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ORIGINAL RESEARCH

Correlation of Cyclin D1 and Ki 67 Immunoexpression in Head and Neck Squamous Cell Carcinomas

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ABSTRACT

Background: Head and neck cancer is the sixth most prevalent cancer, constituting 3% of all localizations. In 48% of instances, oral cancers are squamous cell carcinomas. Epidermal growth factor receptor (EGFR) is an ErbB family tyrosine kinase receptor expressed in solid tumours, including head and neck squamous cell carcinoma (HNSCC). HNSCCs have various clinicopathological characteristics and a dismal prognosis.

Martial and Methods: 160 HNSCCs were studied in the Department of Pathology, Mallareddy Medical College for women 18 months [Nov 2020-April 2022] for the immunoexpression of Cyclin D1 and Ki 67 with relation to histopathological grade and various clinical parameters such as age, gender, history of smoking, alcohol consumption, tobacco chewing, anatomical site involvement, lymph node status, and cancer stage wherever available.

Results: 113 of 160 cases were male; 47 were female. 106 head and neck squamous cell carcinomas (66.25%) were male and drank. 26 of 160 cases were radical neck dissected. 73% of patients had metastatic lymph nodes. 14 cases (66.6%) had >50% immunoreactive cells. 13 of 21 patients (62 %) were Ki-67 immunopositive. Lymph node-positive HNSCC had higher Cyclin D1 and Ki 67 immunoexpression. In 160 of 29 patients, cyclin D1 positive was unrelated to clinical stage. Stage IV tumours (66.6 %) had the highest Ki 67 values, followed by stage III. Higher pathogenic stages enhanced Ki 67 expression (p=0.037). Cyclin D1 and KI 67 immunoexpression correlated with lymph node metastases (p = 0.005 and 0.008, respectively).

Conclusion: The present investigation found a link between Cyclin D1 and Ki 67 immunoexpression correlated with histopathological grade and lymph node status, and Ki 67 with advanced tumor stage.

Keywords: Cyclin D1, and Ki 67, Immunoexpression, Histopathology, Lymphnode metastasis.

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INTRODUCTION

The cell cycle underlying all organisms helps in growth, renewal, and repair. Cancer alter the cell cycle control. Tumors disrupt the signalling mechanism that promotes DNA replication and mitosis. Oncogenes that impact the cell cycle (e.g., cyclins, CDK, and CDI) or stress or damage response genes are examples of these changes (p53).[1,2] These genetic mutations cause cancer, serve as prospective therapeutic targets, and/or serve as diagnostic and prognostic indicators. Cellular proliferation proceeds in an orderly fashion through the cell cycle stages, and at each transition, signalling pathways check for the successful completion of upstream activities before moving on to the next phase. Cyclin D1 is a protein that acts as a G1/S checkpoint. Many epithelial malignancies cause G1/S disruption.[3-5] Cyclin D1 is a proto-oncogene that regulates cell cycle progression from G1 to S. It is required for the growth and progression of several cancer types, including oral epithelial carcinoma. Cyclin D1 expression that is abnormal, whether through rearrangement, amplification, or transcriptional up-regulation, contributes to the loss of normal cell cycle control, which is connected to an increased risk of carcinogenesis (Zhao et al, 2014). In curable cases of squamous cell carcinoma of the head and neck (HNSCC), overexpression of CyclinD1 has been linked to recurrence and decreased overall survival.[1,4,5] This highly labile protein controls the cell cycle's G1/S transition by interacting with cyclindependent kinases, which phosphorylate retinoblastoma protein and increase cell proliferation Cyclin D1, a cell cycle regulator, is overexpressed in HNSCCs. Cyclin D1 substantially corresponds with other prognostic pathological characteristics of squamous cell carcinomas of the head and neck.[6,7] Squamous carcinomas account for 3% of all human malignancies and 90% of oral malignant tumours, with approximately 350 000 new cases diagnosed each year.[8-10] Cigarettes and alcohol are substantial risk factors for the formation of lesions . Men above the age of 40 are more likely to develop lip and lingual squamous oral carcinomas.[11,12] Precancerous lesions, invasive carcinoma, and metastases are all components of oral carcinogenesis.[7,8] Control mechanisms are lost, ensuring proper tissular functioning throughout cell cycle development and tumour cell growth. The expression of proteins involved in biomolecular pathways demonstrates the need for oral squamous carcinoma biomarkers. P53 and p16 are cell cycle regulatory proteins that "arrest" and apoptose cancer cells while also inhibiting their proliferation by maintaining hypophosphorylated retinoblastoma protein.[13,14] There is no concurrence on the timing and prognostic significance of aberrant protein expression.[15] In advanced HNSCCs, the proliferation marker Ki 67 is a prognostic predictor. Ki 67 was traditionally thought to be a terrible omen. This HNSCC study looks into the immunopositivity of Cyclin D1 and Ki 67 and links their expression patterns with a range of clinicopathological variables to help guide patient care and prognosis.

OBJECTIVES

1. To study the expression of Cyclin D1and Ki 67 in Head and Neck Squamous Cell Carcinomas by immunohistochemistry.
2. To correlate the expression of Cyclin D1 and Ki 67 with histopathological grading and other variables such as gender, age, anatomical site, smoking history, alcohol consumption history and stage of cancer (wherever available).

MATERIALS & METHODS

The present study was conducted in the Department of Pathology, Mallareddy Medical College for women. It was a retro-prospective study from the period of November 2020-April

2022 comprising of samplesfrom 160 patients. Institutional ethical committee clearance was obtained.

Study duration: 18 months [November 2020 to April 2022]

Sample size: 160 cases.

Inclusion criteria:

All cases diagnosed as primary squamous cell carcinoma on routine biopsy of head and neck lesions irrespective of age and gender of patient.

Exclusion criteria

1. Head and neck lesions diagnosed as carcinoma in situ.
2. Cases of squamous cell carcinoma deposits in Lymph nodes of Head and Neck region.

Sample collection:

In the purpose of performing a prospective study, biopsies and tissue samples taken from patients who had been diagnosed with squamous cell carcinomas of the head and neck region were investigated. In order to conduct a retrospective investigation, samples that had been previously classified as HNSCCs were embedded in paraffin, and the corresponding hematoxylin and eosin stained sections of those samples were collected from the archives of the pathology department. In each and every one of the cases, the proforma was filled out with all of the clinical particulars that were pertinent to the study.

Sample Processing:

Biopsies and tissue samples were fixed in 10 % Neutral buffered formalin solution for an average period of 24 hours. Resection specimens were also fixed as said above and bits were given from representative areas and processed in Microm histokinette, paraffin embedded using an automated instrument and sections (4-5 microns thickness) were cut using Microm microtome having section transfer system facility. Sections were taken onto slides and stained by routine Hematoxylin and Eosin (H&E) stain (annexure). Hematoxylin and eosin stained sections diagnosed as SCC, were further graded according to descriptive Broder's criteria as well differentiated, moderately differentiated and poorly differentiated tumours. The sections were further subjected to Immunohistochemistry (IHC) for CyclinD1 and Ki-67 using peroxidase –antiperoxidase method and Daminobenzidine (DAB) chromogen. Immunohistochemistry for Cyclin D1 was performed using monoclonal antibodies from Biogenex and Scytek, and for Ki-67 using MIB 1 clone from Dako laboratories. Staining in the basal and suprabasal layer of the normal stratified squamous epithelium in the sections is taken as positive control. Negative control was taken by staining those sections by omitting the primary antibodies. Labelling index percent for Cyclin D1 [Positivity Index -PoI] and Ki-67 [proliferation index PI] were calculated by reporting the number of positive cells (nuclei) to the total number of cells (positive and negative), with the result expressed in percentage. A minimum of 500 cells were counted under 40X objective (High power objective). For the immunoquantification of Cyclin D1 and Ki-67, a semiquantitative evaluation system consisting of three levels : <10%, 10-50%, and >50% was used. The histological grade on the H&E sections and the expression of Cyclin D1 and Ki-67 were correlated along with other clinical parameters.

Statistical Analysis:

Descriptive statistical analysis such as mean, median, proportion was calculated using Microsoft excel. Chi-square test of independence and goodness of fit was employed to determine the probability (p) value. A p value of < 0.05 was considered as statistically significant.

RESULTS

Age distribution

The age of patients ranged from 21 to 90 years with a mean age of 55.8 years. Maximum number of cases was seen in the age group of 51 to 60 years (31.25%) and least number of cases in the age group of 81-90 years (1.2%). Only three cases were seen in the young age group of 21 to 30 years.

Table 1: Age distribution in Head and Neck Squamous cell carcinomas

Age Group In Years	Number of Cases	Percentage E
21-30	5	3.125
31-40	17	10.62
41-50	31	19.38
51-60	50	31.25
61-70	46	28.75
71-80	9	5.62
81-90	2	1.25

Gender distribution:

Out of 160 cases, 113 (70.62%) were males and 47 (29.38%) were females.

Table 2: Gender distribution in Head and Neck Squamous cell carcinomas

Gender	Number of Cases	Percentage
Male	113	70.62
Female	47	29.38
Total	160	100

Distribution of cases among smokers and non-smokers.

One hundred and four cases had a history of smoking, accounting for 65 % of total number of cases with all of them being males (100%). Among the category of non-smokers (56 cases, 35 %), 9 were males and 47 were females.

Table 3: Distribution of cases among smokers and non-smokers

Smoking History		Number of Cases
Smokers	104 (65 %)	M = 104 (100 %) F = 0
Non Smokers	56 (35 %)	M = 09 (16 %) F = 47 (84 %)
Total	160	

Duration of smoking history in patients with HNSCC.

Of the 104 cases that had a history of smoking, maximum number of patients (49cases, 47.1 %) had a history of smoking for a period of 21 to 30 years. Twenty percent of patients had a history of smoking for more than 30 years.

Table 4: Duration of smoking history in patients with HNSCC

Duration of Smoking In Years	Cases	Percentage
0-10	12	11.5
11-20	20	19.2
21-30	49	47.1

31-40	21	20.4
41-50	2	1.9
Total	104	100

Distribution of cases in patients with history of alcohol consumption.

One hundred six cases (66.25%) of head and neck squamous cell carcinomas gave a positive history of alcohol consumption, all of whom were males.

Table 5: Distribution of cases in patients with history of alcohol consumption

Alcohol Consumption History	**Number of Cases**	
Yes	106 (66.25 %)	M = 106 (100 %) F = 0
No	54 (33.75 %)	M = 07 (13 %) F = 47 (87.0 %)
Total	160	

Distribution of cases with respect to history of tobacco chewing.

Ninty four cases had a positive history of tobacco chewing accounting for 58.8 % of cases. There was an almost equal distribution between both the genders.

Table 6: Distribution of cases with respect to history of tobacco chewing

History of Tobacco Chewing		**Number of Cases**
Yes	94 (58.8)	M= 52(55.3 %) F = 42 (44.7 %)
No	66 (41.2 %)	M = 61 (92.4 %) F = 05 (7.6 %)
Total	160	

Distribution of cases with respect to site of the lesion.

Majority of the cases (73 cases, 45.7 %) were seen in the oral cavity, with buccal mucosa 35 [21.9 %] being the commonest site, followed by the tongue 29 [18.12]. Among 31.2 % of pharyngeal Squamous cell carcinoma, the pyriform fossa was found to be the most commonly involved site. Supraglottic SCC was the most frequently encountered laryngeal SCC. Laterality was not considered in the assessment of site as laterality is not applicable in most of these unpaired organs of head and neck region.

Table 7: Distribution of cases with respect to site of the lesion

squamous cell carcinoma in Head and Neck			**Number of cases n[%]**	**Males**	**Females**
LARYNX (n=37) (23.1)%	Epiglottis		3 [1.87]	2	1
	Aryepiglottic fold		1 [0.6]	1	0
	Supraglottis		15 [9.37]	13	2
	True Vocal cord		13 [8.12]	11	2
	False vocalcord		1 [0.6]	1	0
	Glottis		2 [1.25]	2	0
	Cricoid		1 [0.6]	1	0
	Post cricoid		1 [0.6]	0	1
Pharynx (n=50) (31.2 %)	Oropharynx	Tonsil	12 [7.5]	9	3
		Posterior pharyngeal wall	11 [6.9]	9	2

	laryngopha rynx	Pyriformf ossa	19 [11.9]	17	2
		vallecula	8 [5.0]	7	1
Oralcavity (n=73) – (45.7 %)	Tongue		29 [18.12]	19	10
	Base of tongue		9 [5.62]	6	3
	Buccal mucosa		35 [21.9]	20	15

Distribution of cases with respect to histopathological grade of squamous cell carcinoma.

Well-differentiated type was the predominant histologic grade accounting for 71 cases (44.4%), followed closely by moderately differentiated type (67 cases, 41.9%) and then by poorly differentiated type (22 cases, 13.7%).

Table 8: Distribution of cases with respect to histopathological grade of squamous cell carcinoma

Histologic Grade	Number of Cases	Percentage
Well differentiated	71	44.4
Moderately differentiated	67	41.9
Poorly differentiated	22	13.7
Total	160	100

Immunoexpression of Cyclin D1 and Ki 67 proliferation index in study cases.

Table 9: Immunoexpression of Cyclin D1 and Ki 67 proliferation index

Antibody	No of casees			Total
	≤ 10 %	11 – 50 %	> 50%	
Cyclin D 1	49 (30.62%)	40 (25.0%)	71 (44.37%)	160
Ki 67	39 (24.37 %)	45(28.12%)	76 (47.5%)	160

Immunopositivity of Cyclin D1 in >50% of tumour cells was seen in a majorityof 71 cases (44.37%), followed by 49 cases (30.62%) showing ≤10% immunostained cells. The remaining 40 cases (25.0%) showed intermediate score of 11-50% of immunopositivity. Higher Ki 67 proliferative index of >50% was seen in a majority of 76 cases (47.5%), followed by intermediate Ki 67 core of 11-50% in 45 cases (28.12%). A low Ki 67 proliferative index of ≤10% was seen in the remaining 39 cases (24.37 % cases).

Correlation of immunoexpression of Cyclin D1 and Ki 67 with various histopathological grades of Head and Neck Squamous cell carcinoma.

Table 10a: Correlation of Cyclin D1 immunoexpression in relation tohistopathological grade of HNSCC

Grades of SCC	Cyclin D 1 expression			Total	
	≤ 10 %	11 – 50 %	> 50%		
Well differentiated	32 (45.07 %)	21 (29.6 %)	18 (25 %)	71 (100 %)	P = < 0.001
Moderately differentiated	15 (22.4 %)	17 (25.4 %)	35 (52.2 %)	67 (100 %)	
Poorly differentiated	2 (9 %)	2 (9 %)	18 (82 %)	22 (100 %)	
Total	49	40	71	160	

Out of 71 well differentiated Squamous cell carcinomas, majority [32 cases (45.07%)] of cases showed Cyclin D1 positivity index of ≤10%. Among moderately differentiated type, 35 cases (52.2%) showed Cyclin D1 positivity index of > 50%. Eighty two percent (18 out of 22 cases) of poorly differentiated type of Squamous cell carcinoma showed Cyclin D1 positivity index of > 50%. The correlation of Cyclin D1 immunoexpression was found to be statistically significant with respect to the various histopathological grade of HNSCC (p=<0.001).

Table 10b: Correlation of Ki 67 immunoexpression in relation tohistopathological grade of HNSCC

Grades of SCC		**Ki 67 expression**		**Total**	
	≤ 10 %	**11 – 50 %**	**> 50%**		
Well differentiated	24 (33.8 %)	27 (38.0 %)	20 (28.2 %)	71 (100 %)	**P = < 0.001**
Moderately differentiated	13 (19.4 %)	14 (21.0 %)	40 (59.6 %)	67 (100 %)	
Poorly differentiated	2 (9 %)	4 (18 %)	16 (73 %)	22 (100 %)	
Total	39	45	76	160	

Out of 71 well differentiated squamous cell carcinomas, 27 cases (38.0%) showed Ki 67 proliferative index of 11-50% and 24 cases showed ≤10%. Among moderately differentiated grade, most of them i.e., 40 cases (59.6 %) showed Ki 67 proliferative index of > 50%. Majority of the poorly differentiated HNSCC, 73 % (16 out of 22 cases) showed a high Ki 67 proliferative index of > 50% immunoreactive cells. The correlation of Ki 67 immunoexpression was found to be statistically significant with respect to the various histopathological grade of HNSCC (p=<0.001).

Correlation of Cyclin D1 and Ki 67 with age and gender.

Table 11a: Correlation of Cyclin D1 expression in relation to age and gender

Variables		**Cyclin D 1 expression**		**Values**
	≤ 10 %	**11 – 50 %**	**> 50%**	
Age <50 yr s n = 53	12 (22.7 %)	15 (28.3 %)	26 (49.0 %)	0.924
Age >50 yrs (n=107)	22 (20.5 %)	27 (25.2 %)	58 (54.3 %)	
Gender Male (n=113)	28 (24.8 %)	27 (23.9 %)	58 (51.3 %)	0.348
Gender Female (n=47)	8 (17.0 %)	15 (32.0 %)	24 (51.0 %)	

Table 11b: Correlation of Ki 67 expression in relation to age and gender

Variables		**Ki 67 expression**		**Values**
	≤ 10 %	**11 – 50 %**	**> 50%**	
Age <50 yr s n = 53	7 (13.2 %)	17 (32.0%)	29 (54.8 %)	0.677
Age >50 yrs (n=107)	20 (18.7 %)	30 (28.0 %)	57 (53.3 %)	
Gender Male (n=113)	21 (18.6 %)	30 (26.5 %)	62 (54.9 %)	0.366

Gender Female (n=47)	6 (12.8 %)	15 (32 %)	26 (55.2 %)	

As is evident from the above two tables, there is no significant association between immunoexpression of Cyclin D1 and Ki 67 and parameters of age and gender in HNSCC.

Correlation of cyclin D1 and Ki 67 with smoking history, duration of smoking, alcohol consumption and tobacco chewing.

Table 12a: Correlation of cyclin D1 with smoking history, duration of smoking, alcohol consumption and tobacco chewing

Variables		**Cyclin D1 expression**		**P Value**
	≤ 10 %	**11 – 50 %**	**> 50%**	
SmokingHistory Yes (n=104) No (n=56)	 24 (23.0 %) 10 (17.9 %)	 22 (21.2 %) 18 (32.1 %)	 58 (55.8 %) 28 (50 %)	0.232
Duration of smoking (n=104)	9 (28.1 %)	4 (12.5 %)	19 (59.4 %)	
<20 yrs(n=32)				0.067
>20 yrs(n=72)	22 (30.5 %)	19 (26.4 %)	31 (43.1 %)	
Alcohol Consumption Yes (n=106) No (n=54)	 25 (23.6 %) 10 (18.5 %)	 23 (21.7 %) 16 (29.6 %)	 58 (54.7 %) 28 (51.3 %)	0.313
Tobacco chewing Yes (n=94)	19 (20.0 %)	27 (28.7 %)	48 (51.3 %)	0.571
No(n=66)	17 (25.7 %)	16 (24.2 %)	33 (50.1 %)	

Correlation of Cyclin D1 and Ki 67 with Major anatomical sites.

Table 13a: Correlation of Cyclin D1 with Major anatomical sites (160 N)

Anatomic site		**Cyclin D1 expression**		**P value**
	≤ 10 %	**11 – 50 %**	**> 50%**	
Oral cavity n = 73	15 (20.5 %)	23 (31.5 %)	35 (48.0 %)	0.336
Pharynx n = 50	8 (16.0 %)	12 (24.0 %)	30 (60.0 %)	
Larynx n = 37	11 (29.8 %)	8 (21.6 %)	18 (48.6 %)	

Table 13b: Correlation of Ki 67 with different Major anatomical sites (N = 160)

Anatomic site	**Ki 67 expression**			**P value**
	≤ 10 %	**11 – 50 %**	**> 50%**	
Oral cavity n = 73	9 (12.3 %)	27 (37 %)	37 (50.7 %)	0.072
Pharynx n = 50	6 (12.0 %)	15 (30.0 %)	29 (58.0 %)	
Larynx n = 37	10 (27.0 %)	9 (24.3 %)	18 (48.7 %)	

As evidenced by the above two tables, there was no significant differing immunoexpression of Cyclin D1 and Ki 67 in the various major anatomical sites of HNSCC. The indices were found to besimilarly distributed in different anatomical sites.

Correlation of Cyclin D1 and Ki 67 in relation to lymph node metastasis.

Table 14: Correlation of Cyclin D1 and Ki 67 in relation to lymph node metastasis

Anti boby		**No of cases**		**P value**
	≤ 10 %	**11 – 50 %**	**> 50%**	
Cyclin D1 n = 21	4 (19.04 %)	3 (14.34 %)	14 (66.66 %)	0.005

Ki 67 n = 21	2 (9.4 %)	6 (28.6 %)	13 (62.0 %)	0.008

In only twenty six of the 160 study cases, radical neck dissection was performed. Of these 21 cases (73%) showed metastatic deposits in the lymph nodes. The study of Cyclin D1 immunoexpression in these 21 cases showed a majority of them i.e., 14 cases (66.6%) with > 50% immunoreactive cells. Similarly 13 out of 21 cases (62 %) showed a high Ki 67 proliferative index of > 50% immunopositivity. The increasing immunoexpression of Cyclin D1 and Ki 67 in lymph node positive HNSCC was found to be statistically significant.

Correlation of Cyclin D1 and Ki 67 with Pathological stage of HNSCC.

Table 15: Correlation of Cyclin D1 and Ki 67 with Pathological stage HNSCC

Stage (n = 29)	**Cyclin D1 and Ki 67 expression**			**P value**
	≤ 10 %	**11 – 50 %**	**> 50%**	
Cyclin D1 StageI (n=3)	2 (67 %)	0 (0 %)	1 (33 %)	0.222
StageII (n=4)	0 (0 %)	0 (0 %)	4 (100 %)	
StageIII (n=10)	3 (30 %)	3 (30 %)	4 (40 %)	
StageIV (n=12)	1 (8.4 %)	1 (8.4 %)	10 (83.2 %)	
Ki 67 StageI (n=3)	2 (67 %)	0 (0 %)	1 (33 %)	0.037
StageII (n=4)	0 (0 %)	0 (0 %)	4 (100 %)	
StageIII (n=10)	0 (0 %)	4 (40 %)	6 (60 %)	
StageIV (n=12)	1 (8.3 %)	3 (25.1 %)	8 (66.6 %)	

160 out of the 29 cases in which pathological staging could be assessed; immune positivity of cyclinD1 was found not to be significantly associated with any of the stages. However, a significant increase in Ki 67 proliferative index was found in tumours of stage IV, [8 cases, 66.6 %] followed by stage III tumours. Increasing Ki 67 expression with respect to higher pathological stages was found to be statistically significant (p=0.037).

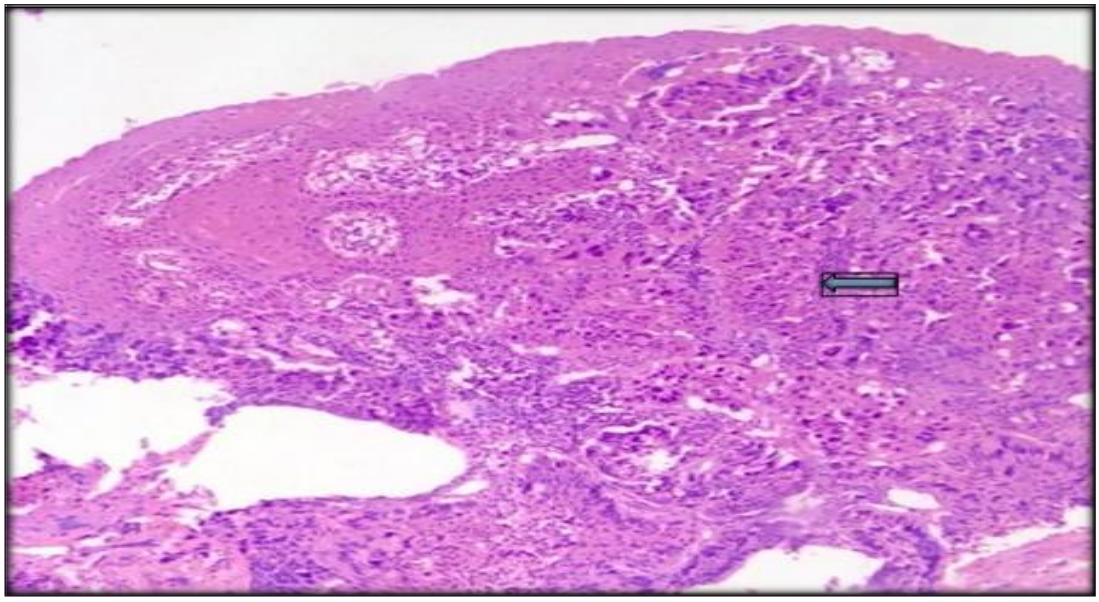

Figure 1: Microphotograph of Squamous cell carcinoma showing invasion into subepithelium [H&E 10x4]

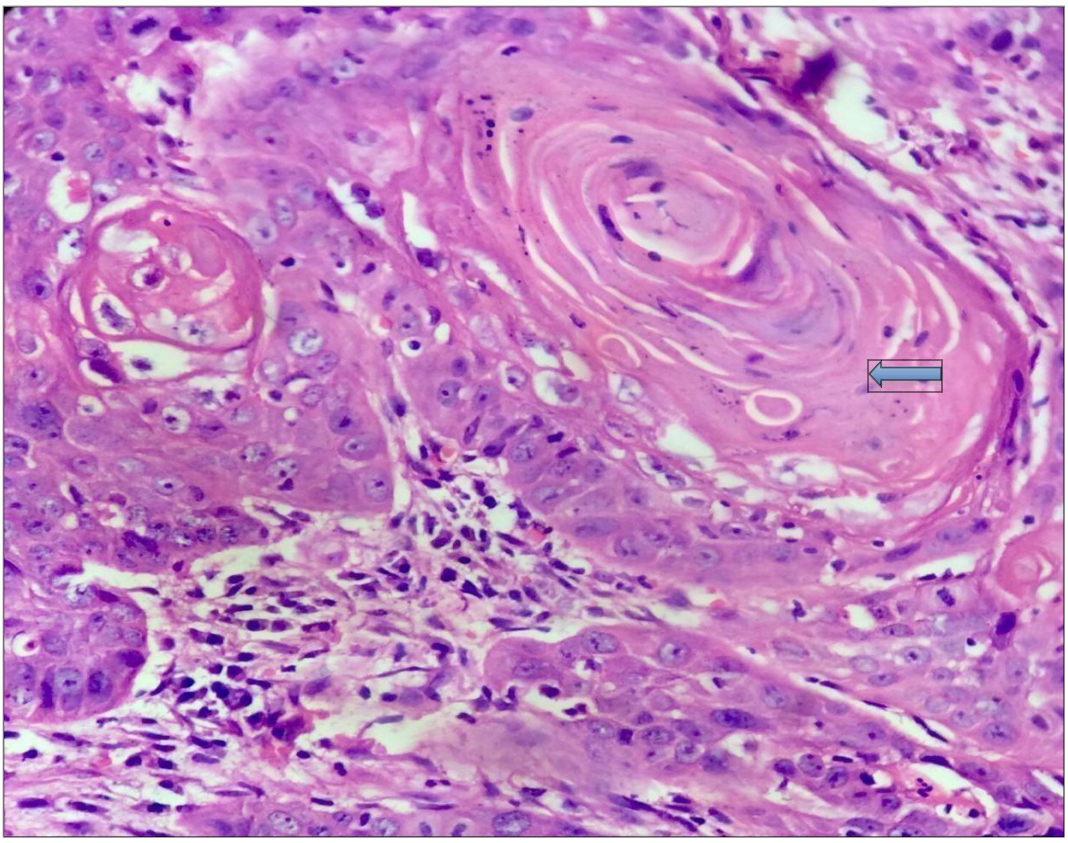

Figure 2: Microphotograph showing keratin pearls in well differentiated Squamous cell carcinoma [H&E 10x10]

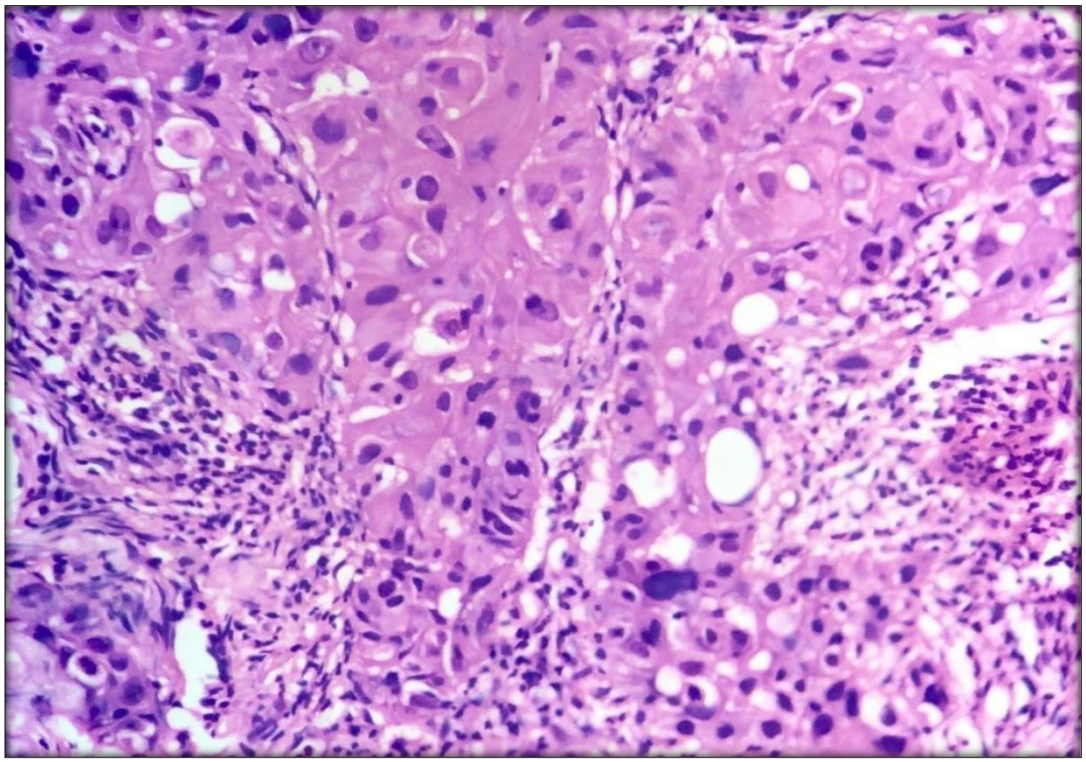

Figure 3: Microphotograph showing moderately differentiated squamous cell carcinoma (H&E-10x40)

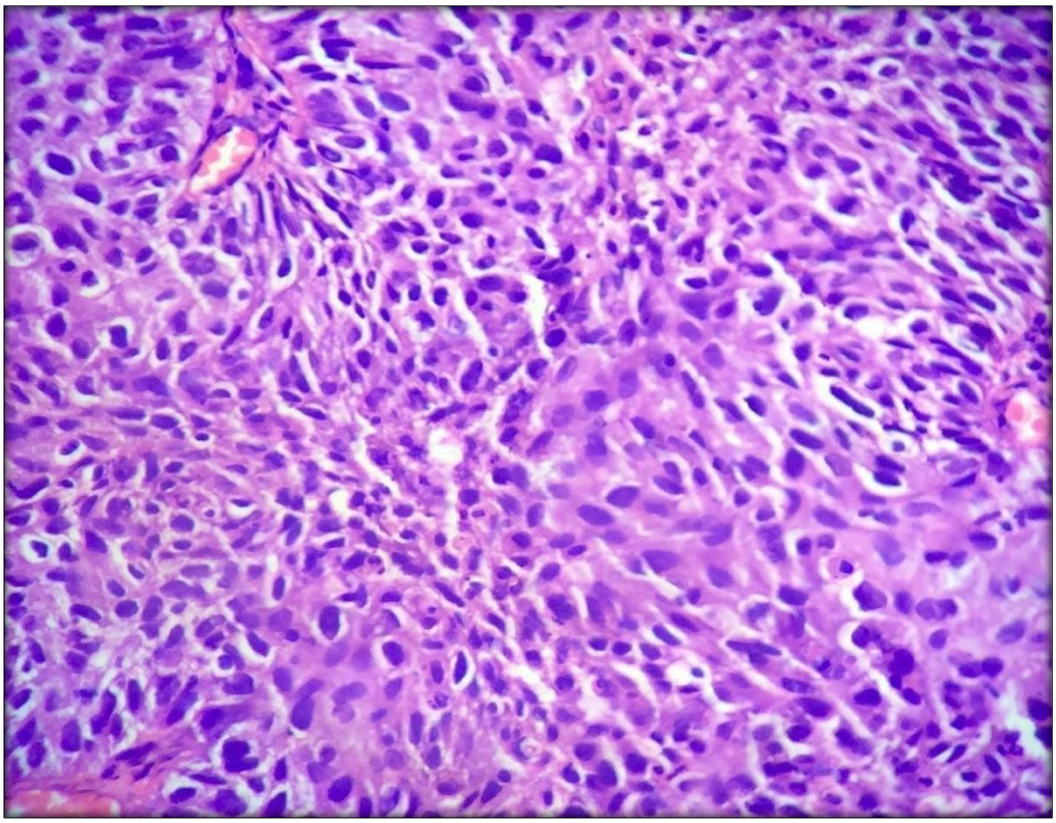

Figure 4: Microphotograph showing poorly differentiated squamous cell carcinoma (H&E 10x40)

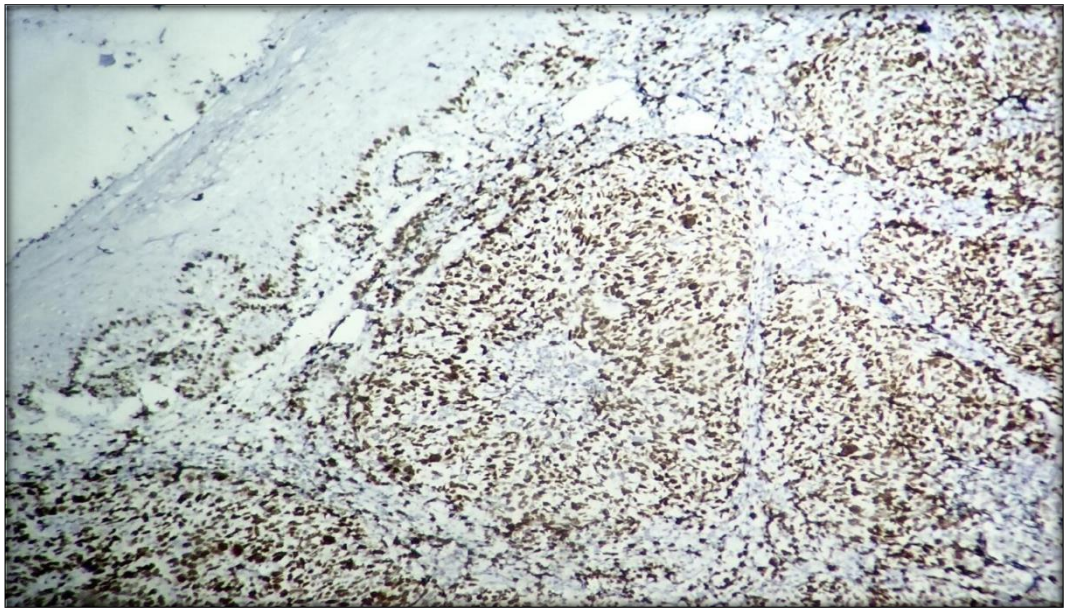

Figure 5: Microphotograph showing nuclear immunoexpression of Cyclin D1in squamous cell carcinoma with overlying normal epithelium showing positivity in basal layer (internal control). (IHC, 10x40)

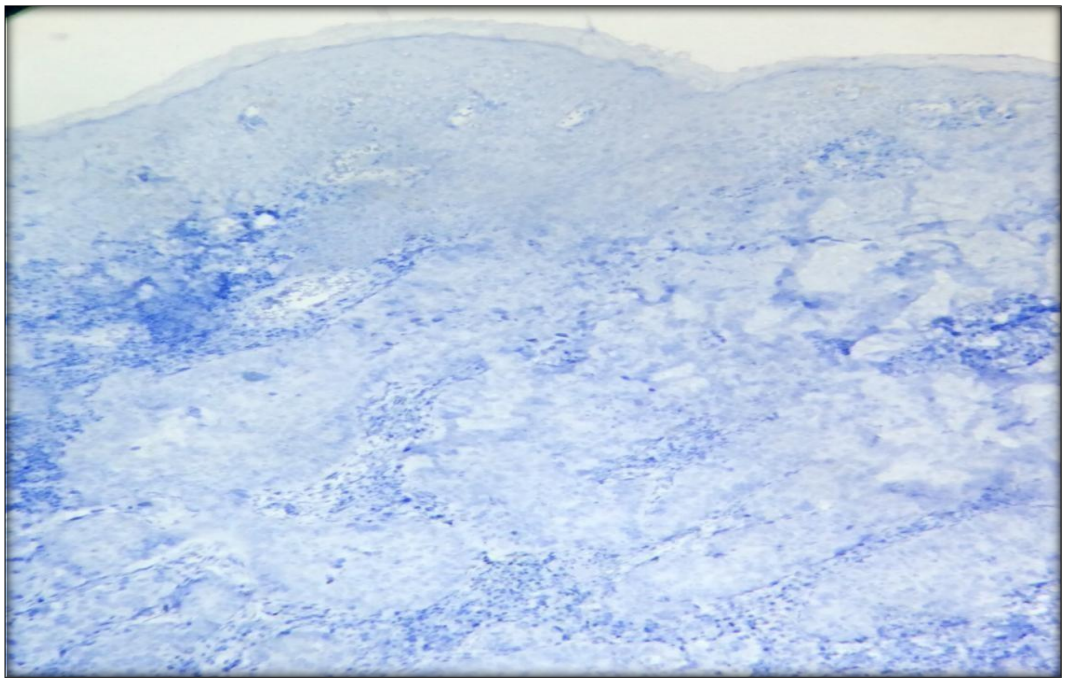

Figure 6: Microphotograph showing IHC stain without the primary antibody (Negative External Control) (10x40)

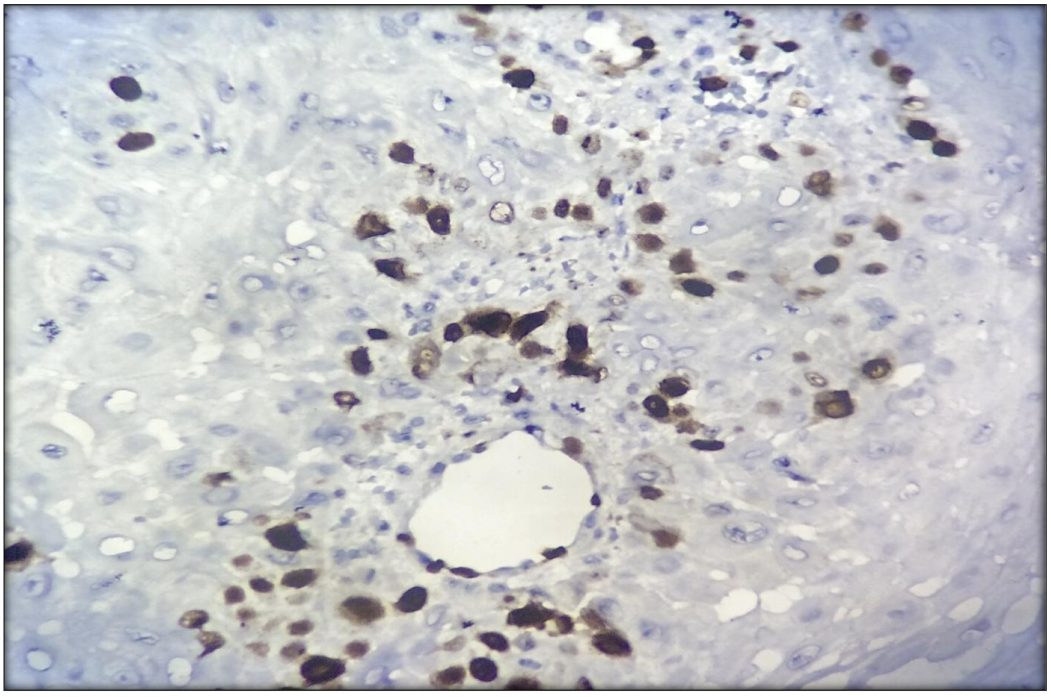

Figure 7: Microphotograph showing nuclear immunoexpression of Cyclin D1 in well differentiated squamous cell carcinoma (score-<10%) (IHC, 10x40)

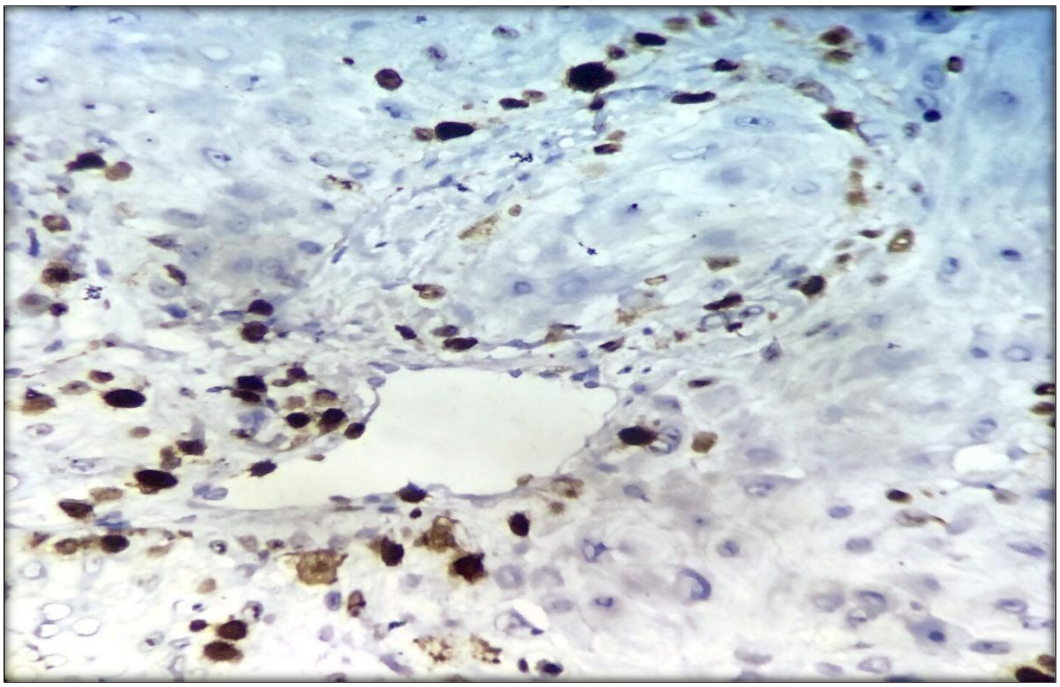

Figure 8: Microphotograph showing nuclear immunoexpression of Ki 67 in well differentiated squamous cell carcinoma (score-<10%) (IHC, 10x40)

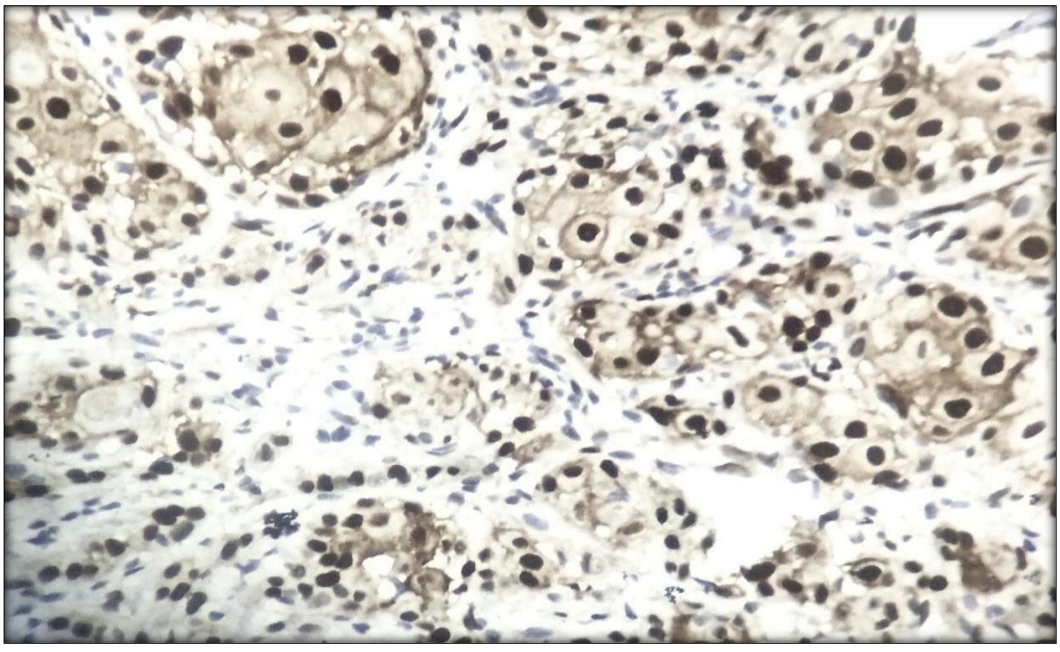

Figure 9: Microphotograph showing nuclear immunoexpression of Cyclin D1 in moderately differentiated squamous cell carcinoma (score 45%)(IHC, 10x40)

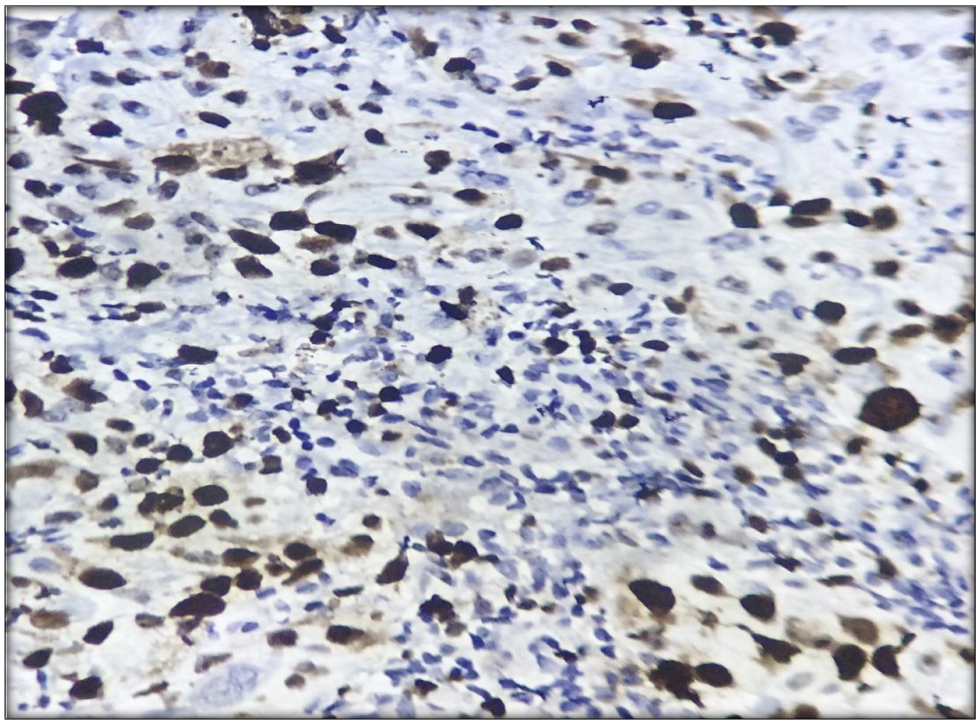

Figure 10: Microphotograph showing nuclear immunoexpression of Ki 67 inmoderately differentiated squamous cell carcinoma (score 40%)(IHC,10x40)

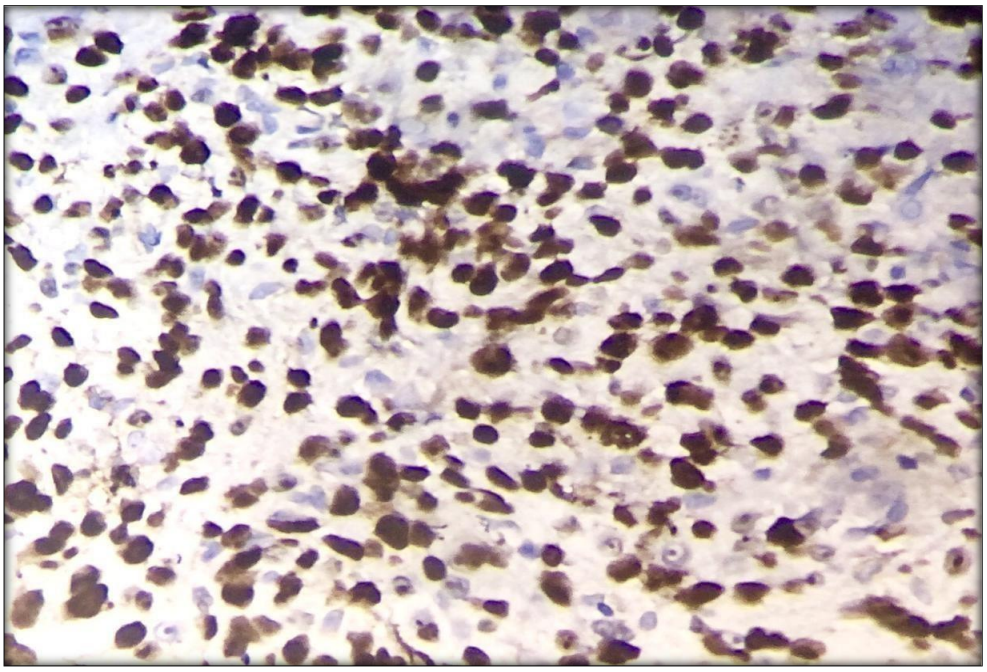

Figure 11: .Microphotograph showing nuclear immunoexpression of Cyclin D1in poorly differentiated squamous cell carcinoma (score-85%)(IHC,10x40)

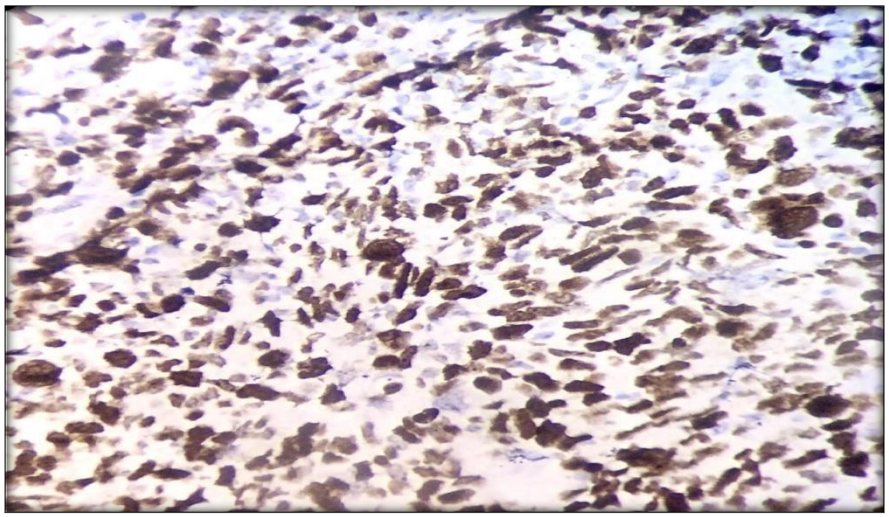

Figure 12: **Microphotograph showing nuclear immunoexpression of Ki 67 in poorly differentiated squamous cell carcinoma (score-95%)(IHC,10x40)**

DISCUSSION

Over the duration of 18 months, 160 cases of primary head and neck squamous cell carcinomas were studied at the Department of Pathology at Mallareddy Medical College for women, Hyderabad (November 2020 to April 2022). On each of the 160 instances, histopathological examination, evaluation for immunoexpression of Cyclin D1 and Ki 67 expression, and linkage with clinical data were also performed. These parameters were compared to those discovered in studies conducted by a number of other authors that were extremely similar.

Age distribution

The vast majority of patients diagnosed with HNSCC are above the age of 50. In our study of 160 patients, we discovered that the average age was 56 years old. Male and female patients were included. This is nearly identical to other research, such as Vicente et al,[16] who discovered that the average patient age was 56.6 years, and Yu et al,[17] who discovered that the average patient age was 61.5 years. This is a perfect representation of how well this compares to other research Mohanapure et al,[18], study. All patients' ages ranged from 35 to 75 years, with a mean of 52.86 years. Olimid et al investigation discovered Males comprised more than three-quarters of the 44 patients with oral squamous cell carcinomas. Males mainly composed more than three-quarters of the 44 patients with oral squamous cell carcinomas.[7]

Gender Distribution:

HNSCC are most frequently seen in men due to their increased susceptibility to potentially dangerous behaviours as a result of their personal habits. A number of research conducted by academics all over the world have revealed a detectable increase in the incidence of HNSCC in the male gender. There was also a male preponderance in the current study, which has previously been observed. According to the Gupta et al study,[19] the majority of cases (70.62 percent) occurred in men aged 40 to 60. (85.1 percent). Female patients constituted 29.38 % of the total. The male-female ratio in our study is 5.7:1. The majority of cases occurred in the fourth and fifth decades.

A brief history of smoking

The use of tobacco products is one of the most significant risk factors that can lead to the development of HNSCC. Authors such as Zhang et al and Huang et al have conducted extensive research on a large number of cases, and their findings have shown that the

majority of their subjects had a history of smoking. Similarly, 65 percent of our cases were determined to be smokers who had a lengthy and consistent history of smoking,[20,21] Sulaiman et al,[11] investigated oral epithelial cell proliferation markers including labelling index (ki67-LI) and tumour suppressor gene protein (p16) in smokers. Compared to nonsmokers, ki67-LI was high in both cancerous and noncancerous cells, which strengthens the concept that smoking products exaggerate epithelial cell division. This exaggeration was seen in both SCC and NNOE (more evident among the non-neoplastic hyperplastic epithelium). Lopes et al,[23] observed that no correlation was found between Ki-67 immunostaining and age (p = 0.610), gender (p = 0.530), smoking (p = 0.945), primary tumour site (p = 0.163), lymph node metastasis (p = 0.163), or disease stage (p = 0.163). No correlation was found between Ki-67 immunostaining and age (p = 0.610), gender (p = 0.530), smoking (p = 0.945), primary tumour site (p = 0.163), lymph node metastasis (p = 0.163), or disease stage (p = 0.163).

A record of past alcohol consumption.

Recent research carried out by the aforementioned authors reveals that alcohol use is a factor in the etiopathogenesis of head and neck squamous cell carcinoma (HNSCC). According to the findings of Kaminagakura et al.[23] investigation of 90 cases, 67.7 percent of the participants had a history of considerable alcohol intake. The current study indicated that 66.25 % percent of the patients who participated in the study admitted to having a considerable history of alcohol drinking. This was another finding of the study. In the L. P. Dragomir et al,[15] investigation, the age of diagnosis was 40–60 (61.8%), mostly men (85.3%), with exposure to risk factors (tobacco and/or alcohol and position on the lips) (61.8 percent). Literature shows comparable results, but with a growing occurrence in females, patients are exposed to many possible risk factors, tobacco and alcohol on top.

Chewing tobacco's history

Chewing tobacco is one of the many forms of tobacco use that has been proven as being an important risk factor in the development and progression of HNSCCs. According to the data presented in the table above, researchers discovered that greater than three-quarters of their patients had a previous history of chewing tobacco. According to the findings of the current research, 58.8 percent of the cases had a good history of tobacco chewing. This finding is in agreement with the findings of other writers,[15,23] The Human Head and Neck Squamous Cell Carcinoma is the sixth most common cancer in the world. The oral cavity, the pharynx, and the larynx are the principal anatomical locations in the head and neck region that are affected by SCC. Other sites such as the skin of the external ear, the scalp, and the nasal region come in second and third, respectively. When the authors of this study restricted their focus to oral cavity SCCs, they discovered that the tongue and the lip are the most common places where these cancers occur. Michalides et al,[24] discovered that the hypopharynx was the most often affected site among all of the head and neck SCCs that they researched. In their study of laryngeal SCCs, Ashraf et al,[25] noticed that the glottic region was the one that was impacted the most frequently. In the current investigation, which involved 160 instances of HNSCCs including all of the major anatomical sites, it was discovered that the majority of cases grouped in the oral cavity (45.7 percent), followed by the pharynx, and then finally by the larynx. Gupta et al,[19] found most patients (92.6%) chewed tobacco, then smoked (77.78%) and drank alcohol (55.56 percent). Most cancers were in buccal mucosa (53.7 percent). All cases underwent Ki 67 and p53 immunohistochemistry. Ki 67 was expressed in 92 instances and p53 in 72. 58 patients had both Ki-67 and p53.

Grade According to Histopathology:

It has been demonstrated that the histopathological grade has independent prognostic value. The modified Broder's grading system, which sub-classifies SCC into well-differentiated, moderately differentiated, and poorly differentiated cancer grades, was utilised in the investigations that were carried out by the authors who are listed in the table that is located above. There was a significant amount of variation in the distribution of tumour grades throughout the many investigations that were carried out on patients from various geographic locations. The current study discovered that the majority of cases, which accounted for 44.4 percent, had awell-differentiated pattern, while the least number of cases, which accounted for 13.7 percent, displayed a poorly differentiated pattern. This is consistent with the findings of Vicente et al,[16] as well as Ashraf et al,[20].

As shown by the numerous international research carried out by the aforementioned authors, a growing level of Cyclin D1 immunoexpression is observed to coincide with an increasing level of tumour grade. Over-expression of Cyclin D1 was found in 82 percent of poorly differentiated tumours in the current study, which reveals similar patterns to those seen in previous research. According to the findings of the majority of the studies that were discussed earlier, a high Ki 67 proliferation index was discovered in the majority of the poorly differentiated HNSCC (73 percent) and in the majority of the moderately differentiated SCC (61.9 percent). The current investigation discovered an increase in the immunoexpression of the Ki-67 proliferative index in tumours that displayed a worsening of their histological differentiation.

Cyclin D1 and Ki 67 levels have a correlation with age

The current investigation discovered that there was no significant association between the expression of Cyclin D1 [p=0.924] and Ki 67 [p=0.677] and age. This is confirmed by the findings of other studies like the one done by Zhang et al, which found that Cyclin D1 had a p value of 0.559 and Ki 67 had a p value of 0.446. Analysis of the Comparative Correlation between Cyclin D1 and Ki 67 with Gender. The current investigation demonstrated no statistically significant link between gender and either Cyclin D1 (p = 0.348) or Ki 67 (p = 0.366). This is consistent with the findings of researchers such as Vicente et al.and Zhang et al.[16,20]

In the Colturato et al,[28] also reported, 113 individuals (male, 95; female, 18; mean age, 60.4) with OSCC (85 cases) and OPSCC (28 instances) were identified. Immunohistochemistry was used to analyse chromogranin-A, synaptophysin, CD56, CD57, CD99, Ki-67, and cyclin D1. chromogranin-A (8.3% OSCC; 0% OPSCC), synaptophysin (9.5% OSCC; 0% OPSCC), CD99 (28.8% OSCC; 3.8% OPSCC), and CD56 were positive (1.2 percent OSCC; 11.1 percent OPSCC). Negative in OSCC and OPSCC, Ki-67 was 43.6% and 42.0%, and cyclin D1 was 33.7% and 26.6%.

Analyses of the Correlation between Cyclin D1 and Ki 67 with Regards to Smoking History and Duration

Several researchers, including Zhang et al,[16] and Vicente et al,[20] find at the correlation between smoking history and Cyclin D1 and Ki 67, however they discovered no significant link. These findings are supported by the results of the current investigation, which showed that there was no significant connection between smoking history and either Cyclin D1 (p=0.277) or Ki 67 (0.148).

History of alcohol intake and its correlation with the cyclin D1 and Ki 67 genes

In accordance with the findings of Vicente et al,[16] and Zhang et al,[20] the present investigation discovered that there was no significant association between alcohol use history and either Cyclin D1 (p = 0.393) or Ki 67 (p = 0.582).[14,15]

Cyclin D1 and Ki-67 levels have a correlation with the amount of time spent chewing tobacco.

Basnaker et al,[26] conducted a study in which they investigated the expression of Cyclin D1 in 20 cases with oral squamous cell carcinomas (OSCCs) and 20 cases with normal mucosal tissue. Both sets of participants had a history of tobacco smoking. Cyclin D1 was discovered to be expressed in 80 out of 100 cases of normal mucosa as well as in 70 percent of OSCCs, according to the investigators' findings. The findings of this study led the authors to the conclusion that the expression of Cyclin D1 in OSCCs that were associated with tobacco chewers provides evidence that tobacco chewing may generate mutations in the Cyclin D1 gene. The discovery that normal cases had higher levels of cyclin D1 expression than OSCCs do lends credence to the hypothesis that mutations in cyclin D1 occur early on in the process, even before dysplasia manifests itself. The current study demonstrated expression of Cyclin D1 and Ki 67 in HNSCCs; however, there was no statistically significant link between tobacco usage and the expression pattern of Cyclin D1 and Ki 67. Both genes were expressed in HNSCCs.[20]

Analysis of the relationship between cyclin D1 and ki 67 and key anatomical areas

The current investigation discovered that Cyclin D1 and Ki 67 levels do not have a significant association with important anatomical regions. The findings of additional research, such as those conducted by Vicente et al.13, Zhang et al,[16] and Raju et al,[29] lend credence to this assertion.

Cyclin D1 and Ki-67 levels have a correlation with the presence of lymph node involvement.

The presence of metastases in lymph nodes is an essential component of the staging system as well as an essential factor in determining the patient's prognosis. Cyclin D1 overexpression was found to have a significant association with regional lymph node metastasis (p=0.002), according to research conducted by Huang et al.16, who examined 264 SCCs. Regarding Cyclin D1, Vicente et al,[16] found results that were quite similar to those found in our study (p = 0.00005). On the other hand, they discovered that there was no statistically significant link in regard to the Ki 67 index. In the current research, out of the 21 instances that demonstrated regional lymph node metastasis, 83.2 percent of those patients showed an overexpression of Cyclin D1, and 66.6 percent of those cases showed a high Ki 67 proliferative index.

Radovi, et al,[30] study Cyclin D1 was not expressed, however HER-2 and bcl-2 were in one case each. p53 expression was reported in 8 instances (57,1 percent).

Statistically significant correlations (p0.05) were identified between lymphovascular invasion of tumour tissue and nodal metastasis; proliferation Ki67 index and tumour differentiation, i.e. size. Other analysed parameters showed no significant statistical correlation. p53 expression was not associated to any of the studied factors, suggesting an independent mechanism.

CONCLUSION

This study emphasises the immunohistochemical use of two essential markers, the cell cycle regulating Cyclin D1 and the proliferation marker Ki 67, both of which show neoplastic progression: in the routine diagnosis of head and neck squamous cell carcinomas.Cyclin D1 and Ki 67 immunoexpression is reported to be more prevalent in poorer grades of tumour differentiation, metastatic lymph node deposits, and advanced stages of the disease.Cyclin D1 and Ki 67 immunoexpression can be quite useful in assessing neoplastic behaviour in head

and neck squamous cell carcinoma and may act as independent prognostic indicators with treatment implications.

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