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A clinicopathological study of esophageal squamous cell carcinoma with special reference to Cyclin-D1 in tumour cells

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Abstract

Introduction: Esophageal squamous cell carcinoma (ESCC) is one of the lethal carcinomas with a high incidence rate in Asia and it stands at 5th position in India in terms of incidence. The cyclin-D1 gene plays an important role in its carcinogenesis. We aimed to analyze the various clinicopathological parameters associated with ESCC. CyclinD1 expression and its role as a prognostic marker are also evaluated. Cyclin D1 being a marker for cell proliferation was used in this study. The primary objectives of the current investigation were to investigate the expression of Cyclin-D1 in ESCC and to establish a relationship between the expression patterns of Cyclin-D1 and the histopathological features of the ESCC.

Materials and methods: We examined 134 samples of ESCC in the Department of Pathology, Silchar Medical College and Hospital and categorized them histologically as well-differentiated, moderately differentiated, and poorly differentiated. For assessing the expression of Cyclin-D1, immunohistochemistry was done in all these cases.

Results: Out of 134 cases, 38.8% were in 6th decade of life. Males were more commonly affected than females. The association between anemia, clinical features, habits, and ABO-Rh grouping was analyzed. The low-income population was found to be associated with its incidences. 63.4 % of cases were moderately differentiated, followed by 34.3% well differentiated and 2.2% poorly differentiated carcinoma. The Middle third of the esophagus was involved commonly. Nodal metastasis was found in 97.5% of cases and 17.5% distant metastasis. Cyclin–D1 expression was seen in 43.5% of well-differentiated carcinoma, 65% of moderately differentiated squamous cell carcinoma, and 66.7% of poorly differentiated carcinoma.

Conclusion: The study found an association between ESCC and low-income groups and males in their 6th decade. However, moderately-differentiated squamous cell carcinoma was identified as the most common type in this study. Furthermore, the finding that Cyclin-D1 expression was more prominent in poorly differentiated carcinoma of the esophagus could potentially lead to more targeted treatment options in the future. Overall, this study provides valuable insights into the characteristics of ESCC and can help guide further research in this area.

Keywords: Esophageal squamous cell carcinoma (ESCC), Histological grades, Metastasis, Immunohistochemistry, Cyclin-D1

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Introduction

Esophageal carcinoma is a significant health concern in India, ranking fifth in terms of incidence. According to GLOBOCAN 2020, it has an incidence rate of 4.8% and a cancer death rate of 6.9%. This cancer predominantly affects men, with a ratio of 6.1:3.4 compared to women (1). The predominant histological type is esophageal squamous cell carcinoma (ESCC), which is increasing in the Asian subcontinent (2). However, there is also an increasing trend in adenocarcinoma, possibly due to lifestyle changes in high development index (HDI) countries.

In the state of Assam, esophageal carcinoma is the leading cause of death in men and the second leading cause of death in women (3). This underscores the importance of raising awareness about the risks and early symptoms of this cancer for proactive prevention and early diagnosis.

Esophageal squamous cell carcinoma is commonly found in the middle and lower thirds of the esophagus. While its early symptoms are challenging to detect, being aware of the risk factors associated with this cancer is crucial. Clinical presentation typically includes progressive dysphagia, anemia, weight loss, and, in rare cases, esophageal perforation.

Squamous cell carcinoma arises from the squamous lining of the esophagus through a progression from premalignant precursors, which are caused by chronic irritation and inflammation. Well-known risk factors associated with this cancer include smoking tobacco, betel quid, as well as the consumption of smoked, fermented, hot, and spicy foods (4).

Esophageal carcinogenesis involves several genetic alterations, with the CCND1 gene located on 11q13 being a significant contributor to the overexpression of CyclinD1 in ESCC. This overexpression plays a crucial role in the G1/S transition of the cell cycle, affecting cell cycle regulation and leading to oncogenesis. Studies have shown that the use of antisense cyclin D1 can inhibit the proliferation of human esophageal cancer cells, highlighting potential avenues for targeted therapy.

Despite advancements in surgery and adjuvant chemo-radiotherapy, the prognosis for esophageal cancer remains poor. The present study considers various parameters, including age, sex, anemia, clinical features, habits, income, endoscopic findings, ABO-Rh grouping, and histological grades, to better understand their relationships with ESCC. The detailed evaluation of these parameters, along with the assessment of Cyclin D1 as a prognostic marker, aims to enhance the understanding and management of patients with squamous cell carcinoma of the esophagus.

Materials and methods

The present study titled “A clinicopathological study of esophageal squamous cell carcinoma with special reference to Cyclin-D1 in tumour cells” was undertaken to study the clinic-pathological findings in Esophageal carcinoma and to assess the expression of Cyclin-D1 in them.

Place of study

This study was conducted at the Department of Pathology in Silchar Medical College and Hospital, Silchar, Assam, India. The institute's Ethics Committee approved the study (No. SMC/15,121) on 20/10/2022. The study is compliant with the ethical guidelines of the Helsinki Declaration.

Study period

1 year: From September 2021 to August 2022

Type of study

Hospital-based prospective cross-sectional study.

Source of data and sample size

134 Punch Biopsy and Esophagectomised specimens (Figure 1) were submitted for histopathological examination at Silchar Medical College and Hospital. CyclinD1 antibody-based Immunohistochemistry was performed as per IHC protocol.

Inclusion criteria

The study included 134 cases of Primary ESCC. The samples were collected through Punch Biopsy and Esophagectomy procedures (Figure 1).

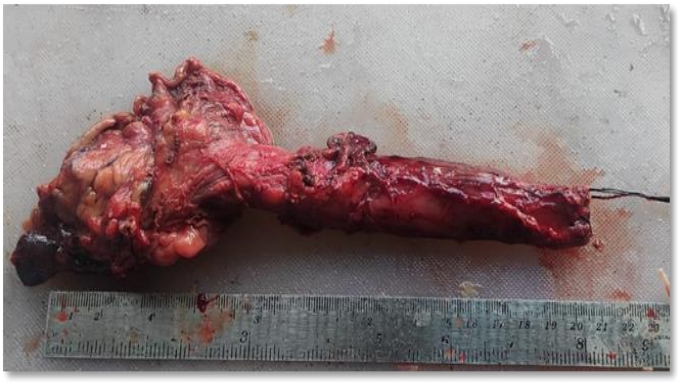

Figure 1. Gross picture of esophagectomised specimen.

Exclusion criteria

- Inadequate biopsy samples
- Inadequate tissue for IHC
- Adenocarcinoma

Parameters studied

I. Detailed clinical history is taken and all routine investigations are done after obtaining patient consent.

II. Hospital records of the patients.

III. Microscopic examination of the tissues.

IV. Immunohistochemistry on paraffin-embedded tissue of histopathologically diagnosed cases.

Methodology

During a year-long study from 2021 to 2022 at Silchar Medical College and Hospital, Assam, India, Department of Pathology, we submitted 134 biopsy/resection specimens for esophageal carcinoma. All regular investigations were performed with patient consent and a thorough clinical history was obtained. The specimens were stained with H&E and then subjected to immunohistochemistry using CyclinD1 antibodies via the IHC methodology.

Immunohistochemical (IHC) Staining for Cyclin-D1

Preparation of slides: To prepare the slides, we first cut paraffin sections and then mounted them on saline-coated slides. The slides were then heated to 65°C to remove the paraffin, followed by immersion in xylene. Once the tissues were rehydrated, we cleaned the slides with distilled water. After that, we washed the slides with Tris buffer and submerged them in a 3% peroxide solution for three minutes to remove any endogenous peroxidase activity.

Antigen detection and antigen retrieval: The process of heat retrieval was carried out using a decloaking chamber along with citrate buffer at a temperature of 95°C for a duration of 40 minutes. After that, the slides were shifted to Tris-Saline buffer to let them cool down to room temperature. To prevent any non-specific immunostaining, the tissue sections were treated with 1% mouse serum. Primary antibodies, such as the Rabbit monoclonal antibody QR022 for CyclinD1, were applied to the sections almost an hour before removal.

Secondary detection of the primary antibody: Following a 10-minute incubation period with biotinylated mouse anti-species antibody, the sections underwent washing in Tris buffer. Subsequently, a Tris buffer solution containing 1mg/mL of the chromogen 3,3'-diaminobenzidine (DAB) and 0.016% fresh H_2O_2 was applied to the slides. Finally, tap water was used to cleanse the DAB from the slides.

Counterstaining: The slides were placed in a mixture of hematoxylin and distilled water in a 1:1 ratio for counterstaining. Once the counterstaining process was complete, the slides were washed in distilled water and then dehydrated by being dipped in ethanol. Finally, after cleaning them with xylene, a coverslip was used to view and report on the slides.

Reporting of Cyclin-D1 immuno-histochemical study

The positive control for this experiment comprises Tonsil tissue, which contains suprabasal squamous epithelial cells, scattered lymphocytes, and endothelial cells. On the other hand, the negative control includes tissues with no primary antibody.

A minimum of 100 tumor cells were scrutinized on each slide to ensure thorough examination.

Criteria followed in this study for Cyclin D1 staining

- **Positive**: Strong nuclear staining in more than 10% of neoplastic cells

- **Negative:** Strong nuclear staining was found in less than 10%.

Statistical analysis: Data was analyzed using IBM SPSS 21.0. Qualitative data was presented as frequency and percentage, and quantitative data as mean (±SD). The chi-square test was used to identify significant associations. A p-value of <0.05 was regarded as statistically significant.

Results

In our study, "A clinicopathological study of esophageal squamous cell carcinoma with special reference to Cyclin-D1 in tumour cells", various clinicopathological parameters are analyzed and presented as under.

The majority of the patients were 51 to 60 years of age (52.1%). This was followed by 38.7% and 25.1% cases belonging to the age range of 61 to 70 years and 20 to 30, respectively. The median age was found to be 57± 9.9 years (Figure 2).

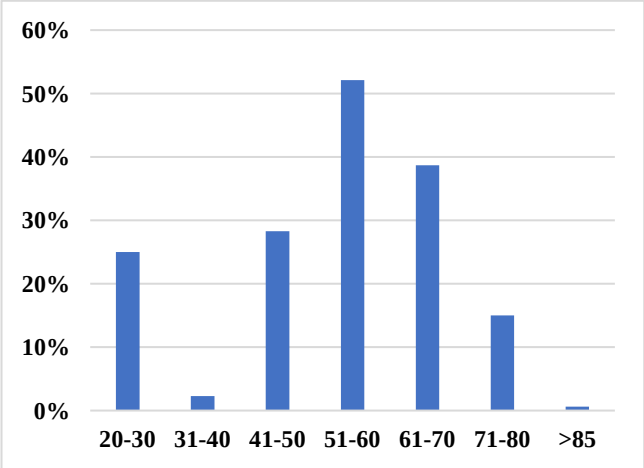

Figure 2. Distribution according to age.

The Male: Female in this study appears to be 1.3: 1 (figure 3). The gender distribution of the study participants holds significant implications for the interpretation of the results and their generalizability to the larger population.

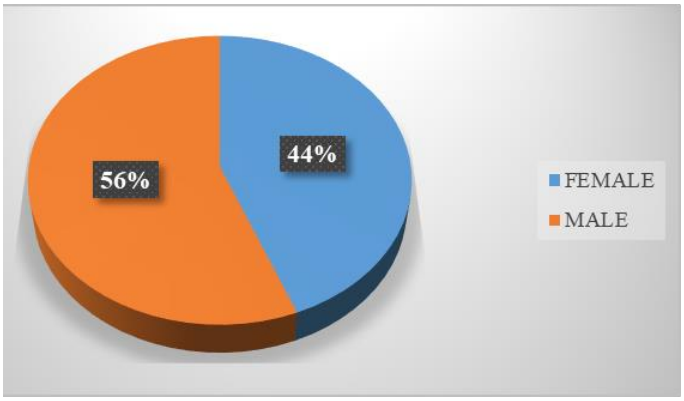

Figure 3. Correlation with sex.

Most of the patients have a monthly income of less than 10,000 rupees (Figure 4). The average monthly income was found to be 6,000 rupees. It was observed that the incidence of ESCC was inversely proportional to income status.

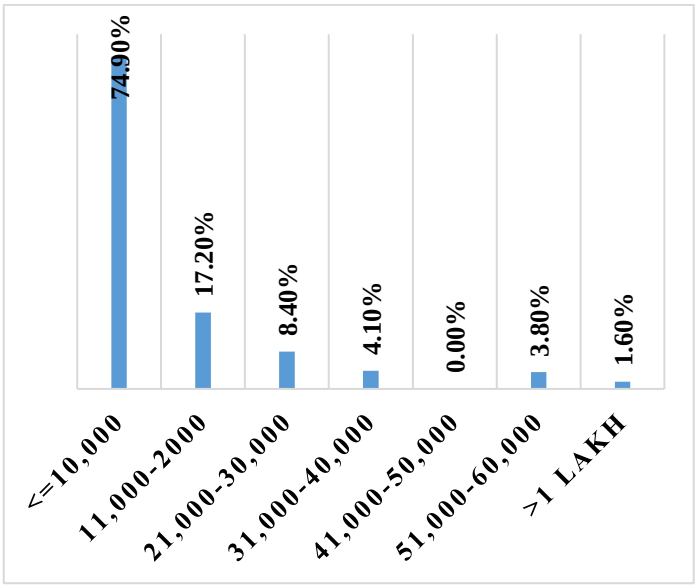

Figure 4. Income status.

In this study, 93.2% of cases had anemia on presentation (Figure 5). For the study, anemia was defined as a hemoglobin level of less than 12g/dl for men and less than 11g/dl for women .

It is worth noting that this affliction is a common condition among 30% of carcinoma patients (11), with causative factors including bleeding in the esophagus, nutritional deficiency, tumoral infiltration in bone marrow, or myelosuppression from carcinoma radiotherapy.

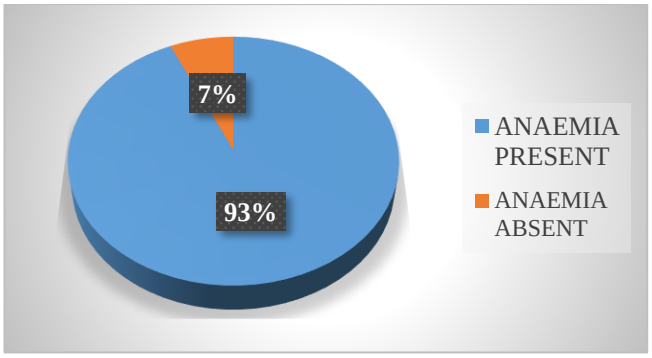

Figure 5. Percentage of anemia in ESCC.

Our study shows the majority (63.4%) cases with dysphagia. The other symptoms that were found were chest pain, cough, vomiting, weight loss, hoarseness of voice, and pain abdomen. (Table 1)

Esophageal dysphagia requires prompt attention to avoid complications like dehydration, malnutrition, respiratory infections, and mortality (13). Early detection and timely management are crucial for better outcomes.

Table 1. Presenting symptoms of ESCC.

Symptoms	Percentage (%)
Dysphagia	63.4
Chest pain	14.9
Cough	12.7
Vomiting	7.5
Weight loss	6
Hoarseness of voice	3
Pain abdomen	2.2

The current study involved the collection of data of blood group from 134 cases, of which only 23.9% (56 cases) had their blood group information available for analysis. Among these, a similar proportion of O+ and B+ cases, each representing 26.8% of the total, were found to be predominant. The study also revealed that A+ and AB+ cases represented 21.4% each, while only 1.8% of B- and AB- cases were identified (Table 2).

Table 2. ABO-RH blood group and ESCC frequency.

Sl.no.	ABO-Rh group	FREQUENCY (n=56) (%)
1	O+	15(26.8)
2	B+	15(26.8)
3	A+	12(21.4)
4	AB+	12(21.4)
5	B-	01(1.8)
6	AB-	01(1.8)

Upper GI-Endoscopy is the gold standard investigation used for determining the segment of the esophagus involved. It appears as ulcer-proliferative growth on Upper GI-endoscopy (Figure 6).

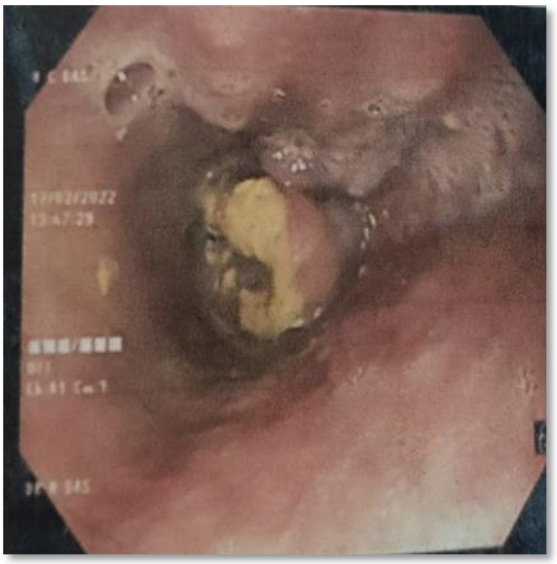

Figure 6. Ulcero-proliferative growth at middle thoracic esophagus on UGI-endoscopy.

According to our study's results, most cases of the ESCC involved the middle third (Figure 7). This observation was derived from the UGI-Endoscopy reports which indicated that 54.2% of the total cases exhibited involvement of the middle third. Subsequently, the lower third (25.2%), upper third (18.3%), and cervical esophagus (2.3%) were found to be involved in decreasing order of prevalence.

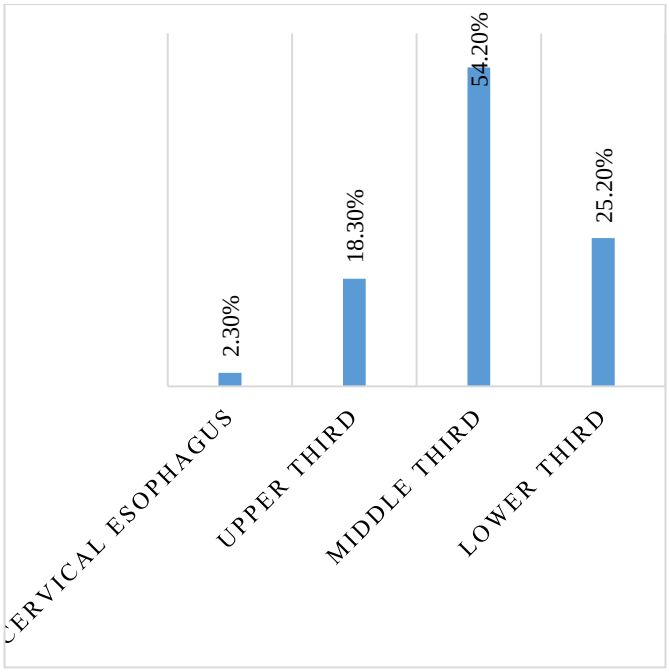

Figure 7. Part of the esophagus involved in ESCC.

In our research (Table 3), we analyzed a majority of cases (63.4%) that exhibited moderately differentiated SCC (Grade II), followed by well-differentiated SCC (Grade I) at 34.3%, and poorly differentiated carcinoma (Grade III) at 2.2%. Our investigation focused on the expression of Cyclin-D1 in these cases, and we discovered a notable correlation (P-value = 0.044). Specifically, Grade III cases demonstrated the highest positivity for Cyclin-D1 at 66.7%, followed by Grade II at 65% and Grade I at 43.5%.

Table 3. Correlation between histological grades of ESCC and cyclin-D1 expression.

Sl.no.	Histological grade	Frequency (n=134) (%)	Cyclin-D1 positive	Cyclin-D1 negative	P-value
1	Grade-I	46 (34.3)	20 (43.5%)	26 (56.5%)	0.044
2	Grade-II	85 (63.4)	56 (65%)	29 (35%)	
3	Grade-III	03 (2.2)	2 (66.7%)	01 (33.3%)	

Discussion

The findings of our study provide valuable insights into the clinicopathological characteristics and Cyclin-D1 expression in ESCC cases within our patient population.

The study discusses age distribution in Figure: 1 depicts a diverse age distribution, with a significant representation in the age group of 51-60 years was consistently found to be involved in studies conducted by Virendra Singh et al. (6). The study conducted by Avninder Singh et al. (7) revealed that mean age of 57±9.9 years and Wang BY et al. (10) revealed that mean age of 56.2±10.35 years were equally involved. This highlights the significance of the study's findings and emphasizes the importance of considering age as a crucial factor in conducting future research.

The male-to-female ratio of 1.3:1, as illustrated in Figure 2, aligns with existing literature by Biswajit Dey et al (8), Avninder Singh et al (7), and Mitra, Tuhin et al (12).

In this study, Figure 3 highlights the income status of the cases and this finding is in line with the study carried out by Nazir A. Dar et al. (9).

A study conducted by Mitra T et al (12) showed dysphagia in the majority of ESCC cases which is in concordance with our study Table 1.

In this study Table 2 highlights the ABO-Rh grouping of the patients and a similar study by Kumar N et al. found that out of 480 patients with esophageal squamous cell carcinoma, 20.4% had blood group A, 12.1% had blood group AB, 47.1% had blood group B, and 20.4% had blood group O. 88.3% had a positive Rh status and 11.7% had a negative Rh status (14).

In this study, Figure 5 highlights the part of the esophagus involved in ESCC. Our study is consistent with the findings of Wang BY et al, wherein a majority of cases of esophageal squamous cell carcinoma (ESCC) showed involvement in the middle third (36.6%) of the esophagus, followed by the lower third (33.4%). A smaller proportion of cases were unknown (19.0%) and showed involvement in the upper third (11.0%) (10).

Also, YC Lin et al found the most affected part in ESCC as the Middle thoracic(56.5%), followed by the Lower thoracic (37.1%) and Upper thoracic (4.8%) (16).

In addition, Yang M et al reported that the middle third of the esophagus was affected in 84.6% of ESCC cases, followed by the lower third (9.6%) and upper third (5.8%) (15).

The similarities between our study and previous research suggest that the middle and lower thirds of the esophagus are the most commonly affected sites of ESCC. These findings may have important implications for this disease's diagnosis, treatment, and prevention.

Histological grading, showcased in Table 3, reveals a predominance of Grade II ESCC, followed by Grade I. In the histological grades of esophageal squamous cell carcinoma (ESCC), Singh V et al. found that the majority of cases were moderately differentiated Grade 2 (66.7%; 50/75), followed by well-differentiated Grade 1 (18.7%; 14/75) and poorly differentiated Grade 3 (14.6%; 11/75) (6). Similarly, Singh A et al. reported that the majority of ESCC cases were Grade II (moderately differentiated) (66%), followed by Grade I (well differentiated) (22%), and Grade III (poorly differentiated) (12%) (7). YC Lin et al. also observed that the majority of ESCC cases were Grade II (56.5%), followed by Grade I (25.8%) and Grade III (17.7%) (16). The findings of these studies align with our current research.

Grade II ESCC displayed a higher frequency of Cyclin-D1 positivity (63.4%) compared to Grade I tumors, with a statistically noteworthy link between Cyclin-D1 expression and tumor grade, which is consistent with the findings of Biswajit Dey et al where 86.7% of patients with ESCC exhibited positive expression of Cyclin D1, while 13.3% tested negative (8). In a separate study, Lin et al. reported a Cyclin-D1 positive expression rate in ESCC of 56.5% (16), while Qi ZL et al. observed a 51.7% Cyclin-D1 positivity rate in their study (17). The results of these studies are consistent with our current research.

In conclusion, the discussion emphasizes the wide range of characteristics found in esophageal squamous cell carcinoma (ESCC) cases. It highlights the importance of Cyclin-D1 as a molecular marker linked to histological grades. The expression of Cyclin-D1 in our study suggests its potential as a valuable biomarker for identifying advanced disease. Recent studies also validate the significance of Cyclin-D1 in ESCC and its impact on overall survival. This study offers valuable insights into the clinicopathological characteristics and Cyclin-D1 expression in ESCC, providing crucial information on potential prognostic markers and guiding further research for a comprehensive understanding of this complex disease.

Conclusion

ESCC is one of the most common types of carcinoma in India. Our study focuses on the clinicopathological aspects of ESCC in the southern region of Assam, India. We found that ESCC is more prevalent in individuals in their 50s and there is a higher incidence in males. The occurrence of ESCC is inversely related to income, and patients often present with dysphagia, leading to an increased incidence of anemia. The majority of the study population has blood groups O+ and B+.

The standard investigation for esophageal carcinoma is UGI-Endoscopy, which typically reveals ulcerative and proliferative growth primarily affecting the middle third of the esophagus. Our study showed that HPE indicated a higher incidence of Grade II ESCC, followed by Grade I. Additionally, the IHC study of Grade III demonstrated the highest positivity for Cyclin-D1, followed by Grade II.

In conclusion, our findings suggest that alterations in Cyclin D1 significantly impact ESCC. This dysregulated protein promotes aggressive behavior in tumor cells. These findings highlight important prognostic markers in ESCC that can be used to predict early diagnosis, prognosis, and personalized therapy decisions for ESCC patients.

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Author contribution

All authors of this research paper have directly participated in the planning, execution, or analysis of

the study. **PH** and **MKD** conceived the idea, designed the study, collected the data, performed the statistical analysis, and wrote the paper. **AD** guided the research project and reviewed the literature. **AT** helped in reviewing the slides and the literature. All authors of this paper have read and approved the final version submitted.

Conflict of interest

The authors declare that they have no competing interests.

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