Original Article

Frequency of Cyclin D1 Insquamous Cell Carcinoma: Does this have any Prognostic Implications?

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ABSTRACT

OBJECTIVE: To evaluate CyclinD1 as a bio-marker for Squamous cell carcinoma and to see it's prognostic role in disease outcome.

METHODOLOGY: This Descriptive cross-sectional study was conducted at section of Histopathology, Department of Pathology & Microbiology at Aga Khan University hospital from September 2016 till August 2017. Convenient sampling technique was used and 85 resection specimens (Paraffin Blocks) of invasive Squamous cell carcinoma of oropharynx of stage III & IV as per TNM classification were included in the study and Immunohistochemical staining for Cyclin D1was performed. Data analysis was done on SPSS version 19.0. Mean, standard deviation, frequencies and Chi square test were applied and p value less than or equal to 0.05 was taken as significant.

RESULTS: More than 50% staining for Cyclin D1 was seen in 78.6%(n=67) of the cases while, 21.2% (n=18) samples showed < 50% staining.

CONCLUSION: In conclusion, our results showed that cyclin D1 is overexpressed in majority of patients with oral SCC and is associated with aggressive clinicopathological features.

KEY WORDS: Squamous cell Carcinoma, CyclinD1, TNM staging.

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INTRODUCTION

Squamous cell carcinoma (SCC) is one of the most common malignant tumors found in human and accounts for almost 90% of malignancies of oral cavity[1]. SCC is reported to be the sixth most common malignant lesion in humans. SCC in oral cavity termed as Oral squamous cell Carcinoma (OSCC) is usually seen in middle to old aged persons[1]. Habit of tobacco usage and alcohol drinking greatly increases the chances of development of OSCC. But studies have shown high incidence of OSCC in persons with no known risk factors[2]. Though, there are many state of the art recent advances are seen in cancer treatment but still overall survival rate of OSCC is significantly constant in last 30 years[3,4]. Currently, the most important prognostic evaluation factor for OSCC is lymph node metastasis (TNM) staging, but in many cases, the tumors with same diagnostic staging responds very differently to all the treatment modalities and hence possess different prognosis[5]. Keeping this in mind, many researches are in process in this regard to identify novel biological and molecular bio-markers which might hold important prognostic role in disease outcome.

OSCC carcinogenesis requires 6-10 genetic events which ultimately lead to disruption of pathways that regulate basic cellular functions, cell cycle and cell death pathways[6]. Hence, it's a multistep process in which several factors such as Tumor suppression genes, Oncogenes etc. are involved[6,7].

Alteration in cell cycle pathways is seen to be involved in different cancers and two important cancer suppressor genes in this regard are Retinoblastoma gene (Rb) and the p53 gene[7,8]. These two regulates transition between different phases of cell cycle at checkpoints. Rb pathway proteins (p16, Rb and Cyclin D1 gene products) are responsible for the regulation of G1-S transition[7-10].

Rb holds the cell cycle in G1 phase, but when Rb is phosphorylated the transition fromG1 to S phase can occur. The phosphorylation of Rb protein is achieved by Cyclin Dependent Kinases (CDKs) CDK4 and CDK6encoded by Cyclin D1 gene located on chromosome 11q13.Cyclin D-CDK4/CDK6 complexes phosphorylates Rb protein as a result, it gets dissociated from the E2F transcription factor and hence process of DNA replication is initiated[7-11]. Researches have shown that overexpression of Cyclin D1 leads to accelerated cell cycle turnover with less requirement of growth factors in many cancers and OSCC is not an exception[12,13]. One study showed that 71.4% of the sample tumors (OSCC) showed low immune-reactivity and 17.1% showed high immune-reactivity for Cyclin D1[14]. Currently the data on cyclin D1 in Patients with carcinoma of oropharynx is scarce and secondly it is comparatively newer modality that shows overexpression in human tumors. If the overexpression is found to be higher in patients with carcinoma of oropharynx then further studies may be undertaken to assess its validity for use of this

modality as screening test.
So, keeping the importance of identification of new biological and prognostic factors which can contribute to the aggressiveness of OSCC we did this study with the objective, to determine the frequency of cyclin D1 overexpression in patients with squamous cell carcinoma of oropharynx.

METHODOLOGY

This Descriptive cross sectional study was conducted at section of Histopathology, Department of Pathology & Microbiology at Aga Khan University hospital and 85 cases of Squamous cell carcinoma of oropharynx were included using Non probability, consecutive sampling technique.
The inclusion criteria was that all resection specimens (tissue blocks) of invasive Squamous cell carcinoma of oropharynx of stage III & IV as per TNM classification (done in collaboration with oncologist) of more than 6 months duration of either gender were included and scanty biopsy specimen, poorly fixed or autolyzed specimen were excluded. Data analysis was done on SPSS version 19.0. Mean, standard deviation, frequencies and Chi square test were applied and p value less than or equal to 0.05 was taken as significant.
IMMUNOHISTOCHEMICAL STAINING FOR CYCLIN D1
The cyclin D1 expression was studied in formalin fixed and paraffin embedded tumor sample by the standard immunohistochemical technique on 5 micron paraffin section using streptavidin –biotin universal detection system using automated immunostianer Autostainer link 48 (DAKO/Denmark). After processing sections were washed and treated with primary antibody of Cyclin D1 [M3642] followed by treatment with biotinylated secondary antibody for 30 min. at room temperature. Slides were made and examined under light microscope. Presence of brown nuclear staining and >50% of the nuclei were labelled as Cyclin D1 expression positive[15]. Variables including age, gender, patient identification and Cyclin D1 expression were obtained on a predesigned form.

RESULTS

Our samples included 13% females (n=11) and 87% male (n=74). On the basisof morphology 98.8% (n=84) were moderately differentiated Squamous cell carcinoma and 1.2% (n=1) was well differentiated. In our samples the most common site was left buccal mucosa, followed by right buccal mucosa and tongue (Figure I). Most of our patients were in T4 stage followed by T3 and in nodal staging most patients were in N0 stage followed by N1(Table I).
As far as staining percentage of Cyclin D1 is concerned, more samplesshowed >50% staining (Table II). Comparison of Cyclin D1 overexpression in different study parameters such as gender, T and N stage and Site of tumor is done by using Chi Square test and is shown in Table III, comparison with age and duration of disease is expressed in Table IV.

FIGURE I: DISTRIBUTION OF SITE

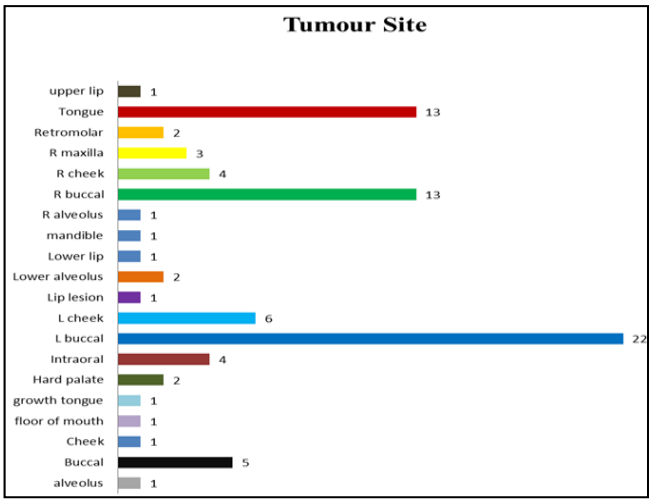

TABLE I: CLASSIFICATION OF T AND N-STAGE IN STUDY PATIENTS

T STAGE	Frequency	Percentage %
T1	2	2.4%
T2	10	11.8%
T3	32	37.6%
T4	40	47.1%
T4a	1	1.2%
Total	85	100.0%
N SATGE	**Frequency**	**Percentage %**
N0	37	43.5%
N1	24	28.2%
N2	10	11.8%
N2b	13	15.3%
N2c	1	1.2%
Total	85	100%

TABLE II: CLASSIFICATION OF OVEREXPRESSION OF CYCLIN D1 IN PATIENTS WITH ORAL SQUAMOUS CELL CARCINOMA (OSCC)

Staining Cyclin D1	Frequency	Percentage %
≤50%	18	21.2%
>50%	67	78.6%
Total	85	100.0%

TABLE III: COMPARISON OF CYCLIN D1 OVEREXPRESSION WITH DIFFERENT PARAMETER OF THE STUDY IN PATIENTS WITH OSCC

De-mographics	Attributes	Cyclin (n=85)		P-Value
		Positive	Negative	
Sex	Male	57(67.1%)	17(20%)	0.293
	Female	10(11.8%)	1(1.2%)	
T Stage	T1	1(1.2%)	1(1.2%)	0.301
	T2	6(7.1%)	4(4.7%)	
	T3	28(32.9%)	4(4.7%)	
	T4	31(36.5%)	9(10.6%)	
	T4a	1(1.2%)	0(0%)	
N Stage	N0	29(34.1%)	8(9.4%)	0.086
	N1	17(20%)	7(8.2%)	
	N2	8(9.4%)	2(2.4%)	
	N2b	13(15.3%)	0(0%)	
	N2c	0(0%)	1(1.2%)	
Tumour Site	alveolus	1(1.2%)	3(3.5%)	0.093
	Buccal	39(45.9%)	7(8.2%)	
	Cheek	0(0%)	1(1.2%)	
	floor of mouth	1(1.2%)	0(0%)	
	Growth tongue	1(1.2%)	0(0%)	
	Hard palate	1(1.2%)	1(1.2%)	
	intraoral	0(0%)	0(0%)	
	Intraoral	4(4.7%)	0(0%)	
	L buccal	0(0%)	0(0%)	
	L cheek	0(0%)	0(0%)	
	Lip lesion	0(0%)	0(0%)	
	Lower alveo-lus	0(0%)	0(0%)	
	Lower lip	0(0%)	0(0%)	
	mandible	1(1.2%)	0(0%)	
	R alveolus	0(0%)	0(0%)	
	R buccal	0(0%)	0(0%)	
	R cheek	2(2.4%)	2(2.4%)	
	R maxilla	2(2.4%)	1(1.2%)	
	Retromolar	1(1.2%)	1(1.2%)	
	tongue	0(0%)	0(0%)	
	Tongue	11(12.9%)	2(2.4%)	
	upper lip	0(0%)	0(0%)	
	Lip	3(3.5%)	0(0%)	

TABLE IV: COMPARISON OF CYCLIN D1 OVEREXPRESSION WITH AGE & DURATION OF DISEASE IN PATIENTS WITH OSCC

De-mographics	Attributes	Cyclin (n=85)		P-value
		Positive	Negative	
Age Groups	**≤50 Years**	39(45.9%)	8(9.4%)	0.297
	>50 Years	28(32.9%)	10(11.8%)	
Duration Disease	**<1 Years**	42(49.4%)	8(9.4%)	0.163
	>1 Year	25(29.4%)	10(11.8%)	

DISCUSSION

Oral squamous cell carcinoma (OSCC) is one of the most prevalent type of malignant disorder and it is the sixth most common cancer worldwide and furthermore in developing countries it is estimated to be the third most common malignancy[16]. Multiple factors are thought to be involved in the carcinogenic pathway of Squamous cell carcinoma in which multiple genetic changes are considered to play the most important role. These mutations either through formation abnormal proteins or through formation of normal proteins in increased amountcauses the loss of control on cell cycle. These mutations are also responsible for the activation of oncogenes and altered functions of tumor suppressor genes. As a result, uncontrolled growth and ultimately invasion of these cells occur which is the hallmark of the malignant disorders[17].

Worldwide, there are 3-10% mortality is because of Oral squamous cell carcinoma (SCC)[18], one of the major reason for this could be late diagnosis of the disease, but with the advent of new diagnostic tools early diagnosis is also becoming possible which would ultimately lead to better prognosis for the patients suffering from this disease[19].

Cyclin D1 is a cell regulator and can serve as an attractive potential marker for tumor aggressiveness. So, it can be utilized as a prognostic tool for Head and Neck SCC across anatomic subsites. There are many methods for analyzing Cyclin D1 expression ranging from routine immunohistochemical expression to sophisticated tools like amplification[20-23]. Some studies suggest that Cyclin D1 is found to be overexpressed by gene amplification or by signaling dysregulation in more than 80% of non–HPV-related head and neck SCC[24,25], which is in concordance with the results of our study where we found 67/85 cases (78.8%) showed Cyclin D1 positivity.

Research studies have evaluated Prognosis of Oral Squamous cell Carcinoma withthe immunohistochemical expression of cyclin D1 and have concluded that the cases which show over expression of Cyclin D1 on Immunohistochemistry exhibits more aggressive behavior and shorter overall survival[26-28]. This is also elaborated by the findings of our study where 40 out of 85 cases which showed

positivity for cyclin D1 were T4 lesions, while 32 while T3, 10 were T2 and two were T1 lesions showing that most cases having Cyclin D1 overexpression belonged to advanced T stages and hence bear poor prognosis.

Similarly for nodal staging 37 out of 85 cases which showed cyclin D1 over expression were N0 lesions, 24 were N1 and 10 were N2 lesions, This shows that cyclin D1 1overexpression are associated with lymph node metastasis and thus poor patient prognosis in oral Squamous cell carcinomas.

On the other hand Lam KY 2000[28] concluded that higher expression of cyclin D1 is seen in oral squamous cell carcinomas (OSCC) with low and intermediate grades of differentiation as compared to that with high-grade of differentiation. In our study 84/85 cases were moderately differentiated squamous cell carcinoma and only one cease was of well differentiated carcinoma hence we could not do this comparison of Cyclin D1 expression in our study.

Our study showed that cyclin D1 overexpression is associated with higher T stage and lymph node metastasis and thus poor patient prognosis in oral Squamous cell carcinomas.

CONCLUSION

In conclusion, are results showed that cyclin D1 is overexpressed in majority of patients with oral Squamous cell carcinoma and it is associated with aggressive clinicopathological features including advanced tumor stage, lymph node metastasis and poor patient prognosis.

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AUTHOR CONTRIBUTIONS

George A: Sample Collection, Analysis
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REFERENCES

1. Renjith S, Chandran S. Current Trends of Drugs Acting on Oral Squamous Cell Carcinoma (OSCC). In: Understanding Cancer Therapies. CRC Press; 2018 Mar 9. p. 13-23.
2. Porter S, Gueiros LA, Leão JC, Fedele S. Risk factors and etiopathogenesis of potentially premalignant oral epithelial lesions. Oral Surg Oral Med Oral Pathol Oral Radiol. 20181; 125(6): 603-11. doi: 10.1016/j.oooo.2018.03.008.
3. Martinez EÁ, López AP, Palacio M, Salas ZC, Grisales H. Survival of cancer patients oral squamous cell treated for the first time in oncology centres in the period 2000-2011, Medellin Colombia. Int J Oral Max Surg. 2017; 46(1): 115. doi:10.1016/j.ijom.2017.02.405.
4. Chou HC, Lin HW, Yang JH, Lin PY, Cheng SJ, Wu YH et al. Clinical outcomes of oral cancer patients who survive for more than 5 years in Taiwan. J Formos Med Assoc. 2019; 118(12): 1616-22. doi: 10.1016/j.jfma.2019.07.022.
5. Moeckelmann N, Ebrahimi A, Tou YK, Gupta R, Low TH, Ashford B, et al. Prognostic implications of the 8th edition American Joint Committee on Cancer (AJCC) staging system in oral cavity squamous cell carcinoma. Oral Oncol. 2018; 85: 82-6.
6. Jain A. Molecular Pathogenesis of Oral Squamous Cell Carcinoma. In: Squamous Cell Carcinoma-Hallmark and Treatment Modalities. Intech Open.; 2019 May 31. P. 75-100.
7. Jia H, Wang X, Sun Z. Exploring the molecular pathogenesis and biomarkers of high risk oral premalignant lesions on the basis of long noncoding RNA expression profiling by serial analysis of gene expression. Eur J Cancer Prev. 2018; 27(4): 370-78. doi:10.1097/CEJ. 0000000000000346.
8. Johnson J, Thijssen B, McDermott U, Garnett M, Wessels LF, Bernards R. Targeting the RB-E2F pathway in breast cancer. Oncogene. 2016 ; 35 (37): 4829-35. doi: 10.1038/onc.2016.32.
9. Li T, Xiong Y, Wang Q, Chen F, Zeng Y, Yu X et al. Ribociclib (LEE011) suppresses cell proliferation and induces apoptosis of MDA-MB-231 by inhibiting CDK4/6-cyclin D-Rb-E2F pathway. Artif Cells Nanomed Biotechnol. 2019; 47(1): 4001-11. doi:10.1080/1691401 .2019.1670670.
10. Fischer M, Müller GA. Cell cycle transcription control: DREAM/MuvB and RB-E2F complexes. Crit Rev Biochem Mol Biol. 2017; 52(6): 638-62.
11. Xiong Y, Li T, Assani G, Ling H, Zhou Q, Zeng Y, et al. Ribociclib, a selective cyclin D kinase 4/6 inhibitor, inhibits proliferation and induces apoptosis of human cervical cancer in vitro and in vivo. Biomed Pharmacother. 2019; 112: 108602.
12. Tyagi N, Deshmukh SK, Srivastava SK, Azim S, Ahmad A, Ahmed AG, et al. ETV4 Facilitates Cell-Cycle Progression in Pancreatic Cells through Transcriptional Regulation of Cyclin D1. Mol Cancer Res. 2018; 16(2): 187-96.
13. Ma J, Ren Y, Zhang L, Kong X, Wang T, Shi Y, et al. Knocking-down of CREPT prohibits the progression of oral squamous cell carcinoma and suppresses cyclin D1 and c-Myc expression. PloS one. 2017; 12(4).
14. Carlos de Vicente J, Herrero-Zapatero A, Fresno MF, Lopex Arranz JS. Expression of cyclin D1 and

ki-67 in squamous cell carcinoma of the oral cavity: Clinicopathological and prognostic significance. Oral Oncol. 2002; 38(3): 301-8. doi: 10.1016/s1368-8375(01)00060-4.
15. Carlos de Vicente J, Junquera Gutiérrez LM, Zapatero AH, Fresno Forcelledo MF, Hernández-Vallejo G, LópezArranz JS. Prognostic significance of p53 expression in oral squamous cell carcinoma: Without neck node metastases. Head Neck. 2004; 26: 22-30.
16. Tokuzen N, Nakashiro KI, Hamakawa H. Detection of somatic mutations in exosomal RNA from human oral squamous cell carcinoma cells [abstract]. In: Proceedings of the American Association for Cancer Research Annual Meeting 2017; 1-5; Washington, DC. Philadelphia (PA): AACR; Cancer Res 2017; 77(13 Suppl): 4461.
17. Mirza D, Raza G, Basit A, Naqvi K, Ahmed S, Abassi ZA. Oral squamous cell carcinoma (OSCC) in Karachi city--a retrospective study. Pak Oral Dent J. 2016; 36(3): 391-4 .
18. Yaaqob MA, Hairon SM, Kannan TP, Rahman NA. Factors associated with mortality of oral squamous cell carcinoma (OSCC) patients managed at Hospital USM. Archi Orofa Sci. 2019; 14(1): 10-12.
19. Sherr CJ, Sicinski P. The D-type cyclins: a historical perspective. In: D-type Cyclins and Cancer. Springer, Cham; 2018. p. 1-26.
20. Li Z, Cui J, Yu Q, Wu X, Pan A, Li L. Evaluation of CCND1 amplification and CyclinD1 expression: diffuse and strong staining of Cyclin D1 could have same predictive roles as CCND1 amplification in ER positive breast cancers. Am J Transl Res. 2016; 8(1): 142.
21. Bahnassy AA, Zekri AR, Saleh M, Lotayef M, Moneir M, Shawki O. The possible role of cell cycle regulators in multistep process of HPV-associated cervical carcinoma. BMC Clinpathol. 2007; 7(1): 4.
22. John RR, Malathi N, Ravindran C, Anandan S. Mini review: Multifaceted role played by cyclin D1 in tumor behavior. Ind J Dent Res. 2017; 28(2): 187.
23. Ansari SS, Sharma AK, Zepp M, Ivanova E, Bergmann F, König R, et al. Upregulation of cell cycle genes in head and neck cancer patients may be antagonized by erufosine's down regulation of cell cycle processes in OSCC cells. Oncotarget. 2017; 9(5): 5797-5810. doi: 10.18632/oncotarget.23537.
24. Smeets SJ, Braakhuis BJ, Abbas S, Snijders PJ, YIstra B, van de Wiel MA, et al. Genome-wide DNA copy number alterations in head and neck squamous cell carcinomas with or without oncogene-expressing human papillomavirus. Oncogene. 2006; 25(17): 2558-64. doi: 10.1038/sj.onc.1209275.
25. Ramos-Garcia P, Gil-Montoya JA, Scully C, Ayén A, González□Ruiz L, Navarro-Triviño FJ, et al. An update on the implications of cyclin D1 in oral carcinogenesis. Oral Dis. 2017; 23(7): 897-912. doi: 10.1111/odi.12620.
26. Michalides RJ, Van Veelen N, Kristel PMP, Hart AAM, Loftus BM, Hilgers FJM, et al. Overexpression of cyclin D1 indicates poor prognosis in squamous cell carcinomas of the head and neck. Arch Otolaryngol Head Neck Surg. 1997; 123(5): 497-502.
27. Ramos-García P, González-Moles MÁ, González-Ruiz L, Ayén Á, Ruiz-Ávila I, Bravo M, et al. Clinicopathological significance of tumor cyclin D1 expression in oral cancer. Arch oral biol. 2019; 99: 177-82. doi: 10.1016/j.archoralbio.2019.01.018.
28. Lam KY, Ng IO, Yuen APW, Kwong DLW, Wei W. Cyclin D1 expression in oral squamous cell carcinomas: clinicopathological relevance and correlation with p53 expression. J Oral Pathol Med. 2000; 29: 167-172.

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