Immunohistochemical Analysis of Expression of Cyclin D1 in Different Grades of Oral Squamous Cell Carcinoma

Sana Fatima [a*], Mervyn Hosein [b], Saima Akram Butt [a], Faraz Baig [c], Rehan Ahmed Siddiqui [d] and Fizza Abidi [a]

[a] Department of Oral Pathology, Ziauddin College of Dentistry, Ziauddin University, Karachi, Pakistan.
[b] Ziauddin College of Dentistry, Ziauddin University, Karachi, Pakistan.
[c] Department of Pathology, Ziauddin University, Karachi, Pakistan.
[d] Department of Research, Ziauddin University, Karachi, Pakistan.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Original Research Article

ABSTRACT

Aims: The present study aimed to evaluate the expression of cyclin D1 in oral squamous cell carcinoma and to find association of expression of cyclin D1 with different histological grades of oral squamous cell carcinoma.
Study Design: It is a cross sectional study.
Place and Duration of Study: Oral squamous cell carcinoma samples were collected histopathology laboratory of Ziauddin Hospital, North Nazimabad Karachi. Immunohistochemistry was performed in multiple disciplinary research laboratory of Ziauddin University, Karachi. The duration of study was from January 2021 to January 2022.
Methodology: The present study included taking biopsy samples from oral squamous cell carcinoma patients. Patients with history of other oral cavity tumors, patients with history of any other treatment of oral cancer were excluded from the study. Immunohistochemistry was performed on a total of 150 samples using anti cyclin D1 antibodies.
Results: A total of 150 samples were part of study. Expression of cyclin D1 was found to be present in 77.3% of cases where as 22.7% of cases did not show expression of cyclin D1. A

Corresponding author: E-mail: sana.fatima@zu.edu.pk;

statistically significant association was shown between different grades of oral squamous cell carcinoma and intensity scoring (p value 0.004). Association between labelling index score for cyclin D1 and different grades of oral squamous cell carcinoma showed statistically significant p value (0.003). Association of total scores of cyclin D1 with different grades of oral squamous cell carcinoma was also found to be statistically significant (p value 0.003).

Conclusion: The present study shows that oncogenic effects on cyclin D1 by mutations caused by oral squamous cell carcinoma can be detected by immunohistochemistry in the form of its overexpression. The association between overexpression of cyclin D1 and different histological grades can be utilize in predicting the oncogenic behavior of oral squamous cell carcinoma.

1. INTRODUCTION

The most common malignancy of oral cavity is oral squamous cell carcinoma (OSCC) which arises from its lining epithelium [1]. It accounts up to 85-95% of all oral malignancies [2]. OSCC burdens the world as sixth leading cause of death accounting for annual 2.5% of all new cancer cases and 1.9% of cancer deaths [3]. The use of tobacco, alcohol and betel nut are some of risk factors that results in development of OSCC [4]. Tobacco and alcohol are found to be independent risk factors for development of OSCC [5]. Treatment modalities for OSCC include surgery with or without postoperative chemotherapy or radiation. The survival rate of OSCC patients has not changed over the past 30 years in spite of advancement in treatment options notably for patients in advance stage so there is dire need to identify tissue based molecular markers that have potential relationship with OSCC which can help in its early diagnosis or prognosis [6].

Tumorigenesis is a multistage process that causes disturbance in cell cycle. This disturbance in cell cycle leads to uncontrolled growth of tumor cells which is the characteristic feature of cancer.

Cyclin d1 gene (CCND1) is located on long arm of chromosome 11q13 [7]. It encodes 36-kda protein cyclin d1 which plays a critical role in regulation of cell cycle by modulating the transition of cell cycle from g1 to s phase. Cyclin D1 forms complexes with cyclin dependent kinases (cdk-4 and cdk-6), by binding and activating these protein kinases , required for phosphorylation of retinoblastoma (prb) protein resulting in release of E2F transcription factor to transcribe genes which are needed by cells to enter into the s phase [8]. Cyclin D1 is overexpressed in a variety of human malignancies such as breast carcinomas [9], hepatocellular carcinomas [10], papillary thyroid carcinomas [11] and OSCC [12]. The overexpression of cyclin D1 can be detected by immunohistochemistry that harmonize well with histopathology, which being the gold standard for microscopic study and detection of OSCC. The present study aims to evaluate the expression of cyclind1 in OSCC and to find its association with different grades of OSCC using immunohisto-chemical method.

2. MATERIALS AND METHODS

This laboratory based immunohistochemical study involved use of 150 formalin fixed paraffin embedded tissue blocks of histopathological diagnosed cases of oral squamous cell carcinoma from department of histopathology, Ziauddin Hospital North Nazimabad, Karachi. Informed consent was taken from patients. Ethical clearance was taken from ethical committee of institute. Cases diagnosed other than OSCC, cases with limited tissue material for accurate diagnosis and cases with extensive necrosis were excluded from study.

2 sections of 3-4 um were cut from each tissue block. One for Hematoxylin and Eosin staining and other for immunohistochemistry. Grading of tumors were done according to Broder's criteria [13].

Immunohistochemical analysis was performed by using tissue sections present on adhesive (saline) slides. The slides were deparafinized and hydrated by placing in series of reagents, each for 1 minute in following sequence: xylene followed by 95% alcohol followed by tap water. Antigen retrieval was performed by using pressure cooker for 7 minutes. The slides were incubated with blocking agent to block endogenous peroxidase activity for 10 minutes then rinsed with tap water. The slides were incubated with primary antibody at dilution of

1:100 (pa5-32373). The slides were rinsed with PSB followed by incubation with secondary antibody and then rinsed again followed by application of (DAB) solution. The sections were washed then counter stained with hematoxylin. Coverslips were placed and slides were examined under light microscope.

For quality control slide of tonsil tissue was used positive control and slide of OSCC without primary antibody was used as negative control.

Staining of nucleus was considered was positive for cyclin D1 expression when present in greater than 1% of cells. The percentage of cells showing nuclear staining was calculated as expression score (ES) 1, 2, 3 and 4 when 1-25%, 25-50%, 50-75% and > 75% cells stained respectively. The staining intensity score (IS) was recorded as 1(mild), 2(moderate) and 3(strong). The total score (TS) was calculated by multiplying ES with is and graded as weak, moderate and strong [14].

3. RESULTS

The obtained data were entered and analyzed on SPSS (version 20.0). In the present study of 150 cases of OSCC 77.3% of cases were immune positive for cyclin D1 whereas 22.7% of cases did not show any expression of cyclin D1. The immune positivity for cyclin D1 was shown by 37.5% of cases of alveolus, 76.59% cases of buccal mucosa, 93.75% cases of tongue and 100% cases of each lip and palate. The cases of OSCC were classified into well, moderate and poorly differentiated tumors among which high immune positivity was shown by moderately differentiated tumors followed by well differentiated tumors and then by poorly differentiated tumors (Table 1).

Association between intensity and differentiation of grades of OSCC was checked using Chi square test which showed a statistically significant P value ($P = 0.004$). (Table 2).

A statistically significant association was also found between labelling index score and different grades of OSCC $P = 0.003$ (Table 2).

Association between total scores of cyclin D1 and different grades of OSCC also showed a statistically significant p value ($P= 0.003$) (Table 3).

Table 1. Immune reactivity of Cyclin D1 according to site and grading of OSCC

Site of OSCC	Subsites of OSCC	Total number	Positive cases	Negative cases	% of positive reactivity
	Buccal mucosa	94	72	22	76.59%
	tongue	32	30	2	93.75%
	alveolus	16	6	10	37.5%
	lip	6	5	0	100%
	palate	2	2	0	100%
Grade of OSCC	Well differentiated	17	9	8	52.94%
	Moderately differentiated	126	105	21	83.33%
	Poorly differentiated	7	2	5	28.57%

**Table 2. Association between grades of OSCC with intensity and expression score for cyclin D1**

Grades of OSCC	Mild intensity	Moderate intensity	Strong intensity	P value	Score 1	Score 2	Score 3	Score 4	P value
Well differentiated	3	3	3		6	3	0	0	
Moderately differentiated	31	40	34	0.004*	63	27	15	0	0.003*
Poorly differentiated	0	2	0		2	0	0	0	
Total OSCC	34	45	37		71	30	15	0	

** Statistically significant, Chi square test applied*

Table 3. Association of total scores of cyclin D1 with site of OSCC and grades of OSCC

|	Subsites of OSCC	Weak	Moderate	Strong	P value
Site of OSCC	Buccal mucosa	55	11	6	
	tongue	6	0	0	
	alveolus	12	12	6	0.00*
	lip	3	0	3	
	palate	2	0	0	
Grades of OSCC	Well differentiated	6	3	0	
	Moderately differentiated	70	20	15	
	Poorly differentiated	2	0	0	0.003*

* Statistically significant, Chi square test applied
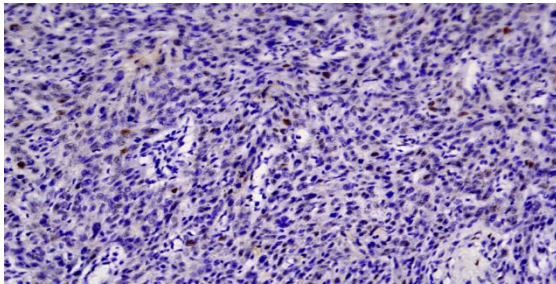
Weak expression of cyclin D1 in OSCC
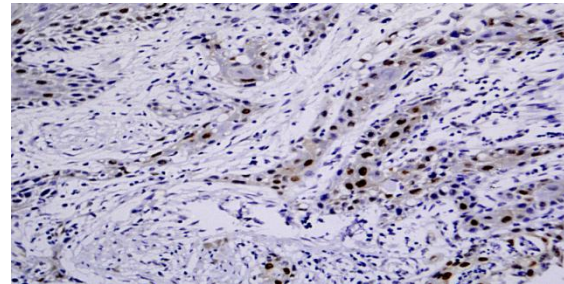
Moderate expression of cyclin D1 in OSCC
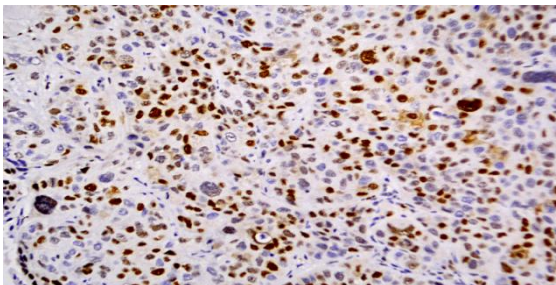
Strong expression of cyclin D1 in OSCC

4. DISCUSSION

The present study was conducted to determine the immunoreactivity and expression of cyclin D1 in OSCC and its association with site of OSCC and different grades of OSCC. The immune positivity of cyclin D1 was determined by presence of brown staining in nucleus. In normal cells the levels of cyclin d1 lowers as the cell progresses to S phase by GSK3β-mediated phosphorylation of THr286 and CRM1-dependent nuclear export followed by degradation in cytoplasm by 26S proteasome in ubiquitin-dependent manner. However in presence of abnormal proliferation and in cancerous cells due to mutation of cyclin D1 it does not remove resulting in nuclear accumulation of cyclin D1 resulting its higher expression [15]. In this study increased expression of cyclin D1 was mainly found in peripheral layers of tumor islands which explains 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 positivity of over expression of cyclin D1 was recorded in 77.3% of cases in present study. Similar results were reported by a study conducted by Punnya et al., in which they found 70.7% of expression of cyclin D1 in OSCC cases [17]. The present study is also in accordance with other studies in which expression of cyclin D1 is reported as 68%, 66.6% and 58.3% in OSCC [18, 19, 20]. Over expression of cyclin D1 can be due to amplification of its CCND1 gene, translocation of chromosomes and other post translational events [21]. Overexpression of cyclin D1 causes dysregulation of cyclin dependent kinases, changes in cell cycle control, less dependency on growth factors, unchecked

proliferation of cells and ultimately tumor growth [22].

However, study conducted by Saawarn et al. [14] reported 45% of expression of cyclin D1 in OSCC cases. A study conducted by Huang et al. [23] found expression of cyclin D1 in only 36% of cases of OSCC. The present study showed a statistically significant association between total score of expression of cyclin d1 with grades of OSCC. Our results are in accordance with other studies by Saawarn et al and Das et al. [14, 19]. However the findings of this study are not in agreement with some previous studies by Dhingra et al. and Zhi-en et al. [24, 25] in which they did not find any significant association between total score of expression of cyclin D1 and grades of tumor. The differences in results might be attributed in differences in technique and type of antibodies used for detection for expression of cyclin D1.

5. CONCLUSION

One of the important parameter of OSCC that helps in understanding the outcome of patients is grading. The present study attempted to evaluate the expression of cyclin D1 and its association with different grades of OSCC. Increase expression of cyclin d1 reflects the proliferative activity of cyclin D1 in OSCC. Observations made in this study may help in better understanding of characteristics of OSCC. It may also contribute in helping the clinicians to provide more appropriate treatment options as well as in wellbeing of patients.

CONSENT

As per international standard or university standard, patients' written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

Ethical approval was obtained from Ziauddin university ethics review committee (reference code: 0040218SFOPATH

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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