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HISTOPATHOLOGICAL EVALUATION AND IMMUNOHISTOCHEMICAL ANALYSIS OF CYTOKERATIN 17 EXPRESSION IN ORAL SQUAMOUS CELL CARCINOMA – A CROSS-SECTIONAL STUDY

Dr Sana Fathima[1], Dr Clement Wilfred D[2]

[1]Lecturer, Department of Biomedical Sciences, Specialization: MD Pathology, Ramaiah Medical College, MSR Nagar, Bengaluru 560054, Email ID: najsan10@gmail.com, ORCID ID: 0000-0001-7614-9483
[2]Professor, Department of Pathology, Specialization: MD Pathology, DNB MNAMS Ramaiah Medical College, MSR Nagar, Bengaluru 560054, Email ID: clement.wilfred@yahoo.com, ORCID ID: 0000-0003-2513-8332

ABSTRACT

Background:

Oral squamous cell carcinoma (OSCC) is one of the most widely occurring cancers, the incidence of which is highest in India, South and Southeast Asian countries. In India, 20 per 100,000 population are affected and over 5 people die every hour because of it. Studies have highlighted the role of tumor markers like CK17 in OSCC and its importance in prognosis. It might be a suitable biomarker for postulating newer treatment strategies. Hence this study is undertaken to evaluate the histopathological features of OSCC and the association of CK17 expression with prognostic factors like tumour stage, grade and lymphatic metastasis.

Objective

1. To evaluate the histopathological features of oral squamous cell carcinoma.
2. To evaluate the immunohistochemical expression of CK17 in oral squamous cell carcinoma and to correlate its expression with pathological prognostic factors like tumor grade, stage and lymph node metastasis.

Methods

This study was conducted on radical resection specimens of OSCC in the Department of Pathology, Ramaiah Medical College and Hospitals, Bengaluru for 2 years. Relevant clinical data were collected from patient files. Tumor morphology and CK17 immunohistochemical staining were studied and correlated with significant findings in each case.

Results

The study included 55 cases of OSCC with male-to-female ratio of 1.6: 1. Mean age was 55.64 years. Most of them were in buccal mucosa (54.4%). Majority were moderately differentiated (50.9%) and presented in an advanced stage. All cases of OSCC (100%) showed CK17 positivity. High CK17 expression was seen in well-differentiated OSCC (80%). Significant association was seen with increasing expression of CK17 and decreasing grade of tumors.

Conclusion

This study provides information about CK17 expression in OSCC which is a little underexplored marker for OSCC. Among the several studies conducted for the same worldwide, very few are done in India, one of the reasons for carrying out this research.

KEYWORDS: Histological grade; TNM stage; CK17 score; Prognosis & Metastasis.

INTRODUCTION

Oral squamous cell carcinoma (OSCC) is the eighth most common cancer in the world with 3% males and 2% females being affected. Head and neck cancers (HNCA) in India account for 30–40% cancers, out of of which 9.4% are oral cancers.

Despite diagnostic and therapeutic advances, the 5-year survival rate of OSCC remains 70–80%, primarily due to recurrences and metastasis to cervical lymph nodes. The key to a good prognosis and low morbidity is early and accurate diagnosis of the primary tumour and early detection of recurrences and metastasis. Despite significant post-operative monitoring, it is difficult to detect recurrences and metastasis. Therefore it is important to identify predictors of disease survival and molecular markers whose expression is related to clinical prognosis and staging.

Oral Squamous Cell Carcinoma

It is a malignant epithelial neoplasm exhibiting squamous differentiation which is characterized by the formation of keratin and/or the presence of intercellular bridges. More than 90-95% of malignant neoplasms of the oral cavity and oropharynx are squamous cell carcinoma. An invasive epithelial neoplasm with varying degrees of squamous differentiation and a

propensity to early and extensive lymph node metastases, occurs commonly in alcohol and tobacco-using adults in the 5th and 6th decades of life.
Cases of OSCC are usually seen in the elderly age group occurring primarily in the 5th–8th decade and 6% of all oral cancers occur in the younger patients (<45 years). Males are more often affected than females because of increased indulgence in both tobacco and alcohol habits in most countries. The male to female ratio is, globally lower for cancer of the oral cavity than for cancer of the oropharynx, thus suggesting that higher exposure to tobacco smoking and alcohol drinking are required to induce oropharyngeal than oral cancer. There is an increase in the global incidence of oral squamous cell carcinoma among people less than 40 years of age.

Risk factors:
Oral cavity is the fourth most common site of carcinoma in the developing world after lung, stomach and liver in males while in females it is the fifth most common cancer after cervix, breast, stomach and lung. Various risk factors are as below:
1. Oral leukoplakia is 'A white patch or plaque that cannot be characterized, clinically or histopathologically, as any other disease'. It has two broad categories, as below.
- Oral leukoplakia of unknown etiology or idiopathic
- Oral leukoplakia associated with tobacco use.

2. Erythroplakia is defined as a bright red patch or plaque that cannot be defined clinically or pathologically as any other condition.
3. OSCC has a very strong etiologic link with the human papillomavirus (HPV).HPV 16 is the most frequently encountered type while HPV 18 is rare in HPV-associated OSCC.
4. Alcohol abuse is the second most common risk factor (after smoking tobacco) for developing an oral/pharyngeal cancer.

Gross morphology:
Grossly OSCC can present as an ulcer, an alteration of mucosal color, or a tumor mass. Ulcerative lesions usually have a crateriform appearance with rolled elevated borders that are firm because of the tumor infiltration along the margins. The cut surface usually has a gray- white glistening appearance.

Microscopy :
Squamous differentiation, often appreciated as keratinization with variable "pearl" formation, and invasive growth are the prerequisite features of SCC. [23]
The tumors are graded according to Broder's classification. [11]
Accordingly, tumors were graded on the basis of degree of differentiation and keratinization of tumor cells into:
- Grade I : Well differentiated tumors, 75-100% of cells are differentiated.
- Grade II : Moderately differentiated tumors, 50-75% of cells are differentiated.
- Grade III : Poorly differentiated tumors, 25-50% of cells are differentiated.
- Grade IV : Anaplastic tumors, 0-25% of cells are differentiated.

CYTOKERATIN 17 gene (CK17)
Cytokeratins (CK) are a family of intermediate filament proteins usually expressed in epithelial cells and have a basic structural function in forming the cytoskeleton, which is important for cell structure integrity and stability. Keratins are also involved in protein synthesis and epithelial cell growth signaling, organelle transport, and cell mobility and proliferation. They are present during differentiation and development of epithelial cells. There are around 54 genes that encode type I (acidic) and type II (basic) keratins, which form heterodimers and show tissue-specific expression.
The two types of cytokeratins are, the low-molecular-weight or acidic type I cytokeratins and the high-molecular-weight or basic or neutral type II cytokeratins. The high-molecular-weight cytokeratins or basic or neutral cytokeratins consist of numerous subtypes. The low-molecular-weight cytokeratins or acidic cytokeratins consist of CK10, CK12, CK13, CK14, CK16, CK17, CK18, CK19, and CK20. The expression of these cytokeratins varies in different organs.
Type I cytokeratin 17 and its varying expression patterns have been indicated in cancer. It was identified as a major keratin of basal cell carcinomas of the skin. Since in keratinocytes CK17 is an inducible keratin in response to stress, injury, or inflammation, it is concluded that squamous cell carcinomas consistently express these keratins. As most normal stratified squamous epithelia lack CK17, its presence in the corresponding tumors may be regarded as neo-expression during tumorigenesis.
On the other hand, CK17 has been demonstrated in certain pathological conditions, including psoriasis, allergic reactions, basal cell carcinoma of the skin, squamous cell carcinomas of the uterine cervix, lung, esophagus, larynx and oral cavity. Prognostic associations for CK17 have been made for gastric adenocarcinoma, breast cancer, intraepithelial cervical cancer, ovarian cancer, hepatocellular carcinoma, and lung, larynx, thyroid, and oral cancer.
In a study conducted by Kitamura et al on 105 OSCC cases, the majority of the tumours were well-differentiated (68.6%) and CK17 was expressed in 96.2 % of the OSCC cases. Further, the authors found that CK17 was significantly expressed in well-differentiated OSCC compared to moderately/poorly differentiated OSCC and concluded that CK17 expression could be associated with the differentiation and the malignancy of OSCC. A study by Mikami et al revealed CK17 immunopositivity in all the 82 cases of OSCC. The authors concluded that emergence of CK17 expression is related to emergence of malignancy.

Hence this study is undertaken to evaluate the histopathological features of OSCC and to examine the association of CK17 expression with significant prognostic markers like tumor stage, grade and lymphatic metastasis.

MATERIALS AND METHODS

This cross-sectional study was conducted on radical resection specimens of oral cavity carcinomas, received in the Department of Pathology, for routine histopathological evaluation from the Department of Surgical Oncology, Ramaiah Medical College and Hospitals, Bengaluru, over a period of 2 years, after obtaining informed consent.

The specimens of OSCC were received in the Pathology Department in 10% formalin. In every case the standard protocol for surgical grossing of specimens was followed. After a detailed gross specimen examination, multiple tissue bits were taken from the tumor, surgical margins and all the lymph nodes. The latter was processed as per standard protocol and paraffin-embedded blocks were cut and stained by hematoxylin and eosin (H & E). The tumors were staged according to the American Joint Committee on Cancer (8th edition, 2017) staging system.

Processing For Immunohistochemistry

Immunohistochemical detection of CK17 was done on 4 μm thick sections, cut from a paraffin block of tumor tissue and taken on a glass slide coated with adhesive (poly-L-lysine). The technique for IHC, using "Dako REALTM EnVision TM detection system" includes antigen retrieval in a citrate buffer in a microwave oven for 15-20 mins, blocking endogenous peroxidase with 3% hydrogen peroxide for 10 mins. The slides were then washed with tris buffered solution (TBS, pH 7.4) for 2 mins and incubation with primary mouse monoclonal antibodies against CK17 (clone E03, DAKO) proteins, linking with rabbit anti-mouse secondary antibody (clone E03, DAKO), enzyme labeling with horseradish peroxidase, developing chromogen with diaminobenzidine (DAB) for 5 mins and counterstaining with hematoxylin.

These slides were examined for cytoplasmic staining of CK17 and were considered as positive when any cytoplasmic staining was detected in > 5% of the tumor cells and <5 % reactive tumor cells were considered negative. The following scores were used to evaluate CK17 expression:

a. 0 (<5% of positive cells),
b. 1+ (5-30% of positive cells),
c. 2+(31-60% of positive cells) and
d. 3+(> 60% of positive cells). [25,28]

For statistical analysis CK17 expression scores of 1 & 2 were grouped as low score/expression and score 3 was considered as high score/expression.

Statistical Analysis:

A study carried out on "Association of CK17 expression in oral squamous cell carcinoma" has revealed a CK17 positivity of 71% .1 Based on the above findings of the study, considering the CK17 expression with a relative precision of 12% and desired confidence level of 95%, it was estimated that minimum of 55 patients were needed for the study.

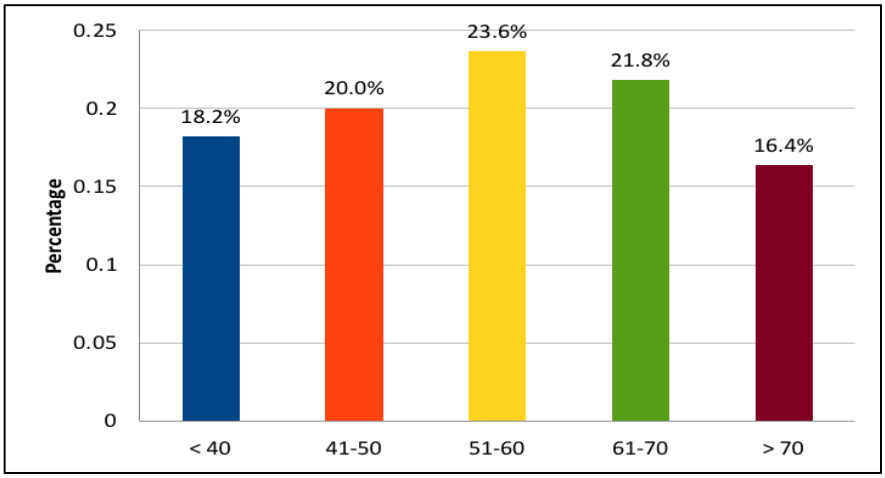
All the quantitative values such as age, tumor size etc. were expressed as mean and standard deviation/median with interquartile range. To evaluate the association of various histopathological and immunohistochemical parameters, Chi-Square test/ oblique McNemar's test of significance was employed.

Results

Age Distribution The patients included in the study were divided into five groups as shown in Table 1. Most patients (23.6%) were in the 5th decade followed by the 6th decade (21.8%).

TABLE 1: AGE DISTRIBUTION OF PATIENTS

AGE GROUP(YEARS)	NO. OF CASES	PERCENTAGE
< 40	10	18.2%
41-50	11	20.0%
51-60	13	23.6%
61-70	12	21.8%
> 70	9	16.4%
TOTAL	55	100.0%



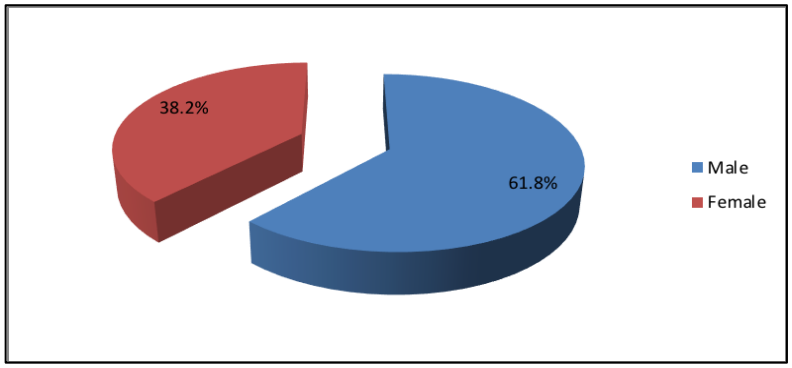
GRAPH 1: AGE DISTRIBUTION IN ORAL SQUAMOUS CELL CARCINOMA

Sex Distribution

In our study, males (61.8%) predominated over females (38.2%) with a male-to-female ratio of 1.6: 1 (as depicted in Table 2).

TABLE 2 : Distribution of sex among cases

SEX	NO. OF CASES	PERCENTAGE
Male	34	61.8%
Female	21	38.2%
Total	55	100.0%



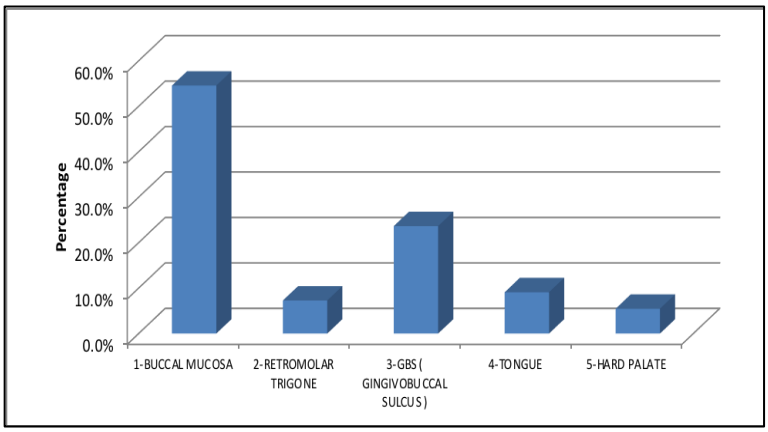
GRAPH 2: PIE CHART SHOWING DISTRIBUTION OF SEX

Anatomical Site. The most common site of tumor occurrence was the buccal mucosa (54.4%) followed by the gingivobuccal sulcus (23.6%) and retromolar trigone (7.3%). There were few cases with the origin of tumor from tongue (9.1%) and others from the hard palate (5.5%) (Table 3).

TABLE 3 : ANATOMICAL SITE OF TUMOUR

Site	No. of cases	Percentage
1-BUCCAL MUCOSA	30	54.5%
2-RETROMOLAR TRIGONE	4	7.3%
3-GBS (GINGIVOBUCCAL SULCUS)	13	23.6%
4-TONGUE	5	9.1%

Site	No. of cases	Percentage
5-HARD PALATE	3	5.5%
Total	55	100.0%



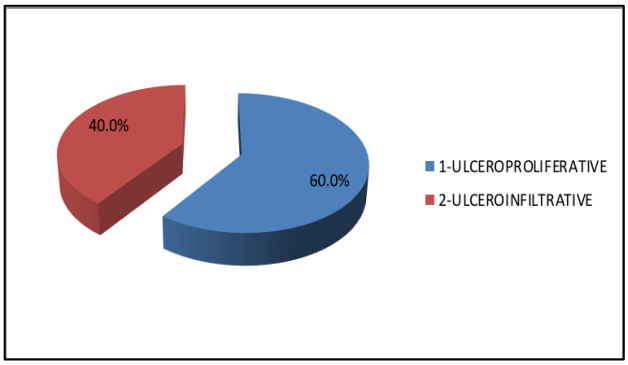
GRAPH 3: ANATOMICAL SITE DISTRIBUTION OF OSCC

Gross Tumour Morphology

The majority of the OSCC in the study were ulceroproliferative (exophytic) (60%) and others ulceroinfiltrative (endophytic) (40%) (Table 4).

TABLE 4 : GROSS APPEARANCE OF OSCC

ULCEROPROLIFERATIVE	33	60.0%
ULCERO INFILTRATIVE	22	40.0%
Total	55	100.0%



GRAPH 4: PIE CHART SHOWING GROSS APPEARANCE OF OSCC

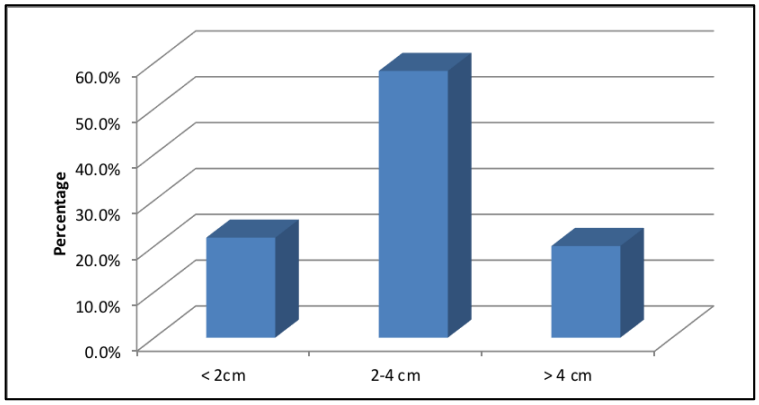
TUMOUR SIZE

The largest size of the tumor is a good prognostic factor in OSCC. It is an important component which determines the pathological stage of the tumor. The majority of the cases in this study were between 2-4 cm in greatest dimension (58.2%), 12 cases (21.8%) were less than 2 cm and 11 cases(20%) were more than 4 cm as shown in Table 5.

TABLE 5 : SHOWING THE LARGEST DIMENSION OF TUMOR

Size (cm)	No. of cases	Percentage
< 2cm	12	21.8%
2-4 cm	32	58.2%

Size (cm)	No. of cases	Percentage
> 4 cm	11	20.0%
Total	55	100.0%

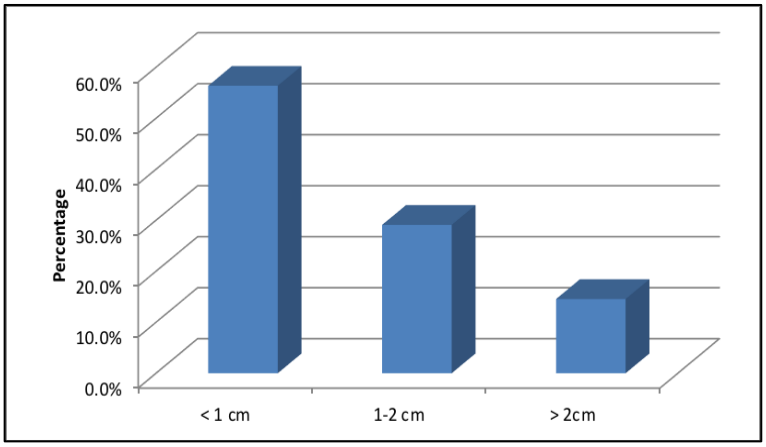


GRAPH 5: BAR GRAPH SHOWING THE LARGEST DIMENSION OF TUMOR

DEPTH OF TUMOUR INVASION Out of 55 cases 31 (56.4%) of them had a depth of invasion of less than 1 cm and 16 cases (29.1%) had a depth of invasion between 1-2 cms, with only 8 cases (14.5%) with a depth of invasion greater than 2 cm (Table 6).

TABLE 6: DEPTH OF TUMOR INVASION IN OSCC

Depth (cm)	No. of cases	Percentage
< 1 cm	31	56.4%
1-2 cm	16	29.1%
> 2cm	8	14.5%
Total	55	100.0%



GRAPH 6: DEPTH OF TUMOR INVASION IN OSCC

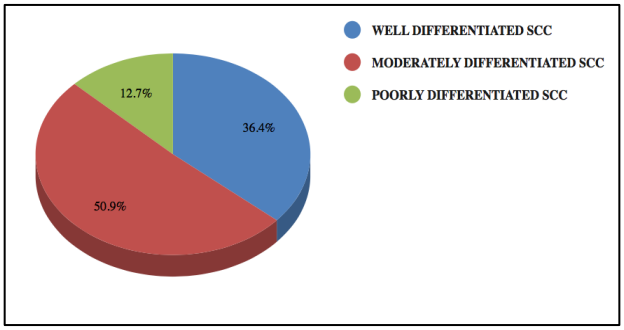
TUMOUR GRADE

In the 55 cases of OSCC 28 (50.9%) were moderately differentiated squamous cell carcinoma, 20 (36.4%) were well-differentiated OSCC and 7 (12.7%) were poorly differentiated OSCC (Table 7)

TABLE 7: HISTOLOGIC GRADES DISTRIBUTION OF OSCC

Histological grade	No. of cases	Percentage
G1-WELL DIFFERENTIATED SCC	20	36.4%

Histological grade	No. of cases	Percentage
G2-MODERATELY DIFFERENTIATED SCC	28	50.9%
G3-POORLY DIFFERENTIATED SCC	7	12.7%
Total	55	100.0%



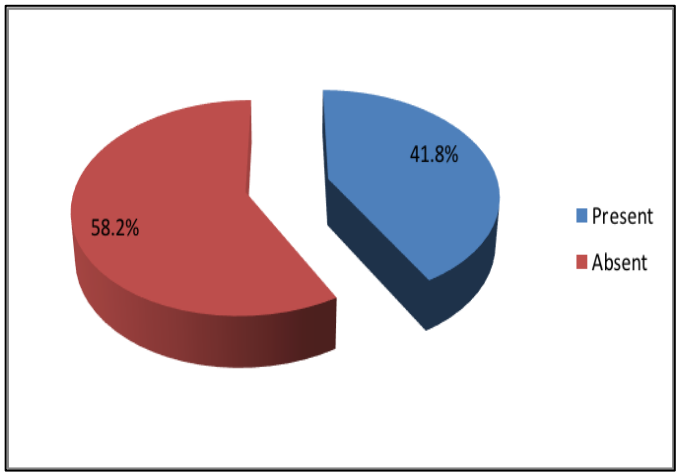
GRAPH 7: HISTOLOGIC GRADE DISTRIBUTION OF OSCC

LYMPH NODE STATUS

Among the set of 55 cases, 23 (41.8%) showed lymph node metastasis as depicted in Table 8.

TABLE 8 : LYMPH NODE METASTASIS IN OSCC CASES

LN status	No. of cases	Percentage
Present	23	41.8%
Absent	32	58.2%
Total	55	100.0%



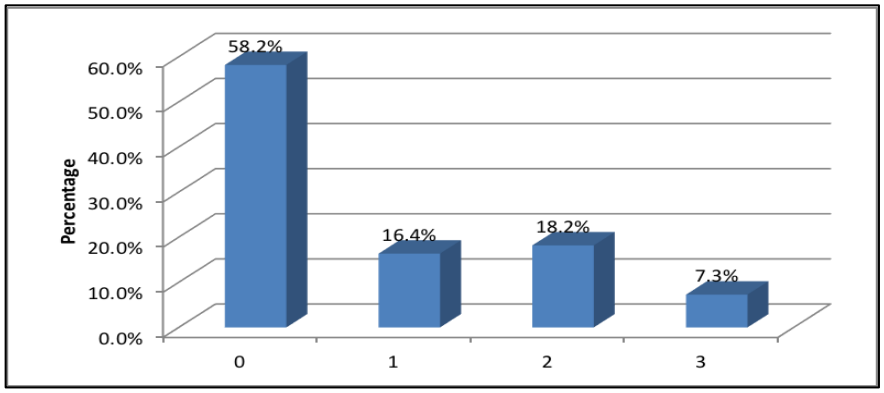
GRAPH 8: PIE CHART SHOWING LYMPH NODE METASTASIS IN OSCC CASES

DISTRIBUTION ACCORDING TO "N" STAGE OF TUMOUR

Out of 55 cases, 32(58.2%) presented in N0 stage followed by 10 (18.2%) in N2 , 9(16.4%) in N1 and 4(7.3%) in N3 stages respectively,
(Table 9).

TABLE 9 : SHOWING "N" STAGE AMONG CASES

N	No. of cases	Percentage
0	32	58.2%
1	9	16.4%
2	10	18.2%
3	4	7.3%
Total	55	100.0%



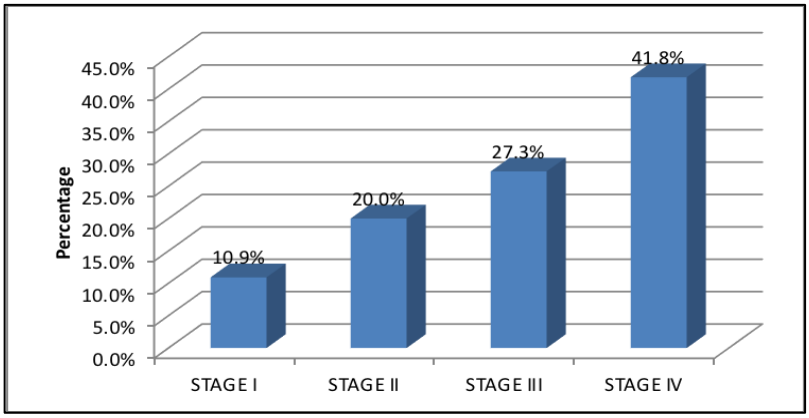
GRAPH 9 : SHOWING DISTRIBUTION OF " N" STAGE AMONG CASES

DISTRIBUTION OF OSCC ACCORDING TO TNM STAGE

Presentation at early stages (TNM stages I and II) was observed in 30.9%, and 69.1% of the cases presented at advanced stages (TNM stages III and IV) as shown in Table 10 below.

TABLE 10 : DISTRIBUTION OF OSCC ACCORDING TO TNM STAGE

Stage	No. of cases	Percentage
STAGE I	6	10.9%
STAGE II	11	20.0%
STAGE III	15	27.3%
STAGE IV	23	41.8%
Total	55	100.0%



GRAPH 10: BAR GRAPH SHOWING THE PATHOLOGICAL STAGE OF CASES

CK17 IMMUNOSTAINING IN OSCC

Cytoplasmic expression of CK17 was seen in all the 55 (100%) cases of OSCC. However, the tumour cells showed varying degrees of cytoplasmic CK17 expression. The percentage of tumour cells expressing CK17 is depicted in Table 11. In 8 cases 65% of the tumour cells showed CK17 expression and in another 8 cases 55% of the tumour cells showed CK17 expression.

TABLE 11 : PERCENTAGE OF TUMOUR CELLS EXPRESSING CK17

PERCENTAGE OF TUMOUR CELLS EXPRESSING CK17	No. of cases	Percentage
15	2	3.6%
20	4	7.3%
25	2	3.6%
40	2	3.6%
45	4	7.3%
50	3	5.5%
55	8	14.5%
60	1	1.8%
65	8	14.5%
70	5	9.1%
75	6	10.9%
80	6	10.9%
85	1	1.8%
90	3	5.5%
Total	55	100.0%

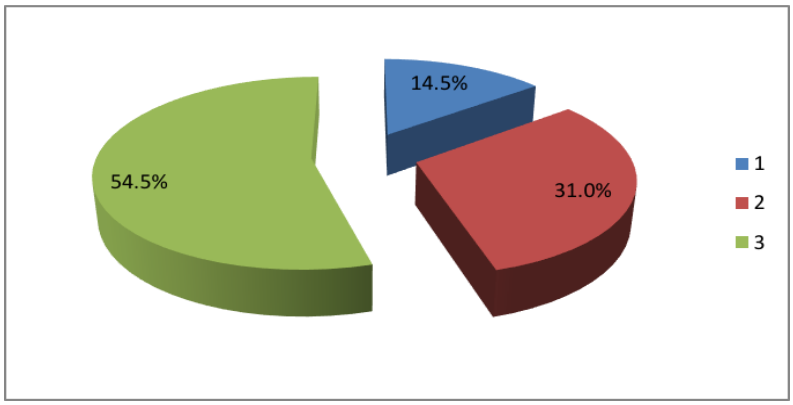
SCORING OF CK17 EXPRESSION IN OSCC

Depending on the percentage of expression of tumour cells there are three scores given, as depicted in Table 12. The majority of the cases (54.4%) showed score 3 CK17 expression followed by score 2 (30.9%) and score 1 (14.5%).

For the purpose of statistical analysis CK17 expression scores of 1 & 2 were grouped as low score/expression and score 3 was considered as high score/expression.

TABLE 12 : SCORING OF CK17 EXPRESSION IN OSCC

CK17 score	No. of cases	Percentage
1(5-30% positive tumour cells)	8	14.5%
2(31-60% positive tumour cells)	17	31.0%
3(>60% positive tumour cells)	30	54.5%
Total	55	100.0%



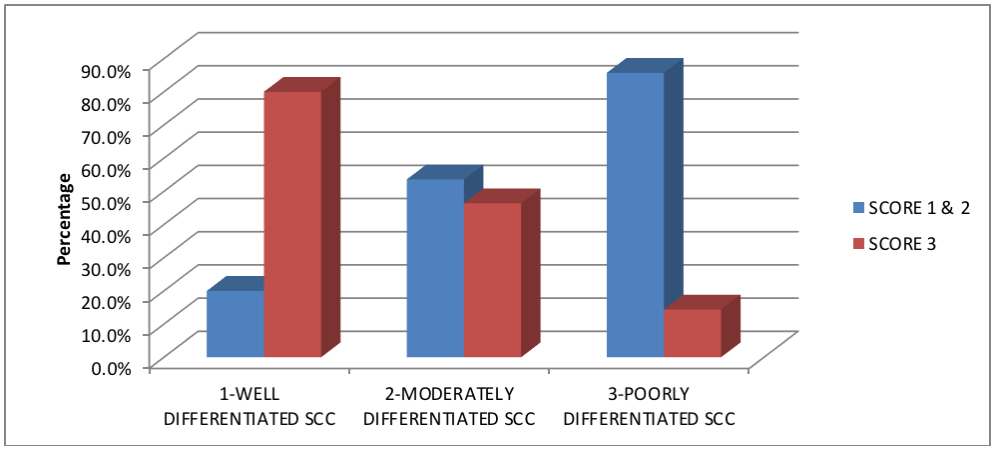
GRAPH 11 : PIE CHART SHOWING SCORING OF CK17 EXPRESSION IN OSCC

ASSOCIATION OF CK17 EXPRESSION WITH HISTOLOGICAL TUMOUR GRADE

Majority of the well-differentiated OSCC (53.3%) showed score 3 CK17 expression whereas 43.3% of moderately differentiated and only 3.3% of poorly differentiated OSCC exhibited score 3 CK17 expression (as depicted in Table 13). There is a statistically significant trend in the expression of CK17 with the histological grade/differentiation of tumor with well-differentiated SCC exhibiting higher score/expression compared to moderate and poorly differentiated SCC (p = 0.005). Thus there is a decreasing trend of CK17 expression with increasing tumour grade.

TABLE 13 : CK17 EXPRESSION IN DIFFERENT HISTOLOGIC GRADES OF OSCC

		CK17 SCORE		Total cases	p-value
		Score 1 & 2 (<60%)	Score 3 (>60%)		
Histological grade/ Differentiation	1-WELL DIFFERENTIATED SCC	4 (20.0%)	16(80%)	20 (36.4%)	0.005
	2-MODERATELY DIFFERENTIATED SCC	15 (53.6%)	13(46.4%)	28 (50.9%)	
	3-POORLY DIFFERENTIATED SCC	6 (85.7%)	1(14.38%)	7 (12.7%)	



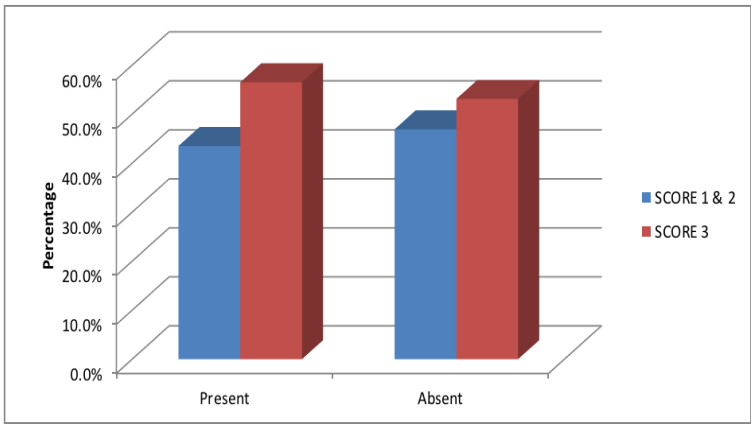
GRAPH 12 : BAR GRAPH SHOWING CK17 EXPRESSION IN DIFFERENT HISTOLOGIC GRADES OF OSCC

ASSOCIATION OF CK17 EXPRESSION WITH LYMPH NODE METASTASIS

Out of 55 cases, 23 showed lymph node metastasis. Of the latter cases 56.5% of them showed score 3 CK17 expression and the rest (43.5%) showed score 1 & 2 CK17 expression as is evident from Table 14 below. There was no statistically significant correlation between lymph node metastasis and CK17 expression in the primary tumour (p = 0.803).

TABLE 14 : ASSOCIATION OF CK17 EXPRESSION WITH LYMPH NODE METASTASIS

		CK17 SCORE		Total	p-value
		SCORE 1 & 2 (<60%)	SCORE 3 (>60%)		
LN status	Present	10(43.5%)	13(56.5%)	23(41.8%)	0.803
	Absent	15(46.9%)	17(53.1%)	32(58.1%)	



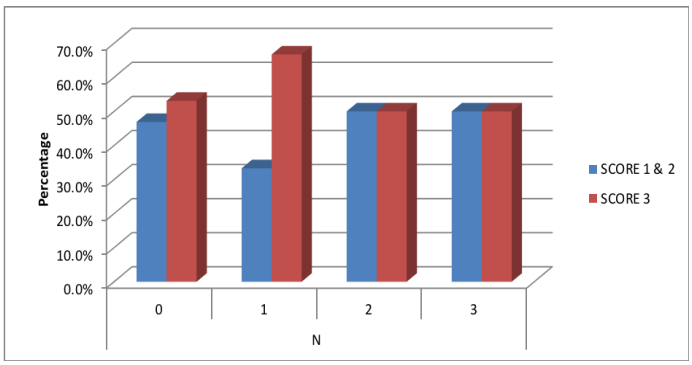
GRAPH 13 : BAR GRAPH SHOWING ASSOCIATION OF CK17 EXPRESSION WITH LYMPH NODE METASTASIS IN OSCC

ASSOCIATION OF CK17 EXPRESSION WITH RESPECT TO "N" STAGE

In our study, 53.1% (17/32) cases of N0 stage showed score 3 CK17 expression whereas 66.7% (6/9) cases of N1 stage showed score 3 CK17 expression. While N3 and N4 stages each exhibited 50% of the cases with score 3 CK17 expressions (depicted in Table 15). There was no statistically significant association between CK17 expression and "N" stage of the tumour (p=0.879).

TABLE 15 : CORRELATION OF CK17 EXPRESSION WITH RESPECT TO "N" STAGE

		CK17 SCORE		Total	p-value
		SCORE 1 & 2 (<60%)	SCORE 3 (>60%)		
N STAGE	0	15(46.9%)	17(53.1%)	32(58.2%)	0.879
	1	3(33.3%)	6(66.7%)	9(16.4%)	
	2	5(50.0%)	5(50.0%)	10(18.1%)	
	3	2(50.0%)	2(50.0%)	4(7.3%)	



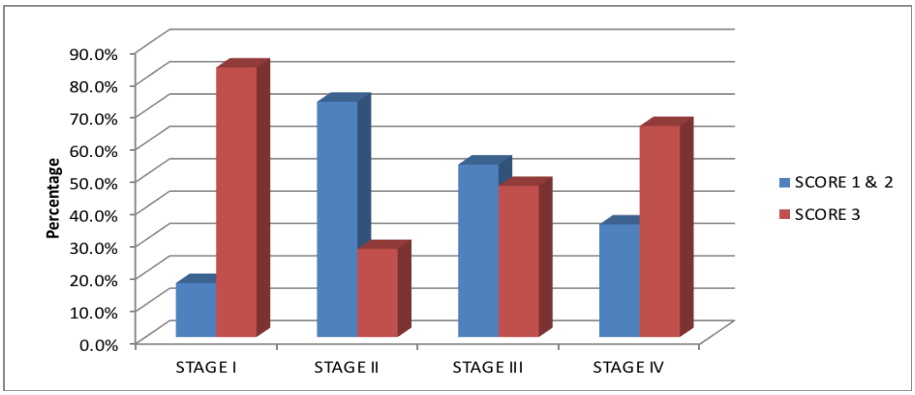
GRAPH 14 : BAR GRAPH SHOWING CORRELATION OF CK17 EXPRESSION WITH RESPECT TO "N" STAGE

CK17 EXPRESSION WITH RESPECT TO TNM STAGE

In our study 57.9% (22/38) cases of advanced TNM stage (III + IV) showed score 3 CK17 expression in contrast to 47.5% (8/17) cases of early TNM stage (I+II) . Thus a relatively higher score of CK17 expression is seen in advanced tumour stage compared to early stage tumours, however this association fell short of statistical significance (p =0.081) (Table 16).

TABLE 16 : CORRELATION OF CK17 EXPRESSION WITH RESPECT TO TNM STAGE IN OSCC

		CK17 SCORE		Total	p-value
		SCORE 1 & 2 (<60%)	SCORE 3 (>60%)		
TNM STAGE	STAGE I	1(16.7%)	5(83.3%)	6 (10.9%)	0.081
	STAGE II	8(72.7%)	3(27.2%)	11(20.0%)	
	STAGE III	8(53.3%)	7(46.7%)	15(27.3%)	
	STAGE IV	8(34.8%)	15(65.2%)	23(41.8%)	



GRAPH 15: BAR GRAPH SHOWING CK17 EXPRESSION WITH RESPECT TO TNM STAGE IN OSCC

DISCUSSION

Oral squamous cell carcinoma (OSCC) is one of the most widely occurring cancers, the incidence of which is highest in India, South and Southeast Asian countries. In India, 20 per 100,000 population are affected every year because of this cancer. [1]

In the present study, 55 resected specimens of the oral cavity were evaluated by light microscopy to determine the histologic grade, tumor stage, lymph node status and subsequently immunohistochemistry was done to find out the CK17 expression of the OSCC and its correlation with histopathological parameters.

Age and Sex Distribution

The mean age of OSCC was 55.64 years (Table 1) which is in accordance with most of the previous studies. Peak incidence was seen in the 5th to 6th decade of life. Similarly, a study by Shenoi R et al showed the majority of their patients in the 5th to 6th decade of life. In our study we observed that 38.2% of the cases were in the <51 years of age group. Similarly, literature review revealed an increasing trend in the younger population. In the study by Abdulla et al, the frequency of OSCC in young individuals (<45 years) was 20.48% [2] The reason for the increasing trend of OSCC cases in young adults could be due to cigarette/bidi smoking and tobacco/paan/betel quid chewing being a common habit in India.

In the present study males were found to be highly susceptible to OSCC (61.8%) when compared to females (38.2%) with male to female ratio of 1.6:1 (Table 2). This is in accordance with studies conducted by Kitamura et al(2017), Kitamura et al(2012), Abdulla et al and Shenoi et al, who reported higher frequencies among males in comparison to females with ratios of 3.75 : 1, 1.02:1 ,1.8:1 and 4.2 :1 respectively.

Anatomical Site

In our study, buccal mucosa was the most common site of the tumor (54.5%) followed by tumors involving the gingivobuccal sulcus (23.6%) , tongue (9.1%), retromolar trigone (7.3%) and hard palate (5.5%) (Table 3). Similarly, Abdulla et al and Acharya et al found that the most common site of tumor involvement was buccal mucosa (32.14% and 28.83 % respectively). In contrast, in a Japanese study conducted by Kitamura et al showed most common site of tumor

occurrence to be the tongue (62.4%). This difference in the anatomical site of tumor might be due to differences in sample size, environmental risk factors and habits.

Gross Tumour Appearance
In the present study exophytic growth was the most common (60%) type of gross tumor appearance when compared to the endophytic type (40%) (Table 4). This is in concordance with another south Indian study conducted by Acharya et al, where 82.3% of OSCC cases presented as exophytic lesions.

Greatest Dimension of the tumour
We observed that the tumor size was <2 cm in 21.8% of the cases, 2-4 cm in 58.2% of the cases and >4 cm in 20% of the cases (Table 5) with the mean maximum diameter of the primary tumor being 2.67 ± 1.04 cm. In a study conducted by Acharya et al similar results were concluded, wherein tumour size of <4cm was observed in 58.56% of the cases and tumor size of >4 cm was observed in 12.6% of the cases.

Distribution Of Histological Tumour Grade
In our study, the majority of the tumors were moderately differentiated (G2) (50.9%) followed by well differentiated (G1) (36.4%) and poorly differentiated (G3) (12.7%) grades (Table 7). In accordance with our study, the study by Abdulla et al showed the majority of the tumors to be moderately differentiated (41.67%) followed by well differentiated (39.29%) and poorly differentiated (5.24%) grades. [2] However, in the study conducted by Sonia et al majority of the tumors (36.8%) were well differentiated.

Lymph Node Status and N Stage
In our study 41.8% of the cases showed lymph node metastasis (Table 8).Out of these cases, 16.4% were in N1, 18.2% in N2 and 7.3% in N3 stages (Table 9). This is in concordance to a study conducted by Sonia et al which concluded that 41.5% of their cases showed lymph node metastasis. Also in another study by Kitamura et al similar results were noted wherein 36.8% of cases showed positive for lymph node metastasis.

Distribution of TNM Stage of OSCC
In our study, the majority of cases (41.8%) presented in stage IV followed by stage III (27.3%), stage II (20.0%) and stage I (10.9%) (Table 10). This corroborated with the studies conducted by Abdulla et al and Shenoi et al which showed maximum presentation in advanced stages (III+IV), i.e., 46.7% and 88.5% of the cases, respectively.
Most patients were diagnosed in advanced stage (stages III and IV),even in other Indian studies and studies conducted abroad. Such presentation is probably due to delayed diagnosis, poverty, lack of education, and screening programs for premalignant and early lesions of OSCC.

CK17 In Oral Squamous Cell Carcinoma
In the current study, the CK17 marker was targeted for immunohistochemical analysis in oral squamous cell carcinoma and its role in cancer prognosis. Keratins are involved in protein synthesis and epithelial cell growth signaling, organelle transport, and cell mobility and proliferation.[24] As most normal stratified squamous epithelia lack CK17, its presence in the respective tumors may be regarded as a factor of neo-expression during tumorigenesis.
Comparison Of CK17 With Other Studies
In our study, the immunohistochemical expression of CK17 was 100%, i.e., all 55 cases showed CK17 expression (Table 11). Similarly in the studies conducted Kitamura et al (2017), Sonia et al, and Mikami et al, the frequency of CK17 was 100% (Table 17).

CK17 And Histological Tumour Grade
In our study, the majority of the well-differentiated OSCC cases (80%) showed high CK17 expression (score 3) when compared to moderately differentiated (46.4%) and poorly differentiated (14.3%) cases. We found a statistically significant trend in the expression of CK17 with the increasing histological grade (p=0.005)(Table 13)
This is in agreement with various other studies (Table 18). [5,24,25,28]
Our finding that CK17 expression decreased with increasing histological grade indicates the role of CK17 in tumor differentiation and prognostication, as already postulated by other studies. It is revealed from previous studies that low CK17 expression levels may serve as a predictor of poor prognosis in OSCC patients and thus aid in the analysis of their disease-free survival. [25]

CK17 Expression And Lymph Node Status
In our study, CK17 was expressed in 41.8% of the lymph node positive cases and 58.1% of lymph node negative cases. However the correlation between CK17 expression and lymph node metastasis was not statistically significant (p=0.803) (Table 13). Similar results were observed in two studies conducted by Kitamura et al (2017) and Kitamura et al (2012) , wherein CK17 expression was seen in 36.8% and 19.04 % of lymph node positive cases respectively. [5][28]

CK17 Expression And N Stage
In our study, score 3 CK17 expression was seen in 53.1%,66.7%, 50% and 50% of N0, N1, N2 and N3 stages respectively (Table 14). There was no statistically significant association between CK17 expression and “N” stage (p=0.879). In the

study conducted by Sonia et al, 61.3% cases of N0 stage and 43.2% cases of N1+N2 stages showed score 3 CK17 expression. The authors found low/decreasing CK17 expression with nodal metastasis, however this was not statistically significant (p = 0.065). [25]

CK17 And TNM Stage

In our study higher frequency of score 3 CK17 (57.9%) was present in advanced TNM-stage tumors when compared with early TNM-stage tumors, where the frequency was 47.05%. However this association fell short of statistical significance (p=0.08) (Table 16) .Similar to our study ,various other studies have not proven any statistical significance with respect to CK17 expression and TNM stage. [28] For instance ,Sonia et al conducted a study in which it was demonstrated that CK17 expression is more in advance stages when compared to early stage tumors and did not show any significant correlation between the stage and CK17 expression scores (0.678). [25]

These disparate results obtained by different studies may reflect the investigation of a relatively small number of tumors as well as the use of antibodies of diverse origin that may have differences in specificity. Another source of variability may also be due to different techniques used (i.e., immunohistochemistry vs RT-PCR techniques).

CONCLUSION

This study elaborates the histopathological characteristics of squamous cell carcinoma and provides information on CK17 immunoexpression.

The age and sex distribution were comparable to a larger extent with other nationwide studies. Majority of the tumors exhibited exophytic gross appearance with buccal mucosa being the commonest anatomical site of origin. The depth of invasion and greatest tumor dimension was <1cm and between 2-4 cms in majority of the OSCC respectively. The predominant histological grade of OSCC was G2 followed by G1. Most of the tumors presented at advanced TNM stage (III + IV).

All the OSCC cases expressed CK17 (100%) with 45.5% of the tumors exhibiting low expression (score 1 & 2) and 54.5% exhibiting high expression (score 3).

There was a statistically significant correlation between CK17 expression and histological tumor grade/differentiation with well differentiated tumors exhibiting high/greater expression when compared to moderate and poorly differentiated tumors. Further, a significant decreasing trend of CK17 immunoexpression was observed with increasing tumor grades.

A statistically significant association between lymph node metastasis and CK17 expression was not observed. Even though advanced TNM-stage tumors showed greater frequency of high CK17 expression (score 3) when compared to early stage tumors, this association fell short of statistical significance.

Because of the statistically significant correlation between decreasing CK17 expression and increasing histological grade, low CK17 immunoexpression can be used as a predictor of poor prognosis in OSCC patients.

However, further studies are needed on larger and varied sample sizes to expound the role of CK17 expression in predicting the survival of patients with OSCC

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Conflict of Interest

"The authors declare no conflict of interest."

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