7%)	
DIFFUSE	0(0%)	0(0%)	0(0%)	3(100%)	3(5.5%)	
UPPER 2/3RD	3(16.7%)	0(0%)	0(0%)	0(0%)	3(5.5%)	

Chi-Square/Fisher Exact Test

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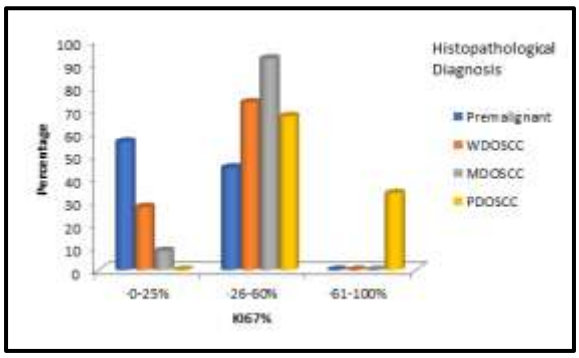

Figure 8: KI 67% of staining in relation to Histopathological Diagnosis of patients studied

In the present study, majority of premalignant lesions, 10(55.6%) cases showed ki67 % expression between 0-25% and 8(44.4%) cases showed ki67% between 26-60%. Most of the cases of WDOSCC 16(72.7%) and MDOSCC 11(91.7%) showed KI67% in the range of 26-60% with 2 (66.7%) cases of PDOSCC. 61-100% of ki67 staining was observed in 1(33.3%) case of PDOSCC. One case of WDOSCC did not show any staining.

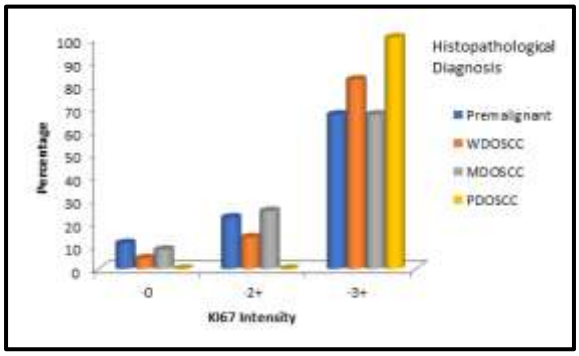

Figure 9: KI 61 intensity of staining in relation to Histopathological Diagnosis of patients studied

The highest intensity of ki67 expression i.e 3+ was observed in 3 cases of PDOSCC (100%), 8 cases of MDOSCC (66.7%) ,18 cases of WDOSCC (81.8%) and 12 cases of premalignant lesions (66.7%). Ki67 intensity of staining of 2+ observed in 3 (13.6%) cases of WDOSCC and 3(25%) cases of MDOSCC and 4(22.2%) cases of premalignant lesions. The intensity of p53 expression was observed to have increased from premalignant lesions to OSCC, however this did not show statistical significance.

7(12.7%) cases showed basal pattern of staining for ki67 and 7(12.7%) cases showed basal with suprabasal pattern of staining which belonged to Premalignant lesions. 21(95.5%) cases of WDOSCC and 11(91.7%) cases of MDOSCC showed peripheral pattern of staining in islands of malignant cells for ki67. All the 3(100%) cases of PDOSCC showed diffuse pattern of staining for ki67. One case of WDOSCC did not show any staining with KI67 and hence no pattern was observed. The pattern of staining in premalignant lesions progressed from non-dysplastic lesions (basal only) to dysplastic lesions (basal +suprabasal and upper two-thirds) and gradually progressed from peripheral pattern of staining in WDOSCC and MDOSCC to diffuse pattern of staining in PDOSCC. This was found to be statistically significant with a P value <0.001.

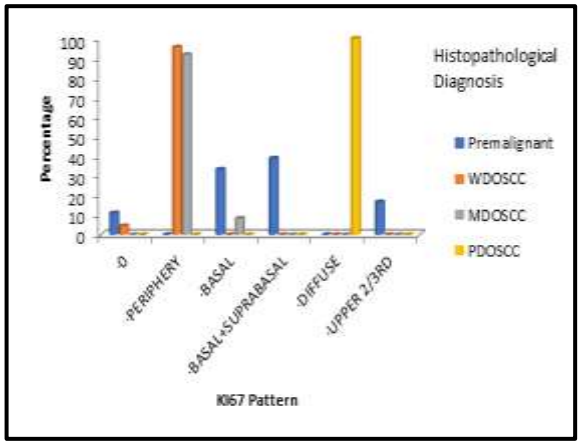

Figure 10: KI 67 pattern of staining in relation to Histopathological Diagnosis of patients studied

Table 9: Frequency distribution of LI score p53 and LI score Ki-67 in relation to Histopathological Diagnosis of patients studied

	Histopathological Diagnosis				Total (n=55)	P value
	Premalignant (n=18)	WDOSCC (n=22)	MDOSCC (n=12)	PDOSCC (n=3)		
LIS Score P53						
0	1(5.6%)	1(4.5%)	0(0%)	0(0%)	2(3.6%)	<0.001**
1	12(66.7%)	3(13.6%)	1(8.3%)	0(0%)	16(29.1%)	
2	4(22.2%)	18(81.8%)	11(91.7%)	0(0%)	33(60%)	
3	1(5.6%)	0(0%)	0(0%)	3(100%)	4(7.3%)	
LIS Score Ki67						
1	10(55.6%)	5(22.7%)	1(8.3%)	0(0%)	16(29.1%)	0.013*
2	8(44.4%)	17(77.3%)	11(91.7%)	0(0%)	36(65.5%)	
3	0(0%)	0(0%)	0(0%)	3(100%)	3(5.5%)	

Chi-Square/Fisher Exact Test

The labelling index score of P53 staining in relation to histopathological diagnosis of patients was found to be statistically significant with a P value <0.001

The labelling index score of KI67 staining in relation to histopathological diagnosis of patients was found to be statistically moderately significant with a P value= 0.013.

The labelling index of P53 and Ki67 was observed to have increased from Premalignant lesions to Oral squamous cell carcinoma and within OSCC it was

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observed to have increased from WDOSCC to PDOSCC.

Table 10: Comparison of Mean of P53% and Ki67 % with Histopathological diagnosis

Variables	Histopathological Diagnosis				Total	P value
	Premalignant	WDOSCC	MDOSCC	PDOSCC		
P53 %	17.78±20.45	27.95±10.07	41.67±14.03	73.33±5.77	30.09±19.96	<0.001**
KI67%	21.38±14.53	29.09±10.65	38.33±10.29	66.67±11.54	30.63±15.87	<0.001**

In the present study, the mean of P53% and Ki67 % with the Histopathological diagnosis was observed to be statistically significant with P values <0.001.

Table 11: Comparison of Co-expression of P53 and Ki67 with LI scores in the present study

LI SCORE- P53	LI SCORE- KI67			Total
	1	2	3	
0	2(12.5%)	0(0%)	0(0%)	2(3.6%)
1	11(68.8%)	5(13.9%)	0(0%)	16(29.1%)
2	3(18.8%)	30(83.3%)	0(0%)	33(60%)
3	0(0%)	1(2.8%)	3(100%)	4(7.3%)
TOTAL	16(100%)	36(100%)	3(100%)	55(100%)

P≤0.001**, Significant, Fisher Exact Test

Table 12: Comparison of Co-expression of P53 and Ki67 with intensity of staining in the present study

P53 Intensity	KI67 INTENSITY			Total
	0	2+	3+	
0	3(75%)	4(40%)	4(9.8%)	11(20%)
2+	0(0%)	2(20%)	5(12.2%)	7(12.7%)
3+	1(25%)	4(40%)	32(78%)	37(67.3%)
Total	4(100%)	10(100%)	41(100%)	55(100%)

P=0.007**, Significant, Fisher Exact Test

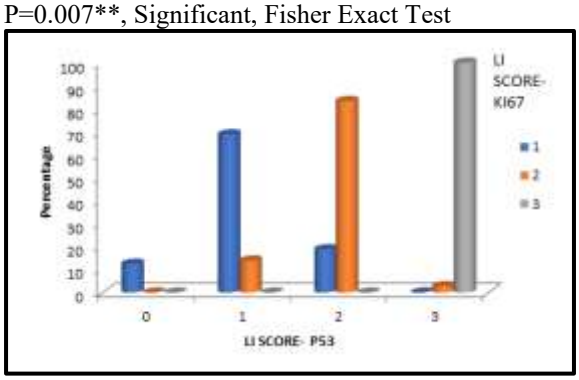

Figure 11: Comparison of Co-expression of P53 and Ki67 with LI scores in the present study

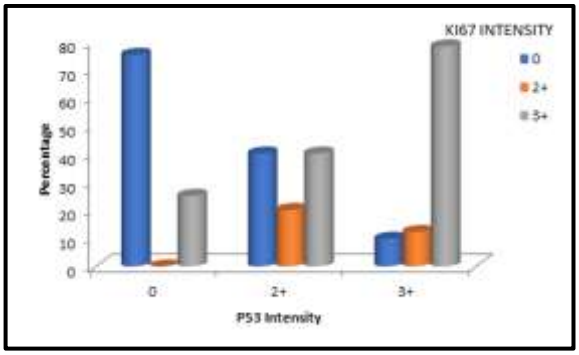

Figure 12: Comparison of Co-expression of P53 and Ki67 with intensity of staining in the present study

In the present study, the Co-expression of P53 and Ki67 with LI scores was found to be statistically significant using Fischer exact test with P value < 0.001

The correlation of intensity of staining by P53 and KI67 was found to be statistically significant with a P value = 0.007.

Table 13: PEARSON CORRELATION between P53 and Ki67

Pearson correlation	R value	P value
P53 VS KI67	0.830	<0.001**

In the present study, the Pearson correlation between P53 and Ki67 showed an R value = 0.83 which is statistically significant with P value < 0.001.

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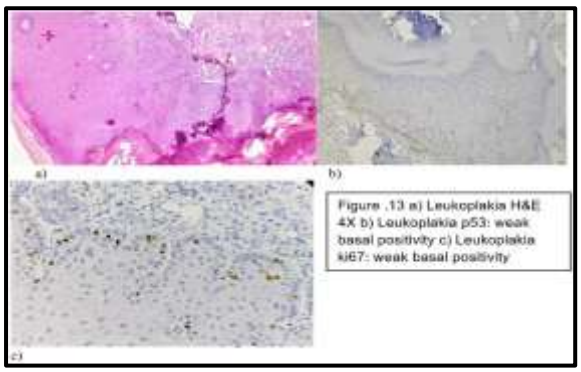
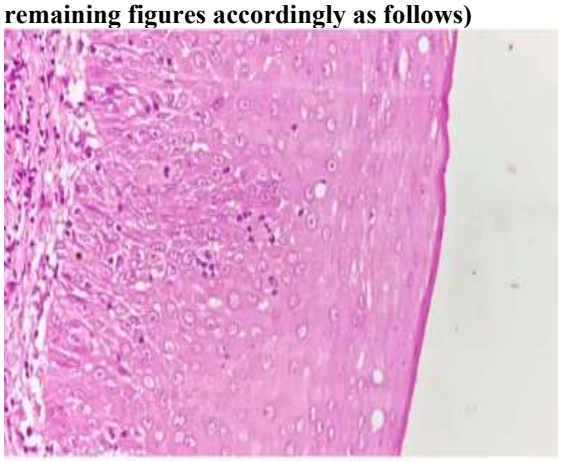
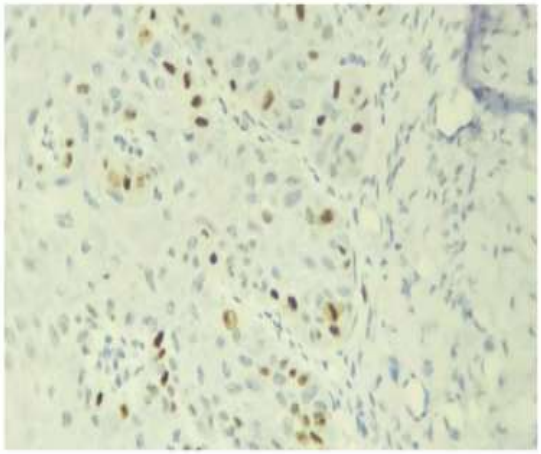


Figure. 13 a) Leukoplakia with atypia: showing hyperplastic stratified squamous epithelium exhibiting atypia in the basal layers (H & E, 100X)
b) Leukoplakia with atypia : showing p53 nuclear staining in the basal layer of the epithelium (IHC p53, 100X) c) Leukoplakia with atypia : showing Ki-67 nuclear staining in the basal layer of the epithelium (IHC ki67, 400X)

Figure. 14: Low grade dysplasia figures and text to be included from word document attached (kindly change the numbers and text of the remaining figures accordingly as follows)





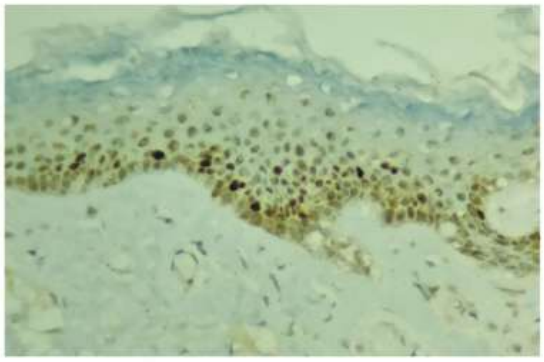


Figure .14:a) Low grade dysplasia: showing stratified squamous epithelium exhibiting dysplasia in the lower one-third (H & E, 400X) b) Low grade dysplasia : showing p53 nuclear staining in the basal and suprabasal layers of the epithelium (IHC P53, 400X) c) Low grade dysplasia : showing ki67 nuclear staining in the basal and suprabasal layers of the epithelium (IHC ki67, 400X)

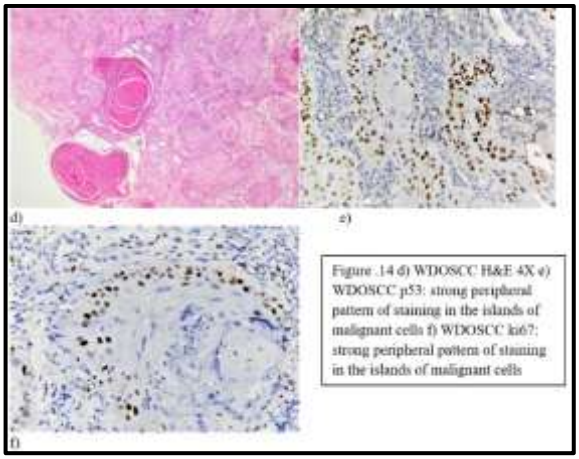


Figure.15: a) Well differentiated OSCC: showing islands and nest of tumor cells exhibiting pleomorphism with keratin pearls (H & E, 100X)
b) Well differentiated OSCC: showing islands and nest of tumor cells exhibiting pleomorphism with keratin pearls (H & E, 100X)
c) Well differentiated OSCC : showing ki-67 nuclear staining in the periphery of tumor islands (IHC ki67, 400X)

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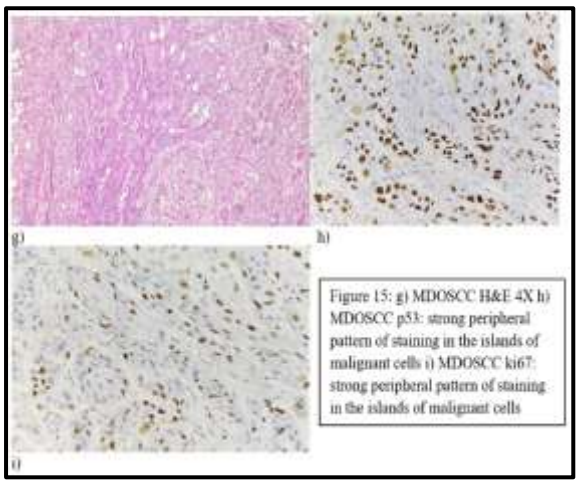

Figure. 16: a) Moderately differentiated OSCC: showing infiltrating tumor cells exhibiting distinct nuclear pleomorphism with focal keratinization and indistinct intercellular bridges (H & E, 100X)
b) Moderately differentiated OSCC: showing p53 nuclear staining in the periphery and part of center of tumor islands (IHC P53, 400X)
c) Moderately differentiated OSCC: showing ki67 nuclear staining in the periphery and part of center of tumor islands (IHC ki67, 400X)

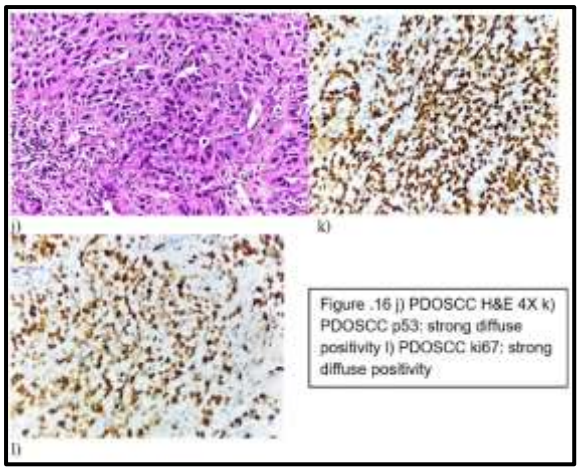

Figure. 17: a) Poorly differentiated OSCC: showing infiltrating tumor cells exhibiting marked nuclear atypia with no/minimal keratinization (H & E, 100X) b) Poorly differentiated OSCC: showing diffuse and intense p53 nuclear staining (IHC P53, 400X) c) Poorly differentiated OSCC: showing diffuse and intense ki67 nuclear staining (IHC ki67, 400X

DISCUSSION

Many studies have been conducted to evaluate the expressions of cell cycle regulatory genes and cell proliferative activity in pre malignant and malignant oral squamous cell lesions both globally and in India due to increased oral cancer burden worldwide and in India. It is one of the most common cancers in South East Asia, and in India the incidence is as high as one third of all malignancies.

In the present study, out of 55 oral cavity specimens received in the Department of Pathology, 18 cases were oral premalignant lesions and 37 cases were of Oral squamous cell carcinoma.

The mean age in this study was 55.67 years, and the peak incidence was in the age bracket of 51-60 years, followed by 41-50 years, which is consistent with studies like Saranath D et al and S. Humayun et al where the mean age was 46 and 40.5 respectively. A study conducted by Kurokawa et al showed mean age as 60.7.[14-16]

Out of all the cases under study 33 (60 %) cases were seen in males and 22 (40 %) cases were seen in females with M: F ratio of 3:2 Showing a male preponderance Similarly, male preponderance was seen in Oral premalignant and OSCC in the studies done by Panjwani S, Verma R et al and Singh S et al while a study by Azizi SA et al showed female preponderance.[17-19]

In the present study, the commonest site of OSCC and Premalignant lesions was the tongue constituting 66.43% and 44.4% respectively. Studies by Azizi SA, Abdulkadir SN, Dave KV (48.71%) and Kumar GK et al (36.86%) the commonest site involved was tongue. Studies done by Saranath et al (48.19%), Verma R et al (43.3%) and Bettendorf O, buccal mucosa was the common site to be involved in OSCC and studies like Singh S et al and Maheshvari V et al and Kumar GK et al (33.01%) showed that in Oral Premalignant lesions, buccal mucosa was the most common site of lesion.[19-22]

Out of 55 cases evaluated in the present study, 37 (67.3%) of the cases belonged to OSCC group and 18 (32.7%) cases belonged to premalignant lesions. This correlated with study conducted by Patel at al in which OSCC constituted 68% of all the lesions studied, 31% of premalignant lesions.[23]

Another study conducted by Owais Gowhar et al on 200 oral biopsies, the most frequent lesions belonged to premalignant lesions (43.3%) followed by Malignant lesions (13.3%).[24]

Within the OSCC cases, the most frequent histopathological grade was well differentiated OSCC (40%) followed by moderately differentiated (21.8%) and least was poorly differentiated (5.5%). Similar frequency of histological grades of OSCC was seen in studies conducted by Bettendorf O. and Herrmann G. et al, Khanna S et al and Juneja S et al while in study by Abdulkhadir SN et al the most frequent histopathological grade was moderately differentiated OSCC.[22,25-27]

In the present study, the mean of the Labelling index of p53 was found to have increased from premalignant lesion to OSCC and this finding was comparable to study by Pandya JA et al. Within the different grades of OSCC in the present study the labelling index increased from WDOSCC (27.9%) to MDOSCC (41.6%) and PDOSCC (73.3%). This was comparable to study by Pandya JA et al where the Labelling index was increased from WDOSCC (42.52%) TO MDOSCC (48.88%) and highest was observed in PDOSCC (70.28%) and was also comparable to other studies like Ayaz Mahmood Dar et al and Verma R et al.[18,28,29]

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In the present study, the mean of the Labelling index of Ki-67 was found to have increased from premalignant lesion to OSCC and this finding was comparable to study Maheshwari V et al, Dwivedi N et al and Takkem A et al. Within the different grades of OSCC in the present study the labelling index of Ki-67 increased from WDOSCC (29%) to MDOSCC (38.3%) and PDOSCC (66.6%). This was comparable to study by Maheshwari V et al where the Labelling index was increased from WDOSCC (48.52%) TO MDOSCC (52.23%) and highest was observed in PDOSCC (58.55%).[30-32]

In the dysplastic lesions in the present study, P53 staining was observed in basal and suprabasal in low grade dysplasia while it involved the upper two-thirds of the epithelium in high grade dysplasia. This is in accordance with study by Pandya JA et al where P53 nuclear staining was evident principally in basal cell layer and suprabasal cell layer, extending till two-third of the epithelium in low grade dysplasia and high grade dysplasia respectively.

Overexpression of inactivated or mutated forms of p53 in oral epithelial dysplasia has been associated with high risk for transformation to early stage OSCC.[29]

Cruz et al. showed that suprabasal p53 immunoexpression patterns are associated with high grades of dysplasia and correlate with progress to oral squamous cell carcinoma.33 Hence the present study was comparible with other studies with respect to pattern of staining in low grade and high grade oral epithelial lesions.

In the present study, P53 staining in well differentiated OSCC and moderately differentiated OSCC was observed in the periphery of the tumor islands and periphery with part of center of tumor respectively and in poorly differentiated OSCC it was diffuse expression of P53, this is accordance to study conducted by Pandya JA et al.[29]

p53 expression was noted in basal and suprabasal cell level as epithelial cells change from normal to dysplastic cells and later to frank carcinoma, thus suggesting a progressive accumulation of mutational errors of TP53 protein.

In the present study, majority of cases of OSCC showed positivity for P53 while 2 cases of well differentiated OSCC and 1 case of moderately differentiated OSCC showed no expression of P53.

Kaur et al. showed that cases with missense mutations in TP53 showed accumulation of protein in premalignant and malignant oral lesions, while those exhibiting nonsense mutations and frameshift mutations did not show detectable levels of TP53 protein.[34]

Hence this could be the reason for negative staining of p53 in 2 cases of well differentiated OSCC and 1 case of moderately differentiated OSCC.

Using the Fisher Exact Test and the Pearson correlation, a positive and substantial association between LI of p53 and Ki-67 was discovered, with a R value of 0.83 in Pearson correlation that was statistically significant with P values 0.001. This suggests that in oral premalignant lesions and OSCC, there is an increase or decrease in p53 LI with an increase or decrease in Ki-67. Nasser et al,[35] Cruz et al,[33] and Kerdpon et al,[36] have all made similar observations. When p53 and ki-67 were expressed separately, Nasser et al showed that they had limited positive predictive value, but their combined expression might help distinguish between dysplastic and non-dysplastic lesions.

The co-expression and connection of p53 and Ki-67 in oral SCC has been reported, implying that changes in p53 cause enhanced cell proliferation and that its co-expression in dysplastic lesions is a marker of cancer progression. In our investigation, p53 and Ki-67 were shown to be co-expressed in 80% of cases, which is comparable to Raju et al,[37] and Patel et al,[23] findings of 80% and 94.87 percent, respectively.

p53 exhibited a good association with tumor progression with significant p values for all the parameters (LI, Intensity and pattern = 0.006, 0.06, <0.001 respectively.)

Ki-67 also exhibited a good association with tumor progression with significant p values for ki-67 LI and pattern of staining (LI and pattern = 0.007, <0.001 respectively.)

Thus, expression of p53 and Ki-67 was reported to grow from Oral Premalignant lesions to OSCC, and within OSCC, from WDOSCC to PDOSCC. Changes in p53 may contribute to enhanced cellular proliferation in premalignant lesions, according to the substantial association between p53 and Ki-67 found in our study. Furthermore, p53 expression was shown to be substantially linked with Ki-67, implying that a mutation in the p53 gene product aided cellular proliferation.

CONCLUSION

One of the most common findings in head and neck cancers, particularly in the oral cavity, is the expression of the p53 and Ki-67 genes. Early in carcinogenesis, p53 mutations can develop, and these mutations are sustained as the cancer progresses to a more advanced stage. Ki-67 is a marker for cell proliferation, which is a key factor in cancer development. Ki-67 and p53 protein overexpression were shown to be associated with increased OSCC histologic grades in the current study. The findings of this study imply that mutations in the p53 and Ki-67 genes may have a role in oral carcinogenesis. The considerable difference in p53 and Ki-67 expression between different histological grades of OSCC shows that both p53 and Ki-67 are more predictive in detecting early alterations in OSCC.

Large-scale studies may shed light on OSCC's possible behavior and clinical course, as well as cancer treatment and prognosis.

As a result, the expression of the p53 protein, as well as Ki-67, may aid in assessing prognosis and play a critical role in preventing genome harm; these genetic

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targets can be used for gene therapy in oral malignancies. As a result, p53 and Ki-67 may be useful tumor biomarkers for diagnosing Oral Squamous Cell Carcinoma as well as determining its severity, aggressiveness, and prognosis. It can also aid in the development of treatment options and the patient's survival.

These antigens' increasing expression and the shift of the oral epithelium from premalignant to malignant stages suggest that they could be useful biomarkers of malignant transformation in oral precancerous lesions and could be employed as intermediate points in cancer prevention programs.

Conflict of interest: None

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