

EXPRESSION OF CYCLIN D1 IN VARYING GRADES OF ORAL SQUAMOUS CELL CARCINOMA: AN IMMUNOHISTOCHEMICAL STUDY

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ABSTRACT

Objective: Cyclin D1 promotes cell cycle progression during G1 phase, a key event in G1-S transition. It is a positive regulator of cell cycle. Overexpression may lead to disturbance in normal cell cycle control and tumor formation. The objective of the study was to evaluate the expression of cyclin D1 in varying grades of oral squamous cell carcinoma (OSCC).

Methods: Nine formalin-fixed paraffin embedded tissues were immunohistochemically analyzed for the expression of cyclin D1 in varying grades of OSCC.

Results: Expression of cyclin D1 was more in poorly differentiated OSCC than moderately and well-differentiated OSCC.

Conclusion: Increased expression of cyclin D1 was seen in poorly differentiated OSCC and thus can be used as a biomarker for the aggressiveness of OSCC.

Keywords: Cyclin D1, OSCC, G1-S transition, Immunohistochemistry.

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INTRODUCTION

Oral cancer is the sixth most common cancer worldwide. More than 90% of all oral cancers are squamous cell carcinoma (SCC) [1]. SCC is a malignant epithelial neoplasm exhibiting squamous differentiation as characterized by the formation of keratin and/or the presence of intercellular bridges [2]. Smoking, alcohol use, smokeless tobacco products, and HPV infections are the various risk factors with smoking and alcohol having synergistic effects [3]. However, individuals with no known risk factors are also seen with the increased incidence of oral SCC (OSCC). New biological markers might add information about the aggressiveness of tumors such as cyclins which is one of cell cycle controlling proteins [4]. Cyclin D1, a 45 kDa protein, is a part of the molecular system that regulates the cell cycle G1 to S transition [5]. Cyclin D1 is a key regulatory protein at G1/S checkpoint of the cell cycle. The G1/S checkpoint is frequently altered in many epithelial tumors [6]. Overexpression of cyclin D1 leads to shortening of the G1 phase and to less dependency on growth factors, resulting in abnormal cell proliferation that in turn might favor the occurrence of additional genetic lesions. This overexpression can be detected by immunohistochemical staining using anti-cyclin D1 antibody [7]. This study was done to evaluate the expression of cyclin D1 in varying grades of OSCC and to correlate its expression with histological differentiation.

METHODS

A total of 9 histopathologically diagnosed, formalin-fixed, paraffin-embedded tissue samples of OSCC were collected from the archives of the Department of Oral and Maxillofacial Pathology and Oral Microbiology, IGGDC, Jammu after gaining an approval by the Institutional Ethical Committee of IGGDC Jammu. Two sections of 4 μ m were cut from each tissue block: one for hematoxylin and eosin staining and other for immunohistochemistry. These blocks were then categorized into different grades of OSCC based on Borders criteria into well-differentiated OSCC - 03 cases, moderately-differentiated OSCC - 03 cases, poorly-differentiated OSCC - 03 cases. Sections were dewaxed, cleared in xylene,

and rehydrated in descending grades of alcohol; antigen retrieval was carried out in a pressure cooker using citrate buffer (10Mm, pH 6.0) for 2-5 min. The slides were slowly allowed to cool down to room temperature. To block endogenous peroxidase activity, the sections were quenched by incubation in 3% hydrogen peroxide solution for 15 min. The sections were incubated with peroxidase blocking reagent for 15 min, followed by incubation with ready-to-use monoclonal cyclin D1 antibody for 1 h at room temperature. The sections were then washed with tris buffered saline (TBS) 3 times for 10 min each, incubated with biotinylated secondary antibody for 30 min at the room temperature in a moist chamber; and washed in TBS 3 times for 10 min. Sections were incubated with streptavidin for 45 min at the room temperature in moist chamber; washed in TBS, and incubated in freshly prepared 3, 3' diaminobenzidine tetrahydrochloride (DAB) solution. DAB was prepared by diluting chromogen (1 drop) in 1 ml of substrate and used as the substrate for localizing antibody binding. Sections were then counterstained with hematoxylin, mounted, and studied under light microscope for immunoreactivity.

Cyclin D1 protein expression was evaluated on the basis of the presence or absence of nuclear staining. Only nuclear positivity (strong brown staining) was assessed quantitatively. Counting of the cells was performed on a research microscope using an eyepiece grid at ($\times 40$) magnification. The grid was having 10 \times 10 boxes. All the nuclear positive cells within the grid were counted and compared among varying grades of OSCC. The results were performed by two individual examiners to remove any interobserver variability.

Statistical analyses

A *t*-test was used for comparison and correlation between varying grades of OSCC. Statistical significance was defined as $p < 0.05$. Statistically significant result was observed between WDOSCC versus MDOSCC ($p < 0.05$) and WDOSCC versus PDOSCC ($p < 0.05$), but a statistically non-significant result was observed between MDOSCC versus PDOSCC (Table 2).

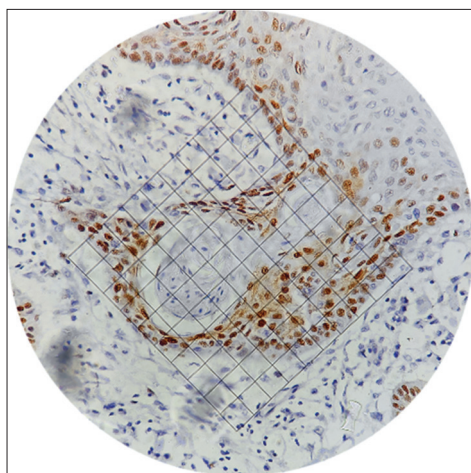


Fig. 1: Cyclin D1 expression in well differentiated squamous cell carcinoma

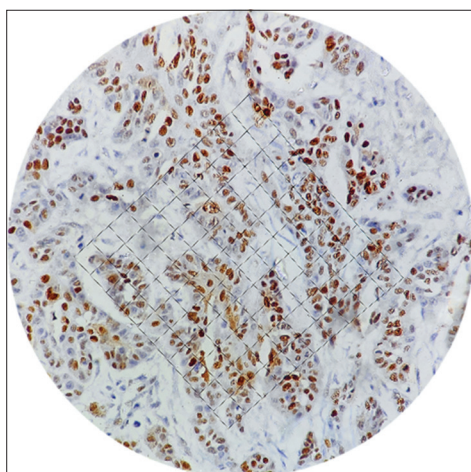


Fig. 2: Cyclin D1 expression in moderately differentiated squamous cell carcinoma

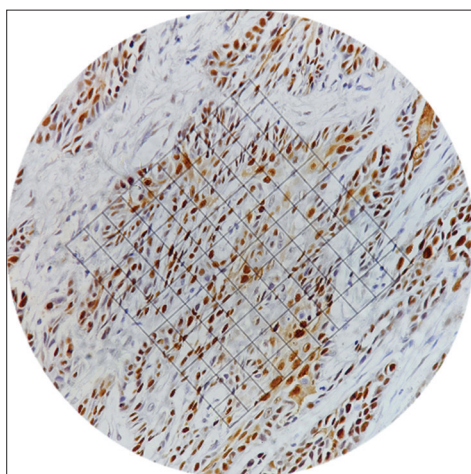


Fig. 3: Cyclin D1 expression in poorly differentiated squamous cell carcinoma (original magnification)

RESULTS

The strong brown nuclear staining of epithelial cells was considered positive. Histological sections with good intensity were assessed

Table 1: Cyclin D1 expression in different grades of oral squamous cell carcinoma

Group	Grades of OSCC	Sample	Mean	Variance	SD
Group 1	WDOSCC	n=3	86	571	23.89
Group II	MDOSCC	n=3	108.3	2032	45.08
Group III	PDOSCC	n=3	129.6	2689.3	51.85

SD: Standard deviation, MDOSCC: Moderately differentiated oral squamous cell carcinoma, WDOSCC: Well-differentiated oral squamous cell carcinoma, PDOSCC: Poorly differentiated oral squamous cell carcinoma

Table 2: Comparison of Cyclin D1 expression in different grades of OSCC

OSCC	Cyclin D1 intensity		Test/Significance
	T	p-value	
Well-moderate	5.08	0.007	Significant
Well-poorly	3.895	0.017	Significant
Moderate-poorly	0.537	0.624	Not significant

p<0.05: Significant. OSCC: Oral squamous cell carcinoma

for cyclin D1 scoring. When the intensity was compared with the histological differentiation, more intensity of cyclin D1 expression was seen in poorly differentiated OSCC than moderately-differentiated oral SCC and well-differentiated oral SCC, respectively.

DISCUSSION

The loss of regulatory control of the cell cycle, leading to unrestrained cell proliferation, is a hallmark of cancer. Cyclins, the regulatory subunits of cyclin-dependent kinases (CDKs), control the passage of proliferating cells through key checkpoints in the cell cycle [8]. Cyclin D1 is the regulatory subunit of the holoenzymes that phosphorylate and, together with sequential phosphorylation by cyclin E/CDK2, inactivate the cell-cycle inhibiting function of the retinoblastoma protein (pRb). pRb serves as a gatekeeper of the G1 phase, and passage through the restriction point leads to DNA synthesis [9]. The high expression of cyclin D1 drives unchecked cellular proliferation promoting tumor growth; thus, the cyclin D1 carries out a central role in the pathogenesis of cancer [10]. Therefore, the overexpression of cyclin D1 or the failure of cyclin D1 degradation both accelerate G1-S transition, helping cancer cells to gain a survival advantage and an uncontrolled proliferation, which further promotes the invasiveness and malignance of cancer [11]. In this study, the expression of cyclin D1 was seen more in poorly differentiated OSCC Table 1. These results were in consistent with the studies done by Angadi *et al.* [7] and Lam *et al.* [12], in which increased expression of cyclin D1 was seen in poorly differentiated OSCC, but the results of our study were in contrast to studies done by Saawarn *et al.* [13], in which the increased expression of cyclin D1 was seen in well-differentiated OSCC. An increased positivity with increasing grade was observed in a study done by Gatoos and Dar, in which significant difference was found between well-differentiated SCC and moderately-differentiated SCC as well as between well-differentiated SCC and poorly differentiated SCC [14]. In our study, the expression of cyclin D1 was less in well-differentiated OSCC (Fig. 1) than moderately differentiated OSCC (Fig. 2) and poorly differentiated OSCC (Fig. 3), and these results were in consistent with the studies done by Huang *et al.*, in which cyclin D1 overexpression was more prevalent in tumors at advanced stage than those at early stage and moderate/poor differentiation than well differentiation [15]. The higher expression of cyclin D1 in high-grade tumors might be explained by the fact that alterations in cyclin D1 are related to an intense proliferative activity and invasiveness capacity of the lesions [4]. A study done by Fatima *et al.* found an increased expression of cyclin D1 in peripheral layers of tumor islands explaining the fact that proliferating cells are mostly present on peripheral areas of tumors and cyclin D1 being the activator

of cell proliferative cycle causes this increased expression in these areas [16]. The high expression of cyclin D1 drives unchecked cellular proliferation promoting tumor growth; thus, the cyclin D1 carries out a central role in the pathogenesis of cancer and is an oncogenic driver in different types of cancers including breast cancer, lung cancer, and melanoma [10]. Thus, in this study, the overall expression of cyclin D1 correlates with poor histological grades of OSCC. The increased cyclin D1 expression with the increasing severity of lesions likely reflects the intense proliferative activity and invasiveness of these lesions.

CONCLUSION

In this study, the increased expression of cyclin D1 was seen with poor histological grades of OSCC. The increased expression of cyclin D1 with poorer prognosis reflects the intense proliferative activity and invasiveness of these lesions. Thus, cyclin D1 may be a useful prognostic marker in cases of OSCC. It can also act as a potential target for molecular intervention studies in the future.

AUTHOR'S CONTRIBUTION

The first three authors have participated in the intellectual content, conception, design, analysis, and interpretation of the data and the 4th author has helped in the writing of the manuscript.

CONFLICT OF INTEREST

Nil.

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