Cyclin D1 Expression in Different Grades of Oral Squamous Cell Carcinoma: A Study Based on Small Biopsy Samples

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Abstract

Background: Cyclin D1 oncogene plays a critical role in various malignancies by regulating the G1/S transition of the cell cycle. Overexpression of cyclin D1 in oral squamous cell carcinoma is associated with poor prognosis and resistance to cisplatin-based conventional chemotherapy.

Objectives: To assess the immunohistochemical expression of cyclin D1 in different grades of oral squamous cell carcinoma (OSCC) and to determine the association of cyclin D1 expression with histopathological grades, age, gender, anatomic site, and the habit of using tobacco, betel quid and areca nut of OSCC patients.

Methods: This cross-sectional observational study was conducted at the Department of Pathology, Sylhet MAG Osmani Medical College, Sylhet during the study period (from March, 2019 to April, 2020 and July, 2021 to April, 2022). A total of 52 small biopsy cases were processed; paraffin blocks were made and stained with routine H&E stain. The sections were examined microscopically and the tumors were graded histologically. Immunohistochemistry was performed by using a commercially available anti-cyclin D1 antibody. Cyclin D1 overexpression was considered when more than 10% of tumor cells displayed nuclear staining with moderate to strong intensity.

Results: Cyclin D1 overexpression was seen in 60% of cases of OSCC. The highest expression was seen in moderately differentiated followed by poorly differentiated and well-differentiated squamous cell carcinoma, with no statistically significant correlation. It did not correlate with age, gender, anatomic site, the habit of using tobacco, betel quid, and areca nut chewing.

Conclusion: In Bangladesh, 60% of cases of OSCC showed cyclin D1 overexpression which was quite similar to the findings of other countries. Further, alarge cohort study on resected samples can be done for overall survival and future targeted therapy against cyclin D1.

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Introduction

In Bangladesh, Oral cancer ranks 3rd, 2ndand 3rd in 5-year prevalence, incidence and mortality rate respectively.[1] The most common histologic type of oral cancer is Oral squamous cell carcinoma (OSCC). It comprises 90% of all Head-Neck squamous cell carcinomas.[2]The use of tobacco and/or the areca nut (betel) yields potentially malignant disorders from which oral cancer develops particularly in developing countries. Alcohol consumption, especially in industrialized nations, plays a significant role in oral carcinogenesis. Other predisposing factors of OSCC include human papillomavirus (HPV 16/18) infection, immunodeficiency, dietary habits, and poor oral hygiene.[3]Cyclin D1 is one of the strongly implicated cyclins in human carcinogenesis. It promotes cell cycle progression during the G1 phase, a key event in the G1-S transition. The cyclin D1 (CCND1) gene is located on 11q13. It encodes a nuclear protein that binds with cyclin-dependent kinases 4 and 6, which phosphorylate and inactivate the retinoblastoma protein (pRb) thereby, allowing the progression of genetically damaged cells in the transition of cell cycles from G1 to S phase.[4] In addition to this, the G1 phase is shortened and dependency on growth factors for cell proliferation is also reduced.[5]Along with cell proliferation, Cyclin D1 also plays important roles in cell growth regulation, mitochondrial activity modulation, DNA repair and cell migration control. CCND1 gene aberration occurs through amplification, chromosomal translocations, mutations, and activation of the pathways involved in cyclin D1 expression.[6] Overexpression of cyclin D1 has been reported in various malignancies including oral, head and neck, esophageal, ovarian, breast, uterine, colon, lung, prostate, lymphoma and Ewing sarcoma.[4,5] It is frequently related to tumor size, nodal involvement, poor differentiation, advanced stage and also non-response to treatment and poor survival.[2,7,8,9] For the systemic treatment of OSCC, Cisplatin is commonly used. However, resistance to cisplatin is noticed and identified as a chief cause of treatment failure. Considering the dual roles of cyclin D1 in promoting cell proliferation and inhibiting cisplatin-induced apoptosis, it would be an important target (Palbociclib-under trial) for future therapy in patients with OSCC.[10,11]

Methods

After the approval of the Institutional Ethical Committee, this cross-sectional observational study was conducted at the Department of Pathology with the collaboration of the ENT and Dentistry Department of Sylhet MAG Osmani Medical College Hospital during the period of March 2019 to April 2022. Histologically diagnosed 52 small biopsy cases of OSCC were included here with the fulfillment of selection criteria. Specimens were fixed in 10% formalin. After conventional processing, paraffin sections of 3-4μm thickness were stained by routine Hematoxylin and Eosin (H&E). Tumors were graded into well, moderate and poorly differentiated OSCC according to WHO criteria.[12] For immunohistochemistry staining, 4 μm tissue sections were taken on Poly-L lysine-coated slide from the paraffin blocks of the tumor. This staining was carried out in theArmed Force Institute of Pathology (AFIP) of Dhaka by using VentanaBanchMark XT automated system. Tonsillar tissue was taken as a positive control (Figure 1) and FLEX Monoclonal Rabbit Anti-Human Cyclin D1 protein Clone EP12 Ready-to-Use (Link) was used as the primary antibody. When more than 10% of tumor cells displayed nuclear staining with intensity scores of moderate and strong, then there was considered to be cyclin D1 overexpression.[8]Only the nuclear staining intensity and distribution were evaluated (Figures 2, 3, 4, 5, 6). Cytoplasmic staining was disregarded. All the collected data were

compiled and analyzed with the SPSS (Statistical Package for Social Science), version 25; using Chi-square and Fisher's Exact test, to assess for statistical significance between the different clinic-pathologic variables. A probability value (p) of <0.05 was considered statistically significant.

Results

Clinicopathologic characteristics of the OSCC patients are listed in Table I. The labeling index scores, intensity of staining and expressions are elaborated in Table II. Cyclin D1 overexpression was seen in 31 cases (60%) of OSCC. The highest overexpression was seen in moderately differentiated followed by poorly differentiated and well-differentiated OSCC (Table III). Cyclin D1 expression did not show any association with histologic grading, age, gender, anatomic site, the habit of using tobacco, betel quid and areca nut chewing (Table I).

Table I: Clinicopathologic parameters of OSCC patients and their association with cyclin D1 expression (N= 52)

Clinicopathologic parameter	Cyclin D1 expression		P value
	Overexpressed	Not overexpressed	
Age			
≤50 years (n=26, 50%)	15 (57.7)	11 (42.3)	
>50 years (n=26, 50%)	16 (61.5)	10 (38.5)	0.347
Mean ± SD (54.37 ± 12.92)			
Range (30-90)			
Gender			
Male (n=28, 53.8%)	15 (53.6)	13 (46.4)	0.403
Female (n=24, 46.2%)	16 (66.7)	08 (33.3)	
Anatomic site			
Buccal mucosa (n=31)	17 (54.8)	14(45.2)	0.160
Tongue (n=09)	08 (88.9)	01(11.1)	
Others* (n=12)	06 (50)	06(50)	
Habit of using tobacco			
Yes 27 (51.9%)	15 (55.6)	12 (44.4)	0.582
No 25 (48.1%)	16 (64)	09 (36)	
Habit of betel quid and areca nut chewing			
Yes (50, 96.2%)	29 (61.7)	18 (38.3)	0.383
No (02, 3.8%)	02 (40)	03 (60)	
Histologic grades			
Well differentiated (n=38, 73%)	21 (55.3)	17 (44.7)	
Moderately differentiated(n=11, 21%)	08 (72.7)	03 (27.3)	0.694
Poorly differentiated (n=03, 06%)	02 (66.7)	01 (33.3)	

SD= Standard Deviation; *Including retromolar area (n=05), palate (n=03), lip (n=02), alveolar mucosa (n=01) and angle of mouth (n=01); Figure within parenthesis indicates the corresponding percentage.

Table II: Cyclin D1 labeling index score, intensity of staining and expression

Cyclin D1 Immunohistochemistry	Number	%
Labeling index score		
≤10% tumor cells	19	36.5
>10% tumor cells	33	63.5
Intensity Score		
Weak	11	21.2
Moderate	32	61.5
Strong	09	17.3
Cyclin D1 Expression		
Overexpression	31	60
Not overexpressed	21	40

Table III: Association between cyclin D1 expression and histologic grades of OSCC

Cyclin D1 expression	Histologic grades of OSCC			p-value
	Well differentiated	Moderately differentiated	Poorly differentiated	
Yes (overexpressed)	21 (55.3)	8 (72.7)	2 (66.7)	
No (not overexpressed)	17 (44.7)	3 (27.3)	1 (33.3)	*0.694
Total	38 (100)	11 (100)	3 (100)	

*Fisher's Exact test was done to measure the level of significance.
The figure within parentheses indicates the corresponding percentage.

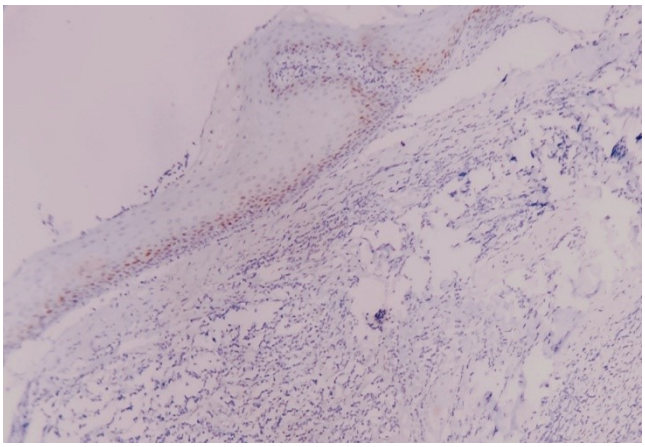

Figure 1. Tonsillar tissue was taken as control showing cyclin D1 expression confined to the basal layers of the epithelium (IHC, 100X magnification).

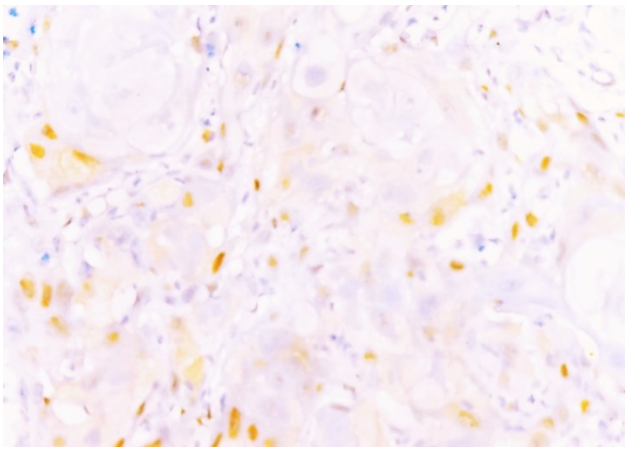

Figure2. Section showing weak intensity of cyclin D1staining (IHC,400X magnification)

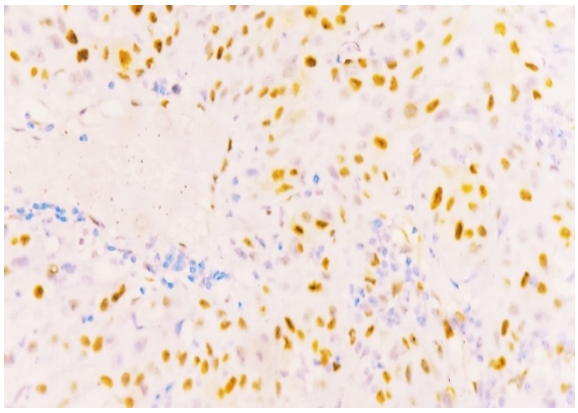

Figure 3. Section showing moderate intensity of cyclin D1 staining (IHC, 400X magnification).

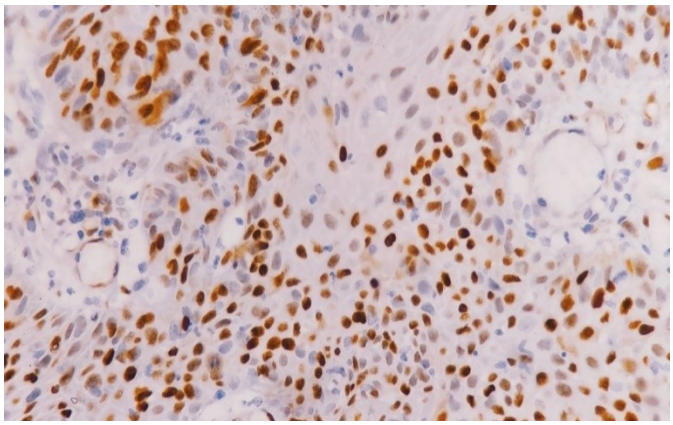

Figure 4. Section showing strong intensity of cyclin D1 staining (IHC, 400X magnification)

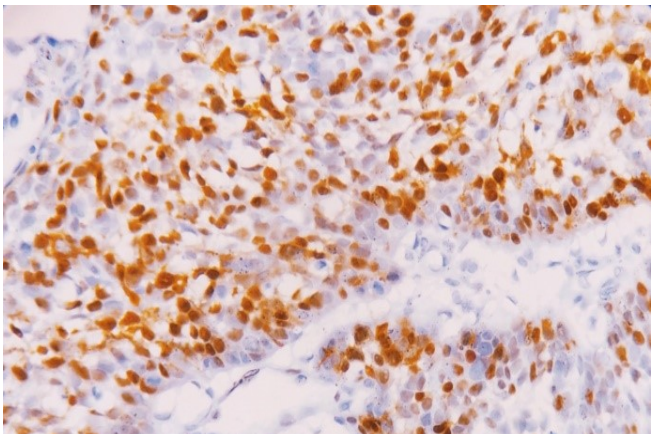

Figure 5. Section showing cyclin D1 positivity in >10% of the tumor cells (IHC,400X magnification).

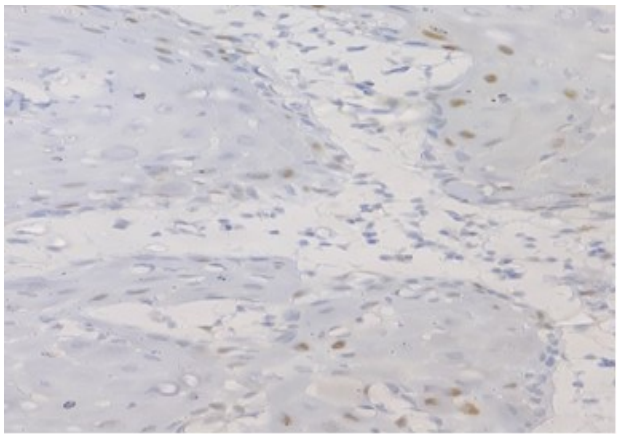

Figure 6. Section showing cyclin D1 positivity in <10% of the tumor cells (IHC,400X magnification).

Discussion

Our results demonstrate that cyclin D1 expression is a frequent event in OSCC (60%) and it did not show any association with histologic grading, age, gender, anatomic site, the habit of using tobacco, betel quid, and areca nut chewing. However, many researchers showed that cyclin D1 expression is frequently related to tumor size, nodal involvement, poor differentiation, advanced-stage and also non-response to treatment and poor survival.[2,7,8,9]

In our study, overexpression of cyclin D1 was 60%. It varies from researcher to researcher and that is 37% to 70.7%. Huang et al. (2012) reported it as 37%. Other investigators like Saawarn et al., (2012); Chowdhary et al., (2016); Gatoo& Dar, (2018); Angadi&Krishnapillai, (2007) and Miyamoto et al., (2003) reported the positivity of cyclin D1 45%, 68%, 58.3%, 70.7% and 65.9%, respectively.[9,13,14-16]

There are certain reasons for these variations of cyclin D1 expression. Among them, asymmetric labeling index scores;

heterogeneity in biopsy and resected specimens, subjective evaluation discrepancies and cut-point dissimilarities are the predominant issues. It has been well-defined that cyclin D1 is expressed mainly in the peripheral layers of tumor islands and not in the cells exhibiting mitosis.[17,18,19]

In the present study, the intensity of the cyclin D1 expression shown by the maximum number of cases was moderate, followed by weak and strong. Highest expression of cyclin D1 was seen in MDOSCC followed by PDOSCC and WDOSCC. It was observed that there is no significant association between cyclin D1 expression and histopathological grades of OSCC. This study was in accordance with Gatoo& Dar, (2018), Dhingra et al. (2017), Chowdhary et al. (2016) and Ohnishi et al. (2014).[2,13,14,20] Whereas, this result was discordant with studies by Huang et al. (2012), Saawarn et al. (2012), Das et al. (2011), Mishra & Das, (2009), Angadi&Krishnapillai, (2007) and Miyamoto et al. (2003).[8,9,15,16,21,22]

Conclusion

Many studies have been done to explore the role of cyclin D1 in OSCC and the fact is that there are so many controversies exist in the scientific literature. In Bangladesh, cyclin D1 protein was overexpressed in 60% of cases of OSCC. Among all prognostic parameters, only histologic grading of OSCC was focused. The interpretation of the above study is insupportable due to its limited sample size based on a small biopsy and therefore the study should be followed further with a large sample size applied in resected specimensto validate our findings as well as staging, nodal metastasis, overall survival and future targeted therapy against cyclin D1.

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