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ORIGINAL RESEARCH ARTICLE

Evaluation of Immunohistochemical Expression of Cyclin D1 and its Clinicopathological Correlation in Oral Carcinoma

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ABSTRACT

Introduction: The present study aimed to evaluate the expression of cyclin D1 in oral carcinoma and to find the association of the expression of cyclin D1 with different histological types, grades and other clinicopathological findings.

Materials and Methods: A 50 formalin fixed paraffin embedded tumor sections, stained with haematoxylin & eosin were graded and immunohistochemistry for cyclin D1 evaluated as expression score (ES), intensity score (IS) and total score (TS) was calculated.

Results: A significant association was seen between total score of cyclin D1 expression with tumor types squamous cell carcinoma (Grade 1 and Grade 2) and verrucous carcinoma.

Conclusion: In the present study for cyclin D1 evaluation, its expression was found in all oral carcinoma cases. A strong association was found between cyclin D1 total score with squamous cell carcinoma (Grade 1 and Grade 2) and verrucous carcinoma. We did not find a significant association between cyclin D1 total score and age, gender, tumor grades, site, laterality, PNI, LVI, pathological and clinical staging.

Keywords: Immunohistochemistry, Cyclin D1, Oral carcinoma.

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INTRODUCTION

Incidence rates for oral cancer vary in men from 1 to 10 cases per 100,000 population in many countries. In south-central Asia, cancer of the oral cavity ranks among the three most common types of cancer. In India, the age-standardized incidence rate of oral cancer is 12.6 per 100,000 population.[1]

The oral carcinoma risk is high in India and other South Asian countries owing to an elevated prevalence of adverse oral habits like tobacco chewing and smoking habits. It is unfortunate that despite innumerable awareness campaigns on the part of the state and through social movements, the occurrence of oral carcinoma is shown a constant surge over the years. As such, oral cancer is a considerable issue in the Indian subcontinent, where it ranks among the top three types of cancer in the country.[2] It is now known that at least 50% of oral cancers, particularly those involving the buccal mucosa, cheek mucosa, and the base of the tongue, harbor oncogenic variants of HPV.[3]

In clinical presentation, the tumor-node-metastasis (TNM) system is the usual prognostic tool for cancers, among which lymph node metastasis is the most significant.[4] The correlation between molecular biomarkers and the aggressiveness of oral carcinoma may offer a considerable advantage for predicting clinical results and determining the most favorable individualized treatment. Lately, consideration has focused on a panel of molecular markers, including cell cycle regulators as probable prognosticators of biological behaviors in oral carcinoma.[5] Carcinogenesis is a course that takes place when numerous factors bring about incoordination in the cell cycle.[6]

The various phases of the cell cycle are regulated by a number of proteins called "cyclins". Cyclin D1 is a 45-kilodalton protein located on chromosome 11q13 and regulates the G1-S phase transition.[7] Cyclins are a group of proteins accountable for the activation of the main cell division transitional points. The activation of precise cyclin-Cdk complex result in a cascade of protein phosphorylation that is required for access through the specific stages of the cell cycle.[8] Cyclin D1 appears to be important in the G1-S transition. The transition from G1 to S is believed to be an extremely important checkpoint in the cell cycle clock, guarded by the product of Rb protein which binds to transcription factor E2F.[9]

Considering the significance of cyclin D1 expression as a helpful aid in understanding tumor progression and prognosis. In this study we attempted to study the cyclin D1 expression in 50 oral biopsy specimens and correlated them with different clinical and histopathological factors. Early diagnosis and treatment of these patients can lead to a better prognosis. Hence, this study will evaluate the immunohistochemical expression of Cyclin D1 in oral

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carcinoma and correlate the expression of cyclin D1 with different grades and types of oral carcinoma with other prognostic factors.

MATERIALS AND METHODS

All the oral carcinoma resection/biopsy specimens submitted to the pathology department (SRMS IMS, Bareilly) were collected. A detailed clinical history and clinical examination were taken from the patient records. The requisition forms accompanying the resected specimen or biopsy sample were assigned a histology lab number, and the patient's clinical details, including name and age, were noted to avoid repetition. The present study included 50 cases, after qualifying inclusion and exclusion criteria, and which were recruited during the above-mentioned time period.

Specimens received were fixed in 10% (v/v) formalin. After conventional processing, paraffin sections of 3 to 4 μm thickness were stained by haematoxylin and eosin (H & E) for diagnosing, grading and staging of the tumor. The cases were further reviewed for various parameters like age, gender, site, histological grades, depth of invasion (DOI), lymphovascular invasion, perineural invasion, and pathological and clinical staging. OSCC specimens were histologically graded as well-differentiated, moderately differentiated and poorly differentiated squamous cell carcinomas according to the differentiation of cells and the resemblance of neoplastic cells to that of the epithelial cells.

IHC was carried out using BioGenex, Fremont CA. The tissue samples were considered positive for cyclin D1 when brown nuclear staining was detected in more than 1% of the cells. The percentage of cells stained will be scored as expression score (ES). The staining intensity was compared with IHC-stained sections of tonsillar tissue, which was taken as a negative control and recorded as an intensity score (IS). Finally, the total score (TS) will be calculated by multiplying ES with IS to produce a semi-quantitative immunohistochemical score, which was graded as cyclin D1 expression will be correlated with different histopathological grades of oral carcinoma and other prognostic findings (Tables 1-3).

Statistical Analysis

Compiled data will be interpreted in the form of numbers, percentage, and statistical test of significance. Data will be presented in the form of tables, charts and graphs. The data entry was done in the Microsoft Excel spreadsheet, and the final analysis was done using the Statistical Package for Social Sciences (SPSS) software, which IBM manufacturer, Chicago, USA, version 25.0 developed.

For statistical significance, $p\text{-}value < 0.05$ was considered statistically significant.

Table 1: Expression score of cyclin D1 staining according to percentage of cells stained

Expression score	*% of cells stained*
1	1–25%
2	26–50%
3	51–75%
4	>75%

Table 2: Intensity score of cyclin D1 expression according to the intensity of staining

Intensity score	*Intensity of staining*
1	Mild
2	Moderate
3	Strong

Table 3: Grading of total score of Cyclin D1 expression (TS=ESxIS)TS= Total Score , ES = Expression Score , IS = Intensity Score 70

Total score	*Grading*
1–4 points	(+) Weak
5–8 points	(++) Moderate
9–12 points	(+++) Strong

RESULTS

In our study, the mean age of patients presenting with oral carcinoma was 49.46 ± 9.29 (29.0–70.0). The majority of cases were males, 43 (86%) and only 7 (14%) females out of 50 oral carcinoma cases. Chief complaints were assessed in 50 patients on the basis of clinical history. All 50 patients complained of ulcerative lesions in mouth, 35 (70%) cases complained of decreased mouth opening and 7 (14%) cases showed decreased appetite out of 50 cases. Amongst the risk factors, 46 (92%) patients had a history of smoking, whereas 32 (64%) patients had a history of tobacco chewing and 10 (20%) patients had a history of areca nut chewing.

Among 50 oral carcinoma cases, buccal mucosa was identified as the most common tumor-originating site in 37 (74%) cases. Most of the tumors were noted on the left side, with 28 (56%) cases. Lymphovascular invasion was noted in 10 (20.0%) cases and a total of 10 (20.0%) cases had perineural invasion. Depth of invasion (DOI) could be assessed in 30 cases as the rest of 20 cases were small biopsies. A maximum number of cases, i.e., 16 (53.3%) cases, had a depth of invasion <10 mm.

Pathological tumor stage pT4 was observed in a maximum in 40.0% of cases. Pathological nodal status was noted majorly in pN0 stage with 63.3% cases. Pathological staging could be done in 30 out of 50 cases because only 30 resection biopsies were available. Clinical tumor stage was majorly observed in CT4 stage with 30% cases. Clinical nodal status was noted majorly in N0 stage

Table 4: Table showing correlation between cyclin D1 total score with types of oral carcinoma

TS grade	Tumor type				Total
	Scc	Percentage (%)	Verrucous carcinoma	Percentage (%)	
Moderate	10	21.28	0	0.00	10
Strong	5	10.64	0	0.00	5
Weak 32		68.09	3	100.00	35
Total	47	100	3	100	50

Statistical Test: Fisher's exact test = 52.901, *p-value* = 0.001

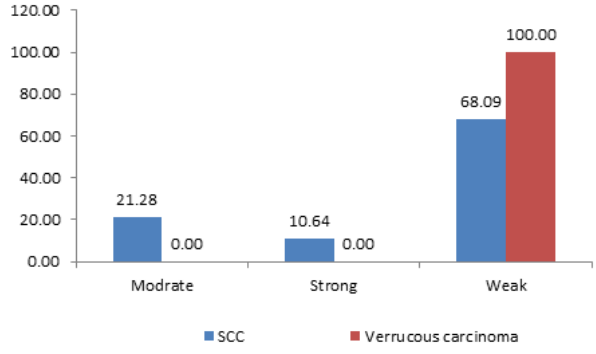

Figure 1: Bar diagram showing relationship between cyclin D1 total score with types of tumor

with 60% cases. Oral carcinoma cases were assessed, a maximum number of cases was found of SCC grade 2 with 35 (70%) cases, followed by SCC grade 1 with 12 (24%) cases and verrucous carcinoma with 3 (6%) cases. On grading of squamous cell carcinoma, a maximum number of cases were of moderately differentiated grade 2 SCC (35;74.5%) followed by well-differentiated grade 1 SCC (12; 25.5%). No case of grade 3 SCC was found in this study.

Our study observed a correlation between cyclin D1 total score and types of oral carcinoma. Weak positivity was seen in all 3 (100%) cases of verrucous carcinoma. Moderate positivity was noted in 10 (21.8%) cases of SCC. Strong positivity was noted in 5 (10.64%) cases of SCC. A strong association was found between the total score of cyclin D1 among verrucous carcinoma and squamous cell carcinoma (Grade 1 and 2) ($p < 0.05$) (Table 4 and Figure 1).

A correlation between cyclin D1 total score and histopathological grades of squamous cell carcinoma was observed**.** There were 12 cases of grade 1 of SCC out of 47 SCC oral biopsy. Most of the cases of grade 1 SCC were observed to have weak staining in 84% of cases (Figure 2 A and B), followed by moderate staining in 8% of cases and strong staining in 8% of cases. grade 2 had 35 cases out of a total 47 SCC biopsies. Most of the grade 2 SCC showed weak staining in 63% cases, followed by moderate staining in 26% of cases (Figure 2 C and D) and strong staining in 11% of cases (Figure 2 E and F). No association was found between the total score of

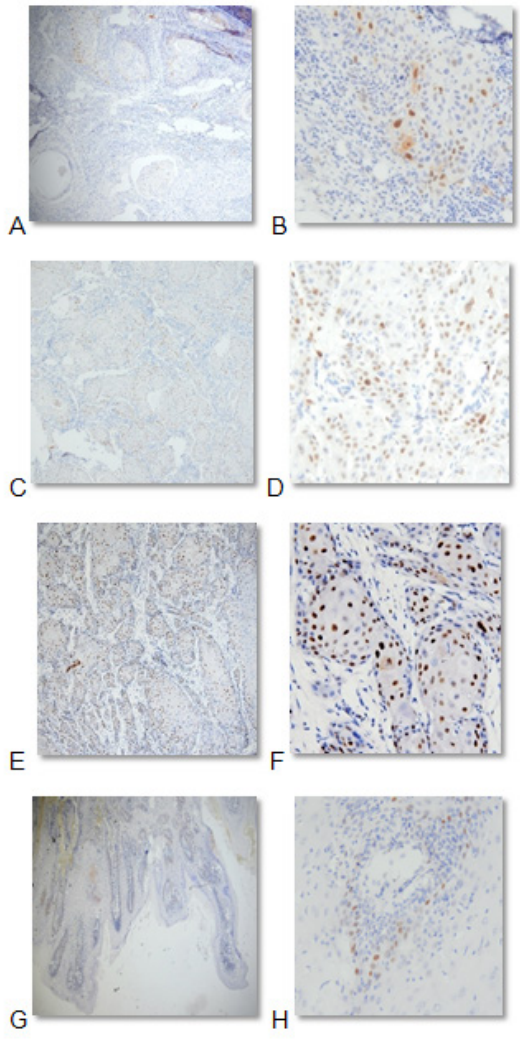

Figure 2: A,B) Cyclin D1 expression in well differentiated squamous cell carcinoma with weak staining at 10x & 40x magnification. C,D) Cyclin D1 expression in moderately differentiated squamous cell carcinoma with moderate staining at 10x & 40x magnification. E,F) Cyclin D1 expression in moderately differentiated squamous cell carcinoma with strong staining at 10x & 40x magnification. G,H) Cyclin D1 expression in verrucous carcinoma with weak staining at 10x & 40x magnification

cyclin D1 and histopathological grades of SCC ($p > 0.05$) (Table 5 and Figure 3)

No significant association was found between Pathological staging and clinical staging with cyclin D1 total score. No significant association was found between LVI, PNI, site, laterality, age groups and gender with cyclin D1 total score either.

Table 5: Table showing correlation between cyclin D1 total score with histopathological grades of squamous cell carcinoma

Ts grade	Grade				Total
	G1	%	G2	%	
Moderate	1	8	9	26	10
Strong	1	8	4	11	5
Weak	10	84	22	63	32
Total	12	100	35	100	47

Statistical Test: Fisher's exact test = 1.899, *p-value* = 0.387

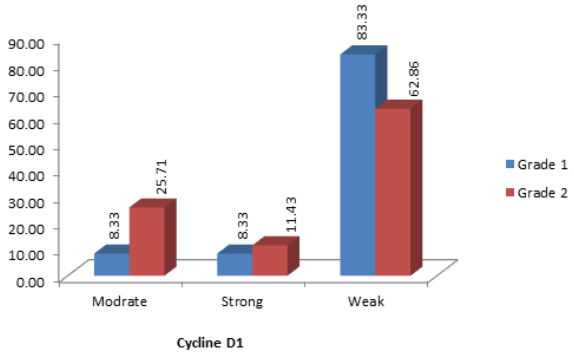

Figure 3: Bar diagram showing correlation of cyclin d1total score with histopathological grades of oral squamous cell carcinoma

DISCUSSION

Oral cancers are a heterogeneous set of malignancies in terms of etiology, biological behavior and prognosis. A primary theory of cancer is that the tumors occur from the accumulation of a number of distinct genetic events that summate to form an invasive cancer. These changes include the activation of oncogenes and inactivation of tumor-suppressor genes, leading to uncontrolled cell proliferation.[10]

Oral cancer can involve labial mucosa, buccal mucosa, floor of the mouth, alveolar ridge, gingiva, anterior two-thirds of the tongue (anterior to the circumvallate papillae), hard palate and retromolar trigone. It is a malignant epithelial neoplasm of the oral mucosa that has multifactorial pathogenesis. The process passes through the numerous phases of potentially malignant changes prior to the development of invasive carcinoma. These preneoplastic changes include mild, moderate, and severe epithelial dysplasia.[11]

As far as histopathological characteristics were concerned, in the present study, we observed 3 (6%) cases of verrucous carcinoma, 12 (24%) cases of Grade 1 SCC and 35 (70%) cases of grade 2 SCC. There was no poorly differentiated squamous cell carcinoma case in the study. Similar findings were noted in Saawarn *et al.*[12] and Sana Fatima *et al.*,[13] who reported most moderately differentiated grade 2 SCC cases. On the contrary, Maryam Nazar *et al.*,[14] had the most common well-differentiated grade 1 SCC cases in their study.

The present study was carried out to study the immunohistochemical reactivity and expression of cyclin D1 and its association with types, histopathological differentiation, clinical and pathological staging of oral carcinoma. The current study found cyclin D1 immunohistochemical expression in 100% of cases. The present study also matches other studies by Dhingra *et al.*,[15] Hala Hanbuli*et al.*[16] and Bakhtiar *et al.*,[17] who reported 100% cyclin D1 reactivity. However, a study conducted by Saawarn *et al.*,[12] reported 45% cases and Shergill *et al.*[18] reported 48.5% cases for cyclin D1 expression in oral carcinoma cases.

To analyze the cyclin D1 expression, we used an objective and comprehensive method, as Dhingra *et al.*[15] reported by multiplying the intensity score with labelling index score and giving total score as an expression divided into weak, moderate and strong grades. In our study, expression was graded as weak 1+ in 35 (70%) cases, moderate 2+ in 10 (20%) cases and strong 3+ in 5 (10%) cases. Similar findings were noted in a study conducted by Dhingra *et al.*,[15] in which most cases showed weak cyclin D1 expression followed by intermediate and strong cyclin D1 expression. Saawarn *et al.*,[12] and Maryam Nazar *et al.*,[14] also showed weak expression in majority of the cases, remarkable to our study. Sana Fatima *et al.*,[13] noted conflicting results, who reported maximum cases of moderate intensity of cyclin D1 expression in oral carcinoma cases. On other hand, Bakhtiar *et al.*,[17] and Malak Ahmed *et al.*,[19] observed strong intensity in maximum cases.

In the present study, we analyzed squamous cell carcinoma (Grade 1 and Grade 2) in 47 (94%) cases and verrucous carcinoma in 3 (6%) cases out of total 50 oral biopsy specimens. The cyclin D1 expression shown by a maximum number of cases was weak, followed by moderate and strong**.** Weak expression was seen in mostly in SCC with 32 (68.1%) cases. Moderate expression was noted in 10 (21.2%) cases of SCC and strong expression in 5 (10.6%) cases of SCC**.** All three cases of verrucous carcinoma showed weak cyclin D1 expression. A strong association was found between cyclin D1 total score expression with squamous cell carcinoma (Grade 1 and Grade 2) and verrucous carcinoma (p 0.05). In contrast to our study Angadi *et al.*[20] observed no statistical significance in cyclin D1 expression between SCC and oral VC and T. R. Menaka *et al.*, [21] reported intense staining in maximum cases of VC.

In our study, there were 12 cases of grade 1 of SCC out of 47 oral biopsies of SCC. Most of the grade 1 SCC cases were observed to have weak staining in 10 (83%) cases, followed by moderate staining in 1 (8%) case and strong staining in 1 (8%) case. Grade 2 SCC had 35 cases out of total 47 squamous cell carcinoma biopsies. Most of

the grade 2 SCC, showed weak staining in 22 (63%) cases, followed by moderate staining in 9 (26%) cases and strong staining in 4 (11%) cases. There is no association between total score of cyclin D1 expression with histopathological grades of squamous cell carcinoma ($p > 0.05$). A similar result was observed by Dhingra *et al.*,[15] who found no significant relation between total score and the tumor grade. In contrary to our study, Sana Fatima *et al.*,[13] showed a statistically significant association between different grades of the OSCC and intensity scoring (*p-value* 0.004), and Saawarn *et al.*[12] reported increase in cyclin D1 expression with increasing differentiation. Highest expression was noted in WDSCC, followed by MDSCC and PDSCC.

We did not find a significant association of cyclin D1 expression with age, gender, risk factors, tumor grade, site, laterality, PNI, LVI, pathological and clinical stage. The findings in present study mostly emphasize the role of cyclin D1 and its progression in oral carcinoma. However, like previous studies, the present study also suffers from the problem of a small sample size owing to which other clinicopathological relationships could not be extensively explored. Hence, further studies with a larger sample size are recommended.

CONCLUSION

In the present study for cyclin D1 evaluation, its expression was found in all oral carcinoma cases. A strong association was found between cyclin D1 total score with squamous cell carcinoma (Grade 1 and Grade 2) and verrucous carcinoma. We did not find a significant association between cyclin D1 total score and age, gender, tumor grades, site, laterality, PNI, LVI, pathological and clinical staging. In conclusion, cyclin D1 might be used as a prognostic indicator for oral carcinoma.

One of the limitations of our study was the constrained sample size and lack of follow-up with patients. Hence, further studies on a larger sample size are recommended. Prognostic value of cyclin D1 is also recommended to be studied in longitudinal studies with follow-up.

COMPLIANCE WITH ETHICAL STANDARDS

Ethical clearance was given by the institutional ethics committee for this study. Written informed consent was obtained from all study participants, who were given permission to participate and publish their data.

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