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A Study of P53 Expression and its Correlation with Grades of Oral Squamous Cell Carcinoma

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Abstract: ***Aims: this study is an attempt to analyze the proportion of P53 expression among the histopathological samples of oral squamous cell carcinoma received at pathology department, to determine any correlation of P53 expression with grades of OSCC and to determine any relation between expression of P53 with demographic (age, gender) and clinicopathological (location) variables of OSCC. Methodology: it is a cross-sectional study with sample size of 55 patients. Inclusion criteria consisted of specimens who are histologically diagnosed as carcinomas of oral cavity which included biopsies and resection specimen of hemiglossectomy / mandibulectomy with neck dissection. Results and conclusion: most of the patients of oral cavity cancer are males (3:1) and most of the patients were belonging to 6th decade. The most common site of tumor was buccal mucosa (37%) followed by tongue (31%). Majority of the patients (57%) were having moderately differentiated squamous cell carcinoma. 85% of cases were positive for P53 and it was found that P53 expression increases with severity of grades of OSCC that is higher P53 expression in poorly differentiated cases. Hence P53 can be included in the routine immunohistochemistry panel. It was found that the age of patient and grade of tumor had significant correlation and strong association was found with % of immunostaining of P53. Hence, P53 may have predictive and prognostic implications in the squamous cell carcinoma of oral cavity.***

Keywords: OSCC, Oral Squamous Cell, Carcinoma

1. Introduction

Oral cancer is the sixth most common cancer worldwideand third most common cancer in developing countries accounting for about 40% of all cancers [(1).] P53 mutations are associated with the development of oral squamous cell carcinomas [(2).]

The etiology of oral squamous cell carcinoma 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 squamous cell carcinoma. [3]

The behaviour of squamous cell carcinoma of oral cavity is difficult to predict solely using conventional clinical and histopathological parameters and due to location of the disease, the multimodal tumour therapy usually prescribed leads to a reduction in quality of life, making the psychosocial consequences of OSCC greater than other malignancies. For these reasons, despite advances in therapeutic strategies, the survival rate of OSCC is still poor. [4]

Tumour suppressor genes, oncogenes, cell proliferation markers, angiogenic markers and cell adhesion molecules have been studied as potential tools to predict the prognosis of patients with OSCC [5].

The p53 is a tumour suppressor gene which has proved to be an important molecule in the development of many tumors. It is located on the short arm of chromosome 17 and encodes a protein of 393 amino acids. The physiological function of p53 protein is that of preventing accumulation of genetic damage in cells either by allowing for repair of damage before cell division or by causing death of the cells. It can be inactivated through various genetic events such as mutation, loss of heterozygosity leading to the loss of tumour suppressor function of protein [6]. The most common p53 alteration is a point mutation confined primarily to exons 5 to 8. This alteration of p53 impairs the ability of the cells to repair and undergo apoptosis leading to uncontrolled cell growth.

Mutation of p53 gene confers single greatest selective advantage favouring cancer formation. [7] Alteration in this gene or inactivation of the wild type of gene product is thought to play an important role in multistep carcinogenesis [8]

Normal p53 protein has a very short half life of 6-20 minutes making it hard to detect in normal tissues. But an altered protein has a half life of about 6 hours so that it can be detected in premalignant and malignant tissue through immunohistochemistry [9]

Aims and Objectives

1) To determine the proportion of p53 expression among the histopathological samples of oral squamous cell carcinoma received at Pathology department.
2) To determine any correlation of p53 expression with grade of oral squamous cell carcinoma.
3) To determine any relation between expression of p53 with demographic (age, gender) and clinicopathological (location) variables of oral squamous cell carcinoma.

2. Materials and Methods

This was **cross-sectional** study to be conducted in Department of Pathology in Dr. S. N. Medical College, Jodhpur.

Inclusion Criteria:

This study was done during the time period from May 2020 to September 2021. This study consisted of specimens which are histologically diagnosed as carcinomas of oral cavity which included biopsies and resection specimens of hemiglossectomy / mandibulectomy with neck dissection. Patients of all ages and both the sexes were included.

Exclusion Criteria:

Tumors with extensive necrosis without sufficient viable tumour cells and benign and inflammatory lesions of oral cavity diagnosed on histopathological evaluation were excluded. Also blocks with inadequate material were excluded. Sample size was calculated using the formula for sample size for estimation of proportion-

$$N = \frac{Z_{\alpha/2}{}^2 \; P\,(1-P)}{E^2}$$

Sample size was calculated to be minimum 32 subjects. For study purpose sample size was enhanced and taken as 55 subjects

3. Methodology

Paraffin blocks of cases that fulfilled the inclusion criteria was selected and issued by the permission of HOD of department of pathology, along with the permission to review the requisition form of these blocks biopsy specimen. Patient name, age, gender, registration number, path number, type of biopsy specimen and its gross feature was noted. Issued blocks were cut serially to 3 to 5 micron thickness using rotatory microtome to prepare slides. Slides were stained with routine hematoxylin and eosin stain and then mounted with DPX to review. After confirming and noting the diagnosis and microscopy details, sections were taken for P53 IHC staining.

About 3-4 mm thick sections were taken from formalin fixed paraffin embedded block from each case. P53 immunohistochemical staining was performed on 3-aminopropyltriethoxysilane-coated slides. Staining and evaluation using mouse monoclonal antibody to P53 was done. Human breast tissue will serve as a positive control. For negative control, Primary antibody was omitted while performing immunohistochemical staining.

The tumours are traditionally graded according to Broder's classification. Accordingly, tumours are graded on the basis of degree of differentiation and keratinisation of tumour cell. The tumor is graded as well, moderately and poorly differentiated according to WHO criteria.

Immunostaining protocol: Automated method on Leica BOND MAX autostainer was used in the study.

Immuhistochemical staining evaluation of p53:

The slides were examined at 100x magnification. Only nuclear staining is considered positive. Nuclear staining for p53 was scored for both proportion and intensity within the tumour parenchyma.

Proportion of staining was grouped into:

- Score 0: No tumor cells stained
- Score 1: <25%
- Score 2: 25-50%
- Score 3: 50-75%
- Score 4: 75-100%

Intensity of staining was grouped into

- Score 0: No staining
- Score 1: Weak staining
- Score 2: Intermediate staining
- Score 3: Strong staining

Finally, Immunoreactivity score for P53 expression was calculated by multiplying the number representing the % of immunoreactive cells by the number representing staining intensity and the cases were categorized in 4 groups shown in Table

Table: Grouping of P53 along with Interpretation group
Immunoreactivity score Interpretation

Group	Immunoreactivity Score	Interpretation
I	0-1	Negative
II	2-4	Weakly positive
III	5-8	Moderately positive
IV	9-12	Strongly positive

Statistical Analysis:
Categorical variables were expressed as frequency and percentage and was analysed using chi-square test. Continuous variables were expressed as Mean and Standard deviation and was analysed using student 't' test. Correlation between 2 variables was determined using Pearson/ Spearman correlation coefficient. P-value less than or equal to 0.05 was taken as statistically significant.

4. Observations and Results

After application of inclusion and exclusion criteria 55 patients were included for the study. Following results and observations were recorded in the study population.

Table 1: Distribution of Age

Age (yrs)	No. of patients	Percentage
30-40	12	21.82
41-50	17	30.91
51-60	8	14.55
≥61	18	32.73
Total	55	100.00

Out of 55 patients, 18 patients (33%) were between age group ≥61 years, 17 patients (31%) were between age group 41-50 years, 12 patients (21%) were between age group 30-40 years and 8 patients (14%) were between age group 51-60 years.

Table 2: Distribution of Gender

Gender	No. of patients	Percentage
Male	41	74.55
Female	14	25.45
Total	55	100.00

Out of 55 patients, 41 patients (75%) were males and 14 patients (25%) were females.

Table 3: Distribution of Habits

Habits	No. of patients	Percentage
Tobacco chewing	27	49.09
Smoker	12	21.82
Smoker with tobacco	10	18.18
No addiction	6	10.91
Total	55	100.00

Table 4: Distribution of Site

Site	No. of patients	Percentage
Buccal mucosa	20	36.36
Tongue	17	30.90
Hard palate	5	9.09
Lower lip	2	3.64
Lower alveolus	2	3.64
Upper alveolus	1	1.82
Gingivobuccal sulcus	2	3.64
Cheek	1	1.82
Tonsil	1	1.82
Tonsillar fossa	1	1.82
Vallecula and epiglottis	1	1.82
Soft palate	1	1.82
Hypopharynx	1	1.82
Total	55	100.00

Out of total 55 cases; 20 patients (36%) presented with a lesion at buccal mucosa, 17 patients (31%) at tongue, 5 patients (9%) at hard palate, 2 patients (4%) at lower lip, 2 patients (4%) at lower alveolus, 2 patients (4%) at gingivobuccal sulcus, 1 patient (2%) at upper alveolus, 1 patient (2%) at cheek, 1 patient (2%) at tonsil, 1 patient (2%) at tonsillar fossa, 1 patient (2%) at vallecula and epiglottis, 1 patient (2%) at soft palate and 1 patient (2%) at hypopharynx.

Table 5: Distribution of Cases of Squamous Cell Carcinoma

Diagnosis	No. of patients	Percentage
Squamous cell carcinoma	54	98.18
Chronic nonspecific tonsillitis	1	1.82
Total	55	100.00

Out of 55 cases, 54 cases (98%) were of squamous cell carcinoma and 1 case (2%) was of chronic nonspecific tonsillitis

Table 6: Distribution of Grade

Grade	No. of patients	Percentage
Well differentiated	19	35.19
Moderately differentiated	31	57.41
Poorly differentiated	4	7.41
Total	54	100.00

Out of 54 cases; 31 patients (57%) were graded as moderately differentiated, 19 patients (35%) were graded as well differentiated and 4 patients (8%) were graded as poorly differentiated.

Table 7: Distribution of P 53 Staining.

P53 staining	No. of patients	Percentage
Positive	47	85.45
Negative	08	14.55
Total	55	100.00

Out of 55 cases; 47 patients (85%) stained positively with P53 and 08 patients (15%) stained negatively for P 53.

Table 8: Distribution of Staining Intensity of P53

Staining Intensity of P53	No. of patients	Percentage
Strong	14	25.45
Moderate	21	38.18
Weak	12	21.81
Negative	8	14.54
Total	55	100.00

Out of 55 patients; 21 patients (38%) showed moderate staining intensity of P53, 14 patients (25%) showed strong staining intensity of P53, 12 patients (22%) showed weak staining intensity of P53 and 8 patients (15%) showed no staining of P53.

Table 9: Distribution of Grade with Age of Patients

Grade	Total		Age (yrs)			
			≤50		>50	
			N	%	N	%
Well	19	35.19	11	57.89	8	42.11
Moderate	31	57.41	17	54.84	14	45.16
Poor	4	7.41	0	0.00	4	100.00
Total	54	100.00	28	51.85	26	48.15

Out of total 54 cases; 31 cases were moderately differentiated. In these 31 cases; 17 cases (55%) were in age group of ≤50 years and 14 cases (45%) were in age group of >50 years.

19 cases were well differentiated; out of which 11 cases (58%) were in age group of ≤50 years and 8 cases (42%) were in age group of > 50 years.

4 cases were poorly differentiated and all (100%) were in age group of >50 years.

Distribution of Grade with Gender

Table 10: Distribution of Grade with Gender

Grade	Total		Gender			
			Male		Female	
			N	%	N	%
Well	19	35.19	13	68.42	6	31.58
Moderate	31	57.41	24	77.42	7	22.58
Poor	4	7.41	3	75.00	1	25.00
Total	54	100.00	40	74.07	14	25.93

Out of total 54 cases; maximum number 31 cases (57%) were moderately differentiated and 19 (35%) and 4 (8%) cases were graded as well and poorly differentiated respectively.

Out of 31 moderately differentiated cases; 24 cases (77%) were male and 7 cases (23%) were females.

Out of 19 well differentiated cases; 13 cases (68%) were males and 6 cases (32%) were females.

Out of 4 poorly differentiated cases; 3 cases (75%) were males and 1 c

Distribution of Habits with Histopathological Grade of OSCC

Table 11: Distribution of Habits with Histopathological Grade of OSCC

Habits	Total		Histopathological grade					
			Well		Moderate		Poor	
			N	%	N	%	N	%
Tobacco	27	50.00	11	57.89	15	48.39	1	25.00
Smoker	12	22.22	5	26.32	6	19.35	1	25.00
Smoker with tobacco	10	18.52	2	10.53	6	19.35	2	50.00
No addiction	5	9.26	1	5.26	4	12.90	0	0.00
Total	54	100.00	19	100.00	31	100.00	4	100.00

Out of 31 moderately differentiated cases; 15 cases (48%) had habit of tobacco chewing. 6 cases (20%) had habit of smoking, 6 cases (20%) had habit of smoking with tobacco chewing and 4 cases (12%) had no addiction.

Out of 19 well differentiated cases; 11 cases (58%) had habit of tobacco chewing.5 cases (26%) had habit of smoking, 2 cases (11%) had habit of smoking with tobacco chewing and 1 patient (5%) had no addiction.

Out of 4 poorly differentiated cases; 2 cases (50%) had habit of smoking with tobacco chewing and 1 patient each (25% each) had habit of smoking and tobacco chewing respectively.

Table 12: Distribution of Grades of OSCC with P53 Staining

Grade	Total		p53 staining			
			Positive		Negative	
			N	%	N	%
Well	19	35.19	14	73.68	5	26.31
Moderate	31	57.41	28	90.32	3	9.67
Poor	4	7.41	4	100.00	0	0.00
Total	54	100.00	46	85.18	8	14.81

Out of 31 moderately differentiated cases; 28 cases (90%) stained positively with P53 and 3 cases (10%) stained negatively with P53.

Out of 19 well differentiated cases; 14 cases (74%) stained positively with P53 and 5 cases (26%) stained negatively with P53.

Out of 4 poorly differentiated cases; all 4 cases (100%) stained positively with P53

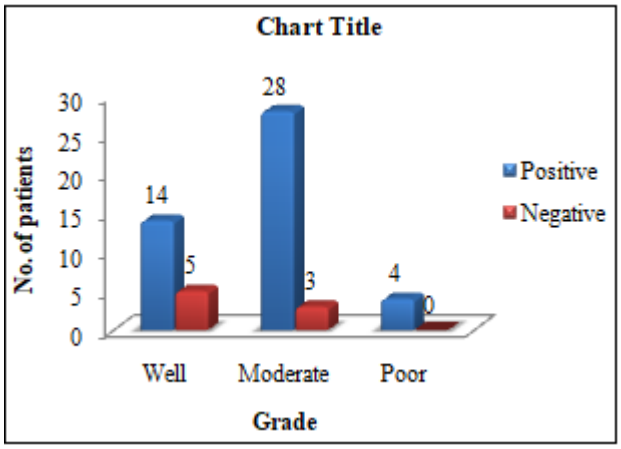


Table 13: Distribution of Grades of OSCC with Total Number of Cases

Grade	Total no of squamous cell carcinoma	Percentage
Well	19	35.19
Moderate	31	57.41
Poor	4	7.41
Total	54	100.00

Out of total 54 cases of oral cavity squamous cell carcinoma; 31 cases (58%) were graded as moderately differentiated. 19 cases (35%) were graded as well differentiated and 4 cases (7%) were graded as poorly differentiated.

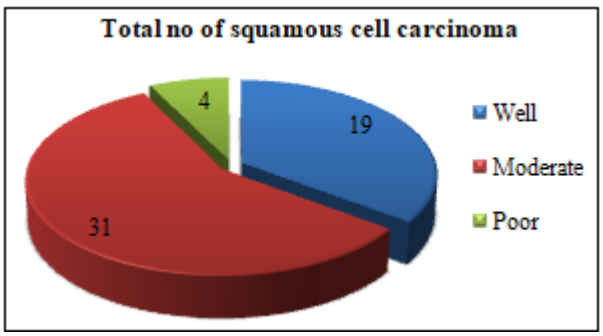


Table 14: Distribution of Grades of OSCC with Intensity of P53 Staining

Grade	Total		Intensity of P53 staining							
			Strong		Moderate		Weak		Negative	
			N	%	N	%	N	%	N	%
Well	15	27.78	2	13.33	2	13.33	6	40	5	33.33
Moderate	31	57.41	7	22.5	19	61.29	3	9.6	3	9.6
Poor	4	7.41	4	100.00	0	0.00	0	0.00	0	0.00
Total	54	100.00	13	24.07	18	33.33	9	16.66	08	14.81

Chi square 27.40 P value 0.0001 (S)

Out of 31 moderately differentiated cases; 19 cases (61%) showed moderate staining for P53, 7 cases (21%) showed strong staining for P53, 3 cases (9%) showed weak staining for P53 and 3 cases (9%) showed negative staining for P53.

Out of 15 well differentiated cases; 6 cases (40%) showed weak staining for P53, 5 cases (34%) showed negative staining for P53 and 2 cases each (13% each) showed strong and moderate staining for P53 respectively.

Out of 4 poorly differentiated cases; all 4 cases (100%) showed strong staining with P53.

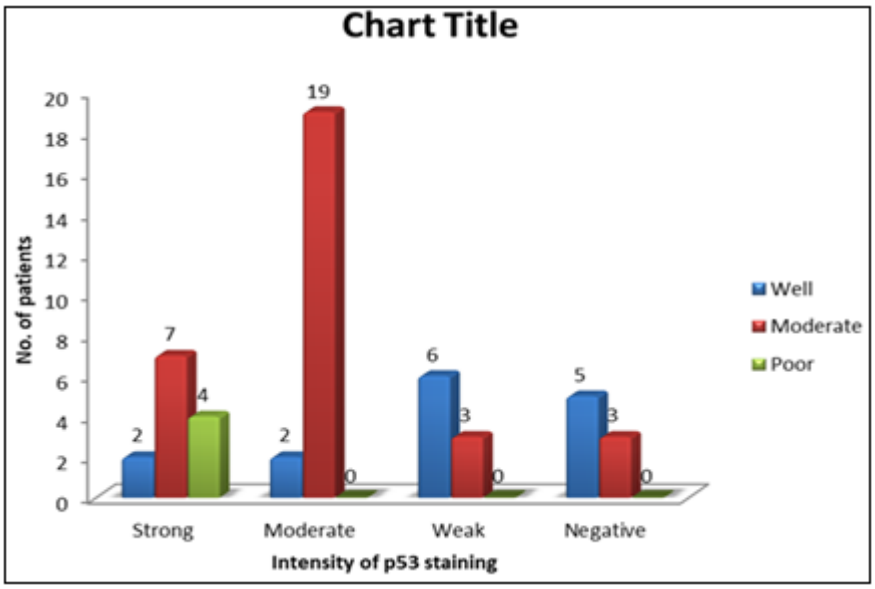


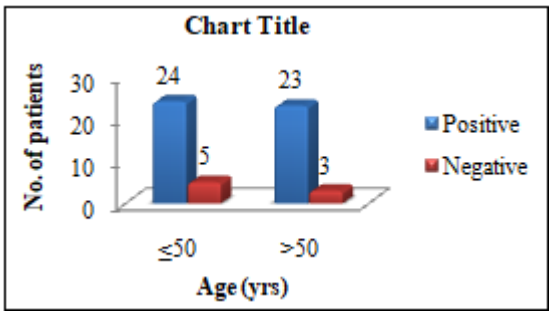
Table 15: Distribution of Age with P53 Staining

Age (yrs)	Total		p53 staining			
			Positive		Negative	
			N	%	N	%
≤50	29	52.73	24	82.75	05	17.24
>50	26	47.27	23	88.46	03	11.53
Total	55	100.00	47	85.45	08	14.54

Fisher exact test, P value 0.708 (NS)

Out of total 55 patients; 29 patients (53%) were in age group ≤ 50 years. Out of these 29 patients, 24 patients (83%) stained positively with P53 and 05 patients (17%) stained negatively with P53.

Out of total 55 patients; 26 patients (47%) were in age group > 50 years. Out of these 26 patients, 23 patients (88%) stained positively with P53 and 03 patients (12%) stained negatively with P53.



Distribution of Gender with P53

Table 16: Distribution of Gender with P53

Gender	Total		p53 staining			
			Positive		Negative	
			N	%	N	%
Male	41	74.55	34	82.92	07	17.07
Female	14	25.45	13	92.85	01	7.14
Total	55	100.00	47	85.45	08	14.54

Out of total 41 male patients; 34 patients (83%) stained positively with P53 and 07 patients (17%) stained negatively for P53.

Out of total 14 female patients; 13 patients (93%) stained positively for P53 and 1 patient (7%) stained negatively for P53.

Table 17: Distribution of Habits with P53

Habits	Total		p53 staining			
			Positive		Negative	
			N	%	N	%
Tobacco	27	49.09	23	85.18	04	14.81
Smoker	12	21.82	10	83.33	02	16.66
Smoker with tobacco	10	18.18	08	80	02	20
No addiction	6	10.91	6	100.00	0	0.00
Total	55	100.00	47	85.45	08	17.77

Chi square 1.306, P value 0.727 (NS)

Out of 27 patients who had habit of tobacco chewing; 23 patients (85%) stained positively with P53 and 04 patients (15%) stained negatively with P 53.

Out of 12 patients who had habit of smoking; 10 patients (83%) stained positively with P53 and 02 patients (17%) stained negatively with P53.

Out of 10 patients who had habit of both smoking and tobacco chewing; 08 cases (80%) stained positively for P53 and 2 cases (20%) stained negatively for P53.

Out of 6 patients who had no addiction; all 6 patients (100%) stained positively with P53.

Table 18: Distribution of OSCC with P53 staining

Diagnosis	Total		p53 staining			
			Positive		Negative	
			N	%	N	%
Chronic nonspecific tonsillitis	1	1.82	1	100.00	0	0.00
Squamous cell carcinoma	54	98.18	46	85.18	08	14.81
Total	55	100.00	47	85.45	08	14.54

Fisher exact test, P value 1.000 (NS)

Out of 54 cases diagnosed with squamous cell carcinoma; 46 cases (85%) showed positive staining with P53 and 08 cases (15%) showed negative staining with P53.

One case of chronic non specific tonsillitis was taken as a positive control and it showed positive staining for P 53.

Table 19

Grade	No. of patients	Mean	SD	Median	Range	IQR (Q1-Q3)
Well	15	37.86	17.31	32	22-70	24-47
Moderate	31	51.83	18.35	52	20-80	39-66.5
Poor	4	87.75	2.63	88	85-90	85.75-90
Total	50	50.52	21.34	45	20-90	32.5-67.75

F value 13.12, P value <0.0001 (S)

Anatomical Correlation:

Out of 20 cases which presented at buccal mucosa; 16 cases (80%) stained positively for P53 while 4 cases (20%) stained negatively for P53.

Out of 17 cases which presented at tongue; 14 cases (82%) stained positively for P53.

Out of 5 cases which presented at hard palate; all 5 cases (100%) stained positively for P53.

Out of 2 cases which presented at lower lip; both 2 cases (100%) stained positively for P53.

Out of 2 cases at lower alveolus; both 2 cases (100%) stained positively for P53.

1 case presented at upper alveolus which stained positively for P53.

2 cases presented at cheek; both (100%) of which stained positively for P53.

1 case presented at tonsil which stained positively for P53.

1 case presented at tonsillar fossa which stained negatively for P53.

1 case presented at vallecula and epiglottis which stained positively for P53.

1 case presented at soft palate which stained positively for P53.

1 case presented at hypopharynx which stained positively for P53.

5. Discussion

P53 (gene location: 17p 13.1) is a tumour suppressor gene acting as a key regulator of the cell's genomic stability, function and homeostasis. P53 aberrant over expression is frequently observed in OCSCC tissues as a result of point mutation or deletion.

The comparison of expression of P53 with histological grade was significant, and was found that P53 expression increases with severity of grades of oral cavity squamous cell carcinoma that is higher P53 expression in poorly differentiated cases [(2)].

Kannan S. et al. [(10),] reported that poorly differentiated tumors had prominent alterations in ki67 and p53 expression than well differentiated ones.

The present study aims to determine the proportion of P53 expression among the histopathological samples of oral squamous cell carcinoma received in pathology department, to determine any correlation of p53 expression with grade of oral squamous cell carcinoma and to determine any relation between expression of p53 with demographic (age, gender) and clinicopathological (location) variables of oral squamous cell carcinoma.

Comparative study of total number of cases studied:

Sample size is an important parameter that can affect the results of the study. A larger study can find many

confounding factors. Carlos et al. (2004) [11] and Riaz et. al (2013) [12] conducted their study on comparatively large sample size (91 and 100 cases respectively). While, Mohamad et. al (2010) [13] had 50 cases, Bhayekar et. al (2016) [14] had 45 cases and Dave et. al (2016) [102] had 40 number of cases. Our study has 55 number of cases.

Comparison of mean age of the cases under study: In the present study, out of 55 patients; 18 patients (33%) were in age group ≥ 61 years, 17 patients (31%) were between age group of 41-50 years, 12 patients (22%) were between age group 30-40 years and 8 patients (14%) were between age group 51-60 years. In our study, the mean age of presentation was 64 years which was comparable to Carlos et. al (2004) [11] and Mohamad et. al (2010) [13] with 60 years as the mean age. Almost all the studies showed that the age of presentation was in 6th decade with the only exception with Riaz et. al (2013) [12] where the mean age of presentation was 42 years.

Comparison of total number of males and females in various studies: All of the studies had predominantly male patients with 75% in the present study. Carlos et. al (2004) [11] and Bhayekar et. al (2016) [14] had 76% and 68% male patients, respectively. While Mohamad et. al (2010) [13] had 98% male patients among their cases.

Comparison of most common site of occurrence of malignancy in various studies: In the present study, it was found that the most common site of tumor was buccal mucosa which was a concordant finding with Bhayekar et. al (2016) [14]. In our study; out of total 55 cases, 20 patients (36%) presented with a lesion at buccal mucosa, 17 patients (31%) at tongue, 5 patients (9%) at hard palate, 2 patients each (3%) at lower lip, lower alveolus and gingivobuccal sulcus and 1 patient each (2%) at upper alveolus, cheek, tonsil, tonsillar fossa, vallecula, soft palate and hypopharynx.

Histological grade	Shin et. al (1994) [16]	Carlos et. al (2004) [11]	Mohamad et. al (2010) [13]	Moniba et. al (2017) [1]	Bhayekar et. al (2016) [15]	Present study
Well	05 (14%)	62 (68%)	27 (50%)	33 (63%)	15 (42%)	19 (35%)
Moderate	24 (67%)	25 (27%)	19 (35%)	16 (31%)	23 (51%)	31 (58%)
Poor	07 (19%)	04 (4%)	08 (14%)	03 (6%)	03 (7%)	04 (7%)
Total	36	91	54	52	45	54

Comparison of grade of squamous cell carcinoma amongst various studies

In our study; out of 54 cases of oral cavity squamous cell carcinoma, 31 cases (58%) were graded as moderately differentiated, 19 cases (35 %) were graded as well differentiated and 04 cases (07%) were graded as poorly differentiated. Findings in the present study are concordant with the studies of Bhayekar et. al (2016) [15] and Shin et. al (1994) [16] in which also maximum cases were graded as moderately differentiated. Rest all studies above showed that well differentiated squamous cell carcinoma was the most commonly encountered histological grade.

Comparison of most common addiction type amongst cases in various studies:

The most common addiction was tobacco chewing in the present study with 49% cases having a positive history. In the present study; out of total 55 cases 27 patients (49%) had a positive history of tobacco chewing, 12 patients (22%) had a history of smoking, 10 patients (18%) had history of both smoking with tobacco chewing and 6 patients (11%) had no addiction.

Comparison of percentage of cases positive for P53 in oral cavity squamous cell carcinoma:

All of the above studies showed that there was a strong positive correlation with the P53 immunostaining and the squamous cell carcinoma of oral cavity except for Carlos et. al (2004) [11] in which the cases showed a weak correlation with P53 immunostaining. The present study showed 85% cases which stained positively with P53 immunostaining which was comparable with Dragomir et. al (2012) [12] having 82.3% positive cases.

Comparison of staining intensity of P53:

In present study; out of 55 cases; maximum number of patients i. e 21 patients (38%) showed moderate intensity of P53 followed by 14 (25%) and 12 (22%) patients who showed strong and weak intensity of P53 respectively. While 8 patients (15%) showed negative staining intensity of P53.

Correlation Of P53 immunoexpression and tumour grade:

Most of the cases included in our study were moderately differentiated constituting 57% of total cases, followed by well and poorly differentiated tumors constituting 35% and 8% respectively. Correlating tumor grade with P53 expression, it was noted that in well differentiated carcinomas, the rate of expression of P53 was 74%; in moderately differentiated carcinomas, the rate of expression was 90% and in poorly differentiated carcinomas, the rate of expression was highest (100%). Thus, an increase in P53 immunoreactivity expression was observed with increase in tumor grade.

This result was in concordant with the study done by Bhattacharya et. al (2017) [17] which stated that a significantly high P53 expression was seen in moderately to poorly differentiated cases.

Correlation of staining intensity of P53 with grades of OSCC:

In present study; all the well differentiated cases had lower staining intensity while moderate to poorly differentiated cases had higher staining intensity. The results of our study are also statistically significant (p-value=0.0001). Out of 15

well differentiated cases, 6 cases (40%) showed weak staining intensity of P53. Out of 31 moderately differentiated cases, 19 cases (61%) showed moderate staining intensity of P53 and out of 4 poorly differentiated cases, all 4 (100%) showed strong staining intensity of P53.

The intensity of immunohistochemical staining was graded based on subjective evaluation of colour exhibited (brown colour) by antigen antibody and chromogen complex.

The results of our study are concordant with the study conducted by Bhattacharya et. al (2017) [17]. In this study also, a statistically significant correlation was found between low immunostaining intensity and P53 expression in cases of leukoplakia and vice-versa in OSCC cases.

Correlation of grade of tumor with age of patients:

In our study; out of 19 cases which were moderately differentiated; 11 cases (58%) were in age group of ≤50 years and 8 patients (42%) were in age group of >50 years. While out of 31 moderately differentiated cases, 55% were in age group of ≤50 years and 45% were in age group of >50 years.

While out of all 4 poorly differentiated cases, all 4 (100%) were in age group of > 50 years, So, it was concluded that as age of patient increases, grade of expression of OSCC also increases. So there is a linear correlation between age of patients and tumor grade.

Correlation of P53 staining with age:

In our study; out of 29 patients which were in age group of ≤50 years; 24 cases (83%) showed positive immunostaining with P53. While out of 23 patients who were in age group of > 50 years; 23 cases (88%) showed positive immunostaining with P53. This states that % of P53 positivity increases as the patient increase in age. On correlating P53 expression with age specific groups; it was found that P53 expression showed highest positivity in patients above 50 years of age. However, no significant correlation was seen between age group with P53 expression statistically (pvalue being 0.708).

Correlation of P53 staining with gender

In our study; out of 41 male patients; 34 cases (83%) stained positively with P53. While out of 14 female patients; 13 cases (93%) stained positively with P53. However, no significant correlation was seen between gender and P53 expression statistically (p value being 0.663)

Correlation of P53 staining with personal habits:

In our study; out of maximum 27 patients with a habit of tobacco chewing; 23 cases (85%) stained positively with P53. While out of 12 patients with habit of smoking; 10 patients (83%) stained positively with P53.

Out of 10 patients with habit of both smoking and tobacco chewing; 8 cases (80%) stained positively with P53. While out of 6 patients with no addiction history; all 6 cases (100%) stained positively with P53.

However, no statistical significant correlation was seen between personal habits and P53 expression (p value being 0.727).

Correlation of P53 staining with location:

In our study; out of 20 patients which presented with a lesion at buccal mucosa; 16 cases (80%) showed positive staining with P53. While out of 17 patients who presented with a lesion at tongue; 14 patients (82%) showed positive staining with P53. 5 cases presented with a lesion on hard palate; all of which also showed positive staining with P53. 2 cases each presented with lesions at lower lip, lower alveolus and gingivobuccal sulcus; all of which showed positive staining with P53.

However; no statistically significant correlation was seen between location of tumor and P53 staining.

6. Conclusions

This study consisted of total 55 specimens which are histologically diagnosed as carcinomas of oral cavity which will include biopsies and resection specimens of hemi glossectomy / mandibulectomy with neck dissection. Patients of all ages and both the sexes were included and following were the key outcomes of the study.

1) Most of the patients of oral cavity cancers were from 4^{th}-7^{th} decade. Out of 55 patients, majority (33%) of the patients were belonging to 6^{th} decade, with median age being 60 years.
2) Male to female ratio was found to be 3: 1.
3) The most common site of tumor was buccal mucosa (37%) followed by tongue (31%). Other sites included hard palate, lower lip, lower and upper alveolus, gingivobuccal sulcus, cheek, tonsil, tonsillar fossa, vallecula and epiglottis, soft palate and hypopharynx.
4) In the present study, all the cases were having oral cavity squamous cell carcinoma.
5) In the present study, we found majority of the patients (57%) were having moderately differentiated squamous cell carcinomas while 35% cases were of well differentiated carcinomas and 8% were having poorly differentiated squamous cell carcinomas.
6) In the present study, majority of the patients (49%) were having a habit of tobacco chewing.22% had habit of smoking, 18% had habit of both smoking with tobacco chewing and 11% had no addiction.
7) It was found that 58% of the cases of younger age group (≤50 years) were having well differentiated squamous cell carcinomas and 100% of the patients in older age group (>5o years) had poorly differentiated squamous cell carcinoma.
8) We observed that 85% of cases were positive for P53 and the mean positivity for P53 immunostaining was 43.75.
9) In the present study, majority of the cases (90%) of moderately differentiated carcinoma showed positivity for P53, and all 4 cases of poorly differentiated carcinoma were positive for P53.
10) P53 staining percentage had significant correlation with the histological grade of tumor.

11) There was a positive correlation between grading of oral cavity squamous cell carcinoma and P53 immunostaining. It was found that P53 expression increases with severity of grade of oral cavity squamous cell carcinoma that is higher P53 expression in poorly differentiated cases (p value=0.0001).
12) Overall there was a positive correlation between P53 immunostaining percent in oral cavity squamous cell carcinomas.

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