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Original Article

Immuno-histochemical Expression of Cyclin D1 in Oral Squamous Cell Carcinoma, Oral Potentially Malignant Disorders, and Normal Oral Mucosa

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ABSTRACT

Due to a high risk and tendency of OPMDs towards OSCC, its early detection is essential for better survival rate. Several molecular markers are available for diagnosis and prognostic assessments of OPMDs, also evaluating aggressiveness of OSCC. **Objectives:** To assess the immuno-histochemical expression of Cyclin D1 in OSCC, OPMDs, and normal healthy oral mucosa. Cyclin D1 has a significant role in cell cycle control and also strongly linked with the metastatic and poorly differentiated tumour cells. **Methods:** The study comprised of already diagnosed 20 cases of OSCC, 20 cases of OPMDs, and 20 normal oral mucosa cells, as a control. **Results:** Cyclin D1 immuno-reactivity was found positive in 100% cases of OPMDs, and 100% in OSCC but not in normal oral mucosa cells. **Conclusion:** A statistically significant expression of Cyclin D1 was observed in OPMDs which may indicate the probability of their transformation to OSCC.

INTRODUCTION

Oral cancer predominantly arises from epithelium cells in which 90 to 95% are diagnosed as Oral Squamous Cell Carcinoma (OSCC) [1]. OSCC is one of the 10th most prevalent malignancies worldwide, responsible for approximately 300,000 new cases and about 145,000 deaths on annual basis [2]. It is the most frequent reasons of death consisting about 50% of the entire cancers [3]. In the United States, 63 000 new cases of head and neck cancers are likely to occur in 2017 accounting approximately 4% of newly diagnosed cancers [4]. OSCC is among the most leading malignancies in South and Southeast Asian countries where, it is the second common malignancy in Pakistan (both in male and female) but first among males with 15.9% new cases and second after breast cancer in females [5,6]. The commonly affected sites of OSCC are tongue, floor of the mouth, and buccal mucosa [7]. The well-established risk factors involved in its development are tobacco use and aggressive intake of alcoholic beverages [8]. Other possible risk factors can be Human Papilloma Virus (HPV) infections and nutritional deficiencies, among others [9]. Tobacco can be consumed in chewable and non-chewable forms. In South Asia Chewable tobacco is more common in form of naswar, paan, and gutka, among others. In Pakistan, common habits of taking excess amount of areca nut or any sort of smokeless tobacco betel quid, gutka, pan masala, naswar,

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tobacco, main puri, and mawa promotes higher incidence of oral cancer [10]. OSCC is usually preceded by changes in the mucosa for years or as a result of already existing oral lesions commonly known as oral potentially malignant disorders (OPMDs) [11]. These are clinical presentations that carry an increased risk of cancer development in the oral cavity in a clinically definable precursor lesion or in clinically normal healthy mucosa [12]. Oral leukoplakia, erythroplakia, actinic chelitis, oral lichen planus, and oral sub-mucous fibrosis are generally categorized as oral potentially malignant disorders [13]. Incidence of oral potentially malignant disorders ranges between 1-5% world-wide [1] and about 4.32% cases of OPMDs have chance of converting into malignancy [14]. Different diagnostic markers have been identified which are helpful in detection of the lesions that are at risk of malignant transformation. Out of these diagnostic markers, Cyclin D1 is one of the vital protein that plays major role in cell cycle regulation [15]. The CCND1 gene on chromosome band 11q13, encodes CyclinD1 protein and promotes development of cell cycle throughout the G1-S phase. High expression of Cyclin D1 accelerates the G1 phase alteration, leading to the abnormal cell proliferation. Thus it was proposed that cyclin D1 is strongly linked with the metastatic and poorly differentiated tumour cells [16]. Due to high risk and tendency of OPMDs towards malignancy, its early detection is essential for better survival rate. Thus several molecular markers are available for diagnosis of OPMDs however the development of additional, new reliable markers may aid in the precise prognostic assessment of OPMDs and aggressiveness of OSCC. Immuno-histochemical analysis of Cyclin D1 could be helpful in assessing OPMDs prognosis and potential for malignancy. Our study aims to evaluate and assess the expression of Cyclin D1 in OSSC and OPMDs, in addition to analyse the prognostic role of OPMDs.

METHODS

This descriptive cross-sectional research study was conducted in Pathology Department of Peshawar Medical College, which consisted of histologically diagnosed 20 cases of OSCC and 20 cases of OPMDs. The samples were collected through the non-probability convenient sampling technique. Already diagnosed Formalin-Fixed Paraffin Embedded (FFPE) blocks were obtained from the Pakistan Institute of Medical Sciences (PIMS), Histopathology Department's archives. Patients with recurrent tumours and those undergoing chemotherapy or radiation were excluded. 20 normal healthy oral mucosa cases from the alveolar ridge were obtained from the patients coming to Peshawar Dental College for tooth extraction or other dental procedures, after taking informed consent from them. Before starting the study, Ethical approval was taken from the institutional review board under the approval No. Prime /IRB/2019-178 of Prime foundation. Cyclin D1 immunohistochemistry was done on sections with the maximum epithelial content after histopathological examination of various kinds of OPMDs and OSCC. The immunohistochemistry (IHC) slides were subdivided into five groups. Each batch contained one slide of Mantle cell lymphoma as a positive control. Antigen retrieval was accomplished using a microwave oven, during the procedure. For immuno-histochemical labelling of selected sections, a primary antibody (monoclonal mouse antihuman Cyclin D1) and a secondary antibody (Dako EnVisionTM detection system) were applied. During procedure antigen retrieval was carried out using microwave oven. (Monoclonal Rabbit anti human CyclinD1) as primary antibody and (Dako EnVisionTM detection system) as secondary antibody were used for immune-histochemical staining of selected sections. Cyclin D1 immuno-positivity was assessed by the presence of brown colour immunostaining in the nucleus. Scoring criteria for Cyclin D1 immuno-positivity was categorized using scoring method prescribed by Dhingra, 2017 [17]. At 400X magnification, the expression of positive cells was assessed in at least five areas and then was graded as (1) 1-25%, (2) 26-50%, (3) 51-75%, and (4) >75%. Immunostaining intensity of Cyclin D1 was scored as (1) Mild (2) Moderate, and (3) Strong staining. Total Scoring (TS) of CyclinD1 was calculated by multiplying Expression Score (ES) with Intensity Score (IS) to produce an Immuno-Reactivity Score (IRS), which was then graded as (1-4) mild, (5-8) moderate, and (9-12) strong. For the statistical analysis, statistical package for social sciences (SPSS) version 20.0 was used. P-value ≤ 0.05 was considered as statistically significant.

RESULTS

Most of the patients age in OSCC and OPMDs were above 60 years, presenting with mean ages of 56.7 and 57.85 years, respectively. MF in OSCC was 1:1.2 while in OPMDs it was 2:3. Cyclin D1 expression was found positive in all layers of squamous epithelium in OSCC. In (80%) cases of OPMDs, Cyclin D1 staining was restricted to basal layer only while (20%) cases showed staining both in basal and supra-basal layers of squamous epithelium, Table 1.

Staining	OSCC			Total			Total (n%)
	WDSCC	MDSCC	PDSCC		Oral Leukoplakia	Oral Lichen Planus	
Basal Layer Only	-	-	-	20 . (100%)	8	8	16 (80%)
Basal and Supra-basal Layers	-	-	-		2	2	4 (20%)
All Layers	6	7	7		-	-	20 (100%)

Table 1: Allocation of the cases according to groups and pattern of staining for Cyclin D1 immuno-reactivity

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The statistical comparison made among the 3 study groups (OSCC, OPMDs, and Normal Oral Mucosa) for Cyclin D1 immuno-reactivity showed significant results p value (<0.05), Table 2.

CyclinD1 immune-reactivity	OSCC n (%)	OPMDs n (%)	Normal oral mucosa n (%)	Total	P-Value
Positive	20	20	-	40	<0.05
Negative	-	-	20 (100%)	20	
Total	20	20	20	60	

Table 2: Comparison of Cyclin D1 immuno-reactivity status between OSCC and OPMDs normal oral mucosa (*p value by Chi Square test)

Table 3 shows that in OSCC, all the 20 cases (100%) showed strong intensity. All 6 cases (100%) of Well Differentiated Squamous Cell Carcinoma (WDSCC) had strong Cyclin D1 staining intensity. All the 7 cases (100%) of Moderately Differentiated Squamous Cell Carcinoma (MDSCC) expressed strong Cyclin D1 staining. In the cases of Poorly Differentiated Squamous Cell Carcinoma (PDSCC), in all 7 cases (100%) the intensity of staining of Cyclin D1 was strong.

Cyclin D1 staining Intensity	OSCC			Total (n %)
	WDSCC (n %)	MDSCC (n %)	PDSCC (n %)	
Mild (1)	-	-	-	-
Moderate (2)	-	-	-	-
Strong (3)	6 (100%)	7 (100%)	7 (100%)	20 (100%)
Total	6 (30%)	7 (35%)	7 (35%)	20

Table 3: Cyclin D1 staining intensity among the cases of OSCC

Table 4 explains that in all 20 cases of OPMDs, staining intensity of Cyclin D1 was weak in 7 cases (35%), and moderate in 13 cases. Mild staining intensity of Cyclin D1 was found in 4 (40%) cases, and moderate in 6 (60%) cases, out of 10 cases of Oral Leukoplakia. Out of 10 cases of Oral Lichen Planus the staining intensity of Cyclin D1 was observed to be weak in 3 (30%) and moderate in 7 (70%) cases.

Cyclin D1 staining Intensity	OPMDs		Total (n %)
	Oral Leukoplakia	Oral Lichen planus	
Mild (1)	4 (40%)	3 (30%)	7 (35%)
Moderate (2)	6 (60%)	7 (70%)	13 (65%)
Strong (3)	-	-	-
Total	10 (50%)	10 (50%)	20

Table 4: Cyclin D1 staining intensity among the cases of OPMDs

DISCUSSION

In the current study, the most common age group of patients diagnosed with OSCC and OPMDs were over 60 years, with mean age in OSCC was 56.76 years. One such study in Pakistan found similar findings, with the average age of the sample population being 53.13 with a range of 25 to 80 years [18]. But contrary to our cross sectional study results (where the age group for OSCC cases was not limited), mean age of 48.35 years was reported with the age range of 20-85 years in a study conducted in India [19]. In our study, the observed mean age was 57.85 years in case of OPMDs, our results are supported by a research study, done in Brazil who reported that average age for OPMDs had been 60 years [20]. In contrast, a study conducted in Saudi Arabia, found the younger age range of (30-40 years) in case of OPMDs [21]. The differences in age groups can be due to small sample size of our study. Males are generally more affected from OSCC and OPMDs as compared to females. But in the present study, female showed pre-dominancy than males. In this study, M & F in OSCC was 1:1.2, comparable to our findings, studies done in Lebanon, Singapore and Sweden also found similar results in Bulgaria, Austria, Denmark, Ireland, and England [22]. In contrast to our findings, males constituted almost 75% of the study to determine the clinic-pathological significance of cyclin D1 in oral cancers [23]. In case of OPMDs, the male to female ratio in our study was 2:3, similar with Iranian research studies which showed female pre-dominancy [24]. A study done in Brazil in which (males constituted 78% while female constituted 59% of the study population) showing contrast to our findings [25]. In the present study, a statistically significant ($p<0.05$) (Table 1) relation was found between the immune-reactivity score of OSCC, OPMDs and Normal healthy oral mucosa. Similar strong relation of the cancer cells for expression of Cyclin D1 using immune-histochemical methods (to determine its expression in tumour specimens) was reported in a research study conducted in Tokyo, Japan [26]. In present study all the 20 cases of OSCC showed strong staining intensity for Cyclin D1 (Table 2). A study done in India almost showed similar results to our findings, demonstrated that most of the WDSCC cases (80%) showed strong Cyclin D1 staining intensity [27]. However, in contrast to our findings, an another study from India demonstrated that 60% cases of OSCC showed mild to moderate intensity for Cyclin D1. In OPMDs, out of all 10 positive cases of leukoplakia, 40% cases showed mild while 60% cases showed moderate Cyclin D1 staining intensity. Contrary to our results, a study done in India found that 52.38% cases of oral leukoplakia showed mild while 33.33% showed moderate staining intensity for Cyclin D1 expression which showed that intensity of Cyclin D1 increases with decrease in severity of the lesion [28]. The discrepancy in staining intensity of Cyclin D1 can be due to large sample size of the Indian study. After extensive literature research no study could be found of Cyclin D1 expression in oral lichen planus. Similar to our study where the normal oral mucosa showed negative Cyclin D1 expression, a study conducted in India considered the expression of Cyclin D1 to be negative in normal cells i.e. <10% [29]. But in contrast to an another study done in India in which 60% cases of normal oral mucosa showed mild

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staining of Cyclin D1 in basal and para-basal layers of squamous epithelium [27]. A larger sample size for research study in order to establish the effectiveness of Cyclin D1 is recommended. The study should also have follow up to assess role of Cyclin D1 in progression of OPMDs to OSCC as expression of Cyclin D1 was seen both in basal and supra-basal layers of OPMDs but was observed in all layers of OSCC. Thus Cyclin D1 expression significantly changed ROM oral epithelial dysplasia to OSCC. As oral cancer is mostly preceded by oral pre cancer so it specifies that increased expression level of Cyclin D1 could be an early event in Oral cancer progression. Thus, Cyclin D1 marker might be valuable in assessing their prognosis and potential for malignancy.

CONCLUSION

Early detection of the transformation of OPMDs towards OSCC is necessary for the improvement of survival rate. Upon assessment of immune-histochemical expression of Cyclin D1 in OSCC, OPMDs, and normal oral mucosal cells, we were able to find positive expression of Cyclin D1 in OPMDs and OSCC but it didn't show any expression in normal oral mucosal cells. The results thus, indicate the probability of OPMD transformation into OSCC.

REFERENCES

[1] Miranda-Filho A, Bray F. Global patterns and trends in cancers of the lip, tongue and mouth. Oral Oncology. 2020 Mar; 102:104551. doi: 10.1016/j.oraloncology.2019.104551.

[2] Dourado MR, de Oliveira CE, Sawazaki-Calone I, Sundquist E, Coletta RD, Salo T. Clinicopathologic significance of ROCK2 expression in oral squamous cell carcinomas. Journal of Oral Pathology and Medicine. 2018 Feb; 47(2):121-127. doi: 10.1111/jop.12651.

[3] Song X, Yang X, Narayanan R, Shankar V, Ethiraj S, Wang X, et al. Oral squamous cell carcinoma diagnosed from saliva metabolic profiling. Proceedings of National Academy of Sciences U S A. 2020 Jul; 117(28):16167-16173. doi: 10.1073/pnas.2001395117.

[4] Adjei Boakye E, Buchanan P, Hinyard L, Osazuwa-Peters N, Schootman M, Piccirillo JF. Incidence and Risk of Second Primary Malignant Neoplasm After a First Head and Neck Squamous Cell Carcinoma. JAMA Otolaryngology; Head and Neck Surgery. 2018 Aug; 144(8):727-737. doi: 10.1001/jamaoto.2018.0993.

[5] Anwar N, Pervez S, Chundriger Q, Awan S, Moatter T, Ali TS. Oral cancer: Clinicopathological features and associated risk factors in a high risk population presenting to a major tertiary care center in Pakistan. PLoS One. 2020 Aug; 15(8):e0236359. doi: 10.1371/journal.pone.0236359.

[6] Mahmood N, Hanif M, Ahmed A, Jamal Q, Saqib, Khan A. Impact of age at diagnosis on clinicopathological outcomes of oral squamous cell carcinoma patients. Pakistan Journal of Medical Sciences. 2018 Jun; 34(3):595-599. doi: 10.12669/pjms.343.14086.

[7] Tandon A, Bordoloi B, Jaiswal R, Srivastava A, Singh RB, Shafique U. Demographic and clinicopathological profile of oral squamous cell carcinoma patients of North India: A retrospective institutional study. SRM Journal of Research in dental sciences. 2018 Jul; 9(3):114. Doi: 10.4103/srmjrds.srmjrds_21_18.

[8] Dhanuthai K, Rojanawatsirivej S, Thosaporn W, Kintarak S, Subarnbhesaj A, Darling M, et al. Oral cancer: A multicenter study. Medicina Oral, Patologia Oral, Cirugia Bucal. 2018 Jan; 23(1):e23-e29. doi: 10.4317/medoral.21999.

[9] Patel A, Gupta V, Shah S. Role of human papillomavirus and its association with an Indian oral squamous cell carcinoma subjects. 2021. doi: 10.21203/rs.3.rs-574115/v1

[10] Anwar N, Pervez S, Chundriger Q, Awan S, Moatter T, Ali TS. Oral cancer: Clinicopathological features and associated risk factors in a high risk population presenting to a major tertiary care center in Pakistan. PLoS One. 2020 Aug; 15(8):e0236359. doi: 10.1371/journal.pone.0236359.

[11] Sridharan G, Ramani P, Patankar S, Vijayaraghavan R. Evaluation of salivary metabolomics in oral leukoplakia and oral squamous cell carcinoma. Journal of Oral Pathology and Medicine. 2019 Apr; 48(4):299-306. doi: 10.1111/jop.12835.

[12] El-Naggar Adel K, Chan John KC, Grandis Jennifer R, Takashi T, Slootweg Pieter J. WHO Classification of Head and Neck Tumours, WHO/IARC Classification of Tumours. 2017.

[13] Lengare PV, Sinai Khandeparkar SG, Joshi AR, Gogate BP, Solanke SG, Gore SH. Immunohistochemical expression of cyclin D1 in invasive breast carcinoma and its correlation with clinicopathological parameters. Indian Journal of Pathology and Microbiology. 2020 Sep; 63(3):376-381. doi: 10.4103/IJPM.IJPM_106_20.

[14] Sun F, Li N, Tong X, Zeng J, He S, Gai T, et al. Ara-c induces cell cycle G1/S arrest by inducing upregulation of the INK4 family gene or directly inhibiting the formation of the cell cycle-dependent complex CDK4/cyclin D1. Cell Cycle. 2019 Sep; 18(18):2293-2306. doi: 10.1080/15384101.2019.1644913.

[15] Sharada P, Swaminathan U, Nagamalini BR, Vinodkumar K, Ashwini BK, Lavanya V. A semi-

DOI: https://doi.org/10.54393/pbmj.v5i7.674

quantitative analysis of immunohistochemical expression of p63, Ki-67, Cyclin-D1, and p16 in common oral potentially malignant disorders and oral squamous cell carcinoma. Journal of Dr. NTR University of Health Sciences. 2018 Apr; 7(2):120. Doi: 10.4103/JDRNTRUHS.JDRNTRUHS_13_18

[16] 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. Archives of Oral Biology. 2019 Mar; 99:177-182. doi: 10.1016/j.archoralbio. 2019.01.018.

[17] Dhingra V, Verma J, Misra V, Srivastav S, Hasan F. Evaluation of Cyclin D1 expression in Head and Neck Squamous Cell Carcinoma. Journal of Clinical and Diagnostic Research. 2017 Feb; 11(2):EC01-EC04. doi: 10.7860/JCDR/2017/21760.9329.

[18] Sahaf R, Naseem N, Anjum R, Nagi AH, Path FR. A study of 89 cases of oral squamous cell carcinoma presenting at Teaching Hospitals of Lahore, Pakistan. JPDA. 2017 Jan; 26(01):27. Doi: 10.25301/JPDA.261.26.

[19] Singh MP, Kumar V, Agarwal A, Kumar R, Bhatt ML, Misra S. Clinico-epidemiological study of oral squamous cell carcinoma: A tertiary care centre study in North India. Journal of Oral Biology and Craniofacial Research. 2016 Apr; 6(1):31-4. doi: 10.1016/j.jobcr.2015.11.002.

[20] Ferreira AM, de Souza Lucena EE, de Oliveira TC, da Silveira É, de Oliveira PT, de Lima KC. Prevalence and factors associated with oral potentially malignant disorders in Brazil's rural workers. Oral Disorders. 2016 Sep; 22(6):536-42. doi: 10.1111/odi.12488.

[21] Alsuwaiket AA, Kazmi F, Mumtaz M. Preventable Risk Factors Associated with Oral Potentially Malignant Disorders: A Study in Saudi Arabia. Asian Journal of Advances in Medical Science. 2020: 31-9.

[22] El Toum S, Cassia A, Bouchi N, Kassab I. Prevalence and Distribution of Oral Mucosal Lesions by Sex and Age Categories: A Retrospective Study of Patients Attending Lebanese School of Dentistry. International Journal of Dentistry. 2018 May 17; 2018: 4030134. doi: 10.1155/2018/4030134.

[23] Al-Rawi N, Al Ani M, Quadri A, Hamdoon Z, Awwad A, Al Kawas S, et al. Prognostic significance of E-Cadherin, β-Catenin and cyclin D1 in oral squamous cell carcinoma: a tissue microarray study. Histology and Histopathology. 2021 Oct; 36(10):1073-1083. doi: 10.14670/HH-18-363.

[24] Irani S, Esfahani AM, Ghorbani A. Dysplastic change rate in cases of oral lichen planus: A retrospective study of 112 cases in an Iranian population. Journal of Oral Maxillofacial Pathology. 2016 Dec; 20(3):395-399. doi: 10.4103/0973-029X.190911.

[25] Mello FW, Melo G, Meurer MI, Rivero ERC. Intraoral Potentially Malignant Disorders in a Brazilian Oral Pathology Service: Epidemiological, Clinical, and Histopathological Findings. Journal of Oncology. 2018 Mar; 2018:2325808. doi: 10.1155/2018/2325808.

[26] Teshima M, Tokita K, Ryo E, Matsumoto F, Kondo M, Ikegami Y, et al. Clinical impact of a cytological screening system using cyclin D1 immunostaining and genomic analysis for the diagnosis of thyroid nodules. BMC Cancer. 2019 Mar; 19(1):245. doi: 10.1186/s12885-019-5452-4.

[27] Menaka TR, Ravikumar SS, Dhivya K, Thilagavathi N, Dinakaran J, Kalaichelvan V. Immunohistochemical expression and evaluation of cyclin D1 and minichromosome maintenance 2 in oral squamous cell carcinoma and verrucous carcinoma. Journal of Oral and Maxillofacial Pathology. 2022 Mar; 26(1):44-51. doi: 10.4103/jomfp.jomfp_446_21.

[28] Patel SB, Manjunatha BS, Shah V, Soni N, Sutariya R. Immunohistochemical evaluation of p63 and cyclin D1 in oral squamous cell carcinoma and leukoplakia. Journal of Korean Association of Oral Maxillofacial Surgeons. 2017 Oct; 43(5):324-330. doi: 10.5125/jkaoms.2017.43.5.324.

[29] Moharil RB, Khandekar S, Dive A, Bodhade A. Cyclin D1 in oral premalignant lesions and oral squamous cell carcinoma: An immunohistochemical study. Journal of Oral Maxillofacial Pathology. 2020 Aug; 24(2):397. doi: 10.4103/jomfp.JOMFP_164_20.