CYCLIN D1 PROTEIN EXPRESSION IN VARIOUS GRADES OF SALIVARY MUCOEPIDERMOID CARCINOMA

Saadia Muneer Malik, Zainab Niazi*, Mehreen Ali Khan, Shoaib Naiyyar Hashmi*****

Army Medical College/National University of Medical Sciences (NUMS) Rawalpindi Pakistan, *Islamabad Medical and Dental College, Islamabad Pakistan, **Armed Forces Bone Marrow Transplant Centre/National Institute of Blood And Marrow Transplant AFBMTC/ NIBMT/National University of Medical Sciences (NUMS) Rawalpindi Pakistan, ***Combined Military Hospital Kharian/National University of Medical Sciences (NUMS) Pakistan

ABSTRACT

Objective: To assess the expression of Cyclin D1 in Mucoepidermoid Carcinoma (MEC) and its association with various histological grades.
Study Design: Case series.
Place and Duration of Study: AFIP Rawalpindi, from Nov 2017 to Apr 2018.
Material and Methods: A total of 30 cases of MEC were included in this study. Paraffin embedded blocks of patients of both genders, diagnosed with salivary gland mucoepidermoid carcinoma were included as experimental samples while necrotic, scarce and autolysed cases were not included. Tumor was graded as per the grading criteria of Auclair *et al*. Cyclin D1 was applied and the results were analyzed using chi-square test.
Results: Of the 30 selected cases, 21 (70%) were male and 9 (30%) were female patients. According to histological grades, 14 (46.67%) comprised of low grade, 06 (20%) intermediate grade and 10 (33.34%) high grade tumors. Among these cases 6 (20%) were positive and 24 (80%) had altered results. In low grade MEC all 14 (0%) cases had negative results and none was positive. Among the 6 intermediate grade cases 3 (50%) showed positive and 3 (50%) showed altered expression and in high grade tumors 7 (70%) out of 10 had altered results and 3 (30%) showed positive results. A significant association (p-value=0.02) was seen between expression of Cyclin D1 and grades of MEC.
Conclusion: A significant association was seen between Cyclin D1 expression and grades of MEC (p=0.02). Cyclin d1 showed an increase in expression with increase in grade of tumor. Hence it can serve as a potential marker for grading of mucoepidermoid carcinoma.

Keywords: Cyclin D1, Mucoepidermoid carcinoma (MEC), Salivary Glands.

INTRODUCTION

Although only 5% of all the tumors belong to head and neck region but salivary gland tumors constitute asignificant place among maxillofacial pathological lesions[1]. Mucoepidermoid carcinoma is the most commonly encountered lesion among all salivary gland tumors[2-4]. In 1945, Mucopeidermoid Carcinoma was described as a separate tumor for the first time by Stewart[1].

MEC shows a slight predominance for female gender, with a female-male ratio of around 3:2[3,5]. A greater predilection for MEC has been observed for grown ups in 4th - 6th decade, with the maximum prevalence seen during the fifties[2-4]. Parotid gland is most frequently affected major salivary gland with an incidence ranging from 45%-56.9%[4,5] followed by minor salivary glands in the palate with an incidence of 22.9-37.1%[6-8].

In Pakistani population, MEC has a prevalence of 9.5-25.6% compared to 12-40% the world over[4,9]. It is believed that the carcinoma arises from the salivary gland ductal cells.Since these ductal cells have the ability to differentiate into 3 different cell types, therefore, MEC is histologically composed of 3 different cell types: mucous, intermediate, and epidermoid cells. Growth patterns vary from cystic to solid to infiltrative. These parameters have been used into several different grading systems to classify these lesions[1,3].

Correspondence: Dr Mehreen Ali Khan, Consultant Hematologist, AFBMTC Rawalpindi Pakistan (*Email: mehreen35@hotmail.com)*
Received: 01 Apr 2019; revised received: 13 Aug 2019; accepted: 20 Aug 2019

For salivary gland tumors, the histological grade of the lesion is a significant marker of the treatment outcome. For low grade mucoepidermoid tumors, the 5-year survival rateis 92-100%, for intermediate grade 62–92%, and for high grade tumors 0-43%[10]. This diagnosis and prediction of outcome are facilitated by immunohistochemistry. This method is also important in determination of prognosis of neoplasms[11].

The orderly progression of the cells through the different phases of cell cycle, namely, G1, S, G2, and M phases is specifically controlled by a chain of proteins called "cyclins". These proteins act when get bind to the cyclin-dependent kinases (CDK)[12]. Cyclin D1 serves an important role in cell cycle from G1 to S phase. This protein is over expressed in other tumors of the body such as breast, prostate and colon cancers[13]. Cyclin D1 over expression is anindication of poor prognosis in different carcinoma grades[14].

The purpose of this study was to determine the expression Cyclin D1 as immune-histochemical prognostic factor to forecast the biological be-havior of mucoepidermoid carcinoma in the local population, where by expression of Cyclin D1 is linked with more aggressive behavior of lesions and a poor prognosis.

The rationale for this study was that if the aggressiveness of MEC via Cyclin D1 expression was established, then the information might be utilized in institution and customized therapy. It might help the surgeons in treating the high grade tumors more radically, decreasing the chance to recur or to metastasize, thereby, improving the survival of the affected patients. This was a case-series in which Cyclin D1 immunohistochemical marker was applied on diagnosed MEC cases and the pattern of its expression on the tumor is studied, predicting thetumor behavior.

MATERIAL AND METHODS

This descriptive case series was conducted at Armed Forces Institute of Pathology (AFIP), Rawalpindi. Permission was taken from the ethical review board (letter no. MP-ORP 13-7/READ-IRB/746) Keeping confidence level (1-α) at 95%, anticipated population proportion (P) at 0.80,15 and absolute precision (d) at 0.145, a sample size of 30 was calculated. Non-probability consecutive sampling technique was utilized. Paraffin embedded blocks of adult patients of both genders, diagnosed with mucoepidermoid carcinoma of salivary glands were included as study samples. Exclusion criteria was necrotic, scarce and autolysed and poorly oriented tissue samples[30]. Paraffin embedded blocks of MEC were retrieved from record files. Data was collected from the histories produced along with cases. All the paraffin blocks were recut and stained with hematoxylineosin stain. After confirmation of the diagnosis on microscopy, histopathological grades was allotted to the sample lesionsas per the criteria described by Auclair *et al* and Goode[16,17] (1992) which divides MEC into three grades based on following histological points (table-I)[18].

After histological grading, immune-marker Cyclin D1 was applied on these samples as per the standard protocol. Findings were analyzed. Immune reactivity was evaluated and its association with histopathological grading was carried out.

After confirmation of the diagnosis of Mucoepidermoid carcinoma. on microscopy, histopathological grades were assessedas per Auclair *et al* and Goode *et al*'s criteria[16,17] and tumor was classified as low, intermediate or high grade.

Immunohistochemical labeling of cyclin D1 was done using Haas *et al*'s criteria, with some alterations[19]. Tumor cells with positive nuclear reaction were distributed as under:

0-5% positive cells = negative expression (-)

5-20% positive cells = reduced expression (+)

>20% positive cells = positive expression (++)

For statistical analysis purpose, cases having negative and reduced expression were considered as altered and with positive expression were considered as over-expression.

Positive expression (++) = over-expression

Negative (-) or reduced (+) = altered expression

Data was analyzed using SPSS version 20. Descriptive statistics were calculated. Quantitative variables were depicted as mean and standard deviation. Categorical variables such as Cyclin-D1 expression were presented as frequency and percentages. Chi-squared test was used to assess the association of Cyclin-D1 with tumor grade. The $p \leq 0.05$ was taken as significant.

Table-I: Criteria for grading of mucoepidermoid carcinoma.

Parameters	Point value
"Intracystic component <20%"	2
"Presence of Neural invasion"	2
"Presence of Necrosis"	3
"Four or more mitosis present per 10 high power fields"	3
"Presence of Anaplasia"	4
Grades	
Low grade having 0-4 points	
Intermediate grade having 5-6 points	
High grade if points are 7-14	

Table-II: Frequency of Cyclin D1 expression in different grades of MEC.

Grade	>20% (++)	5-20% (+)	0-5% (-)
Low	-	3 (21.42%)	11 (78.58%)
Intermediate	3 (50%)	3 (50%)	-
High	3 (30%)	2 (20%)	5 (50%)

Table-III : Association of Cyclin D1 expression with tumor grades.

Grade	Cyclin D1		*p*-value
	Positive	Altered	
Low	-	14 (100%)	0.02
Intermediate	3 (50%)	3 (50%)	
High	3 (30%)	7 (70%)	

RESULTS

Out of 30 cases, 21 (70%) were male and 9 (30%) were female patients. Patients ranged in age from 17-88 years with a mean age of 47.6 ± 18.6 years. Majority of the cases were from parotid region 18 (60%) and 14 (46.67%) were low grade which make the highest number.

Cyclin D1 was applied on 30 cases of MEC. Among those 6 cases were positive (++) making 20% of the total, 8 were of reduced expression (+) showing 26.67% and 16 were negative (-) getting the highest percentage of 53.34%. The results showed that of 14 cases of low grade MEC, 11 were negative, three of reduced expression and none were positive.

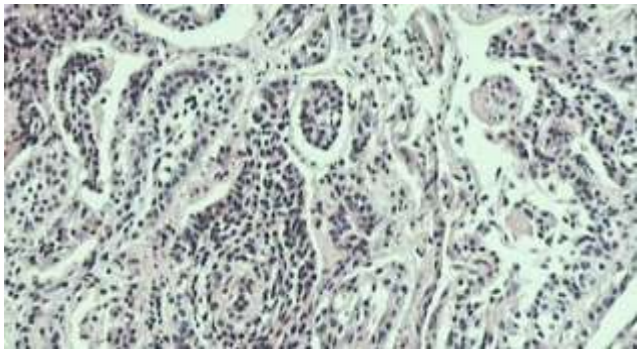

Figure-1: MEC- High Grade showing atypical squamoid cells (H and E stain, 10x 100 magnifications).

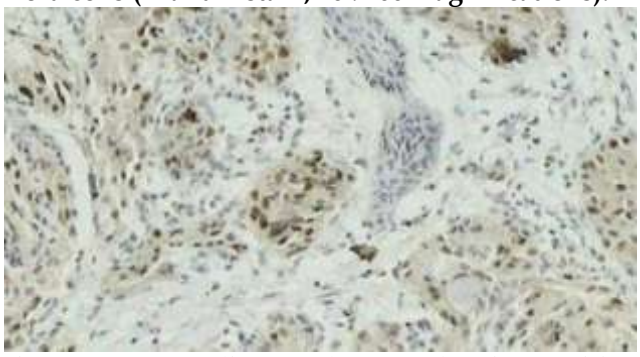

Figure-2: Intermediate grade MEC showing positive (++) nuclear staining in tumor cells (Cyclin D1 immunohistochemical stain, 10 x 100 magnifications).

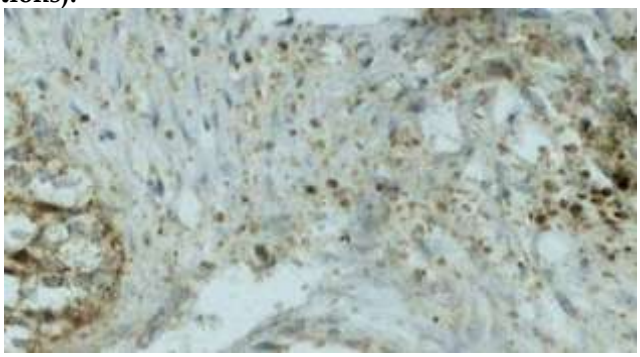

Figure-3: High grade MEC showing positive (++) Cyclin D1 nuclear staining in tumor cells (Cyclin D1 immunohistochemical stain, 40 x 100 magnifications).

Among the six cases of intermediate grade tumor, 50% showed positive results and 50%

showed reduced expression. While among the 10 cases of high grade MEC, 30% were positive, 20% showed reduced expression and 50% were negative. These results were correlated with different grades of tumor (table-II).

According to the analysis criteria proposed for Cyclin D1, of the 30 study samples, 6 were positive and 24 were altered. Among low-grade tumors, all 14 cases showed altered expression and none was positive. Among the 6 cases of intermediate grade, 3 showed positive expression while 3 had altered expression. In high-grade tumors, 7 out of 10 had altered expression and only 3 showed positive expression as shown in table-III and figure-1 to 3.

DISCUSSION

Although uncommon, salivary gland tumors hold asignificant place among all oral and maxillofacial pathological lesions. They constitute approximately 5% of the head and neck malignant lesions, with MEC being the most prevalent[20]. MEC comprised of a mixture of mucous epidermoid and intermediate cells. It is also known to show a variety of clinical behaviours but the prognosis depends on tumor grade[18,20].

In the present study, MEC were slightly more common in younger age group. Of all the MEC cases, 60% patients were aged less than 50 years while 40% were above 50 years. In under 50 years age group, 16.67% tumors were high grade whereas this figure was 58.33% in above fifty years age group. This showed definite disadvantage with increasing age as far as the tumor grade was concerned. Likewise, Ozawa *et al*[5]. (2008) in his study proposed that patients aged 56 years and above had decreased survival. In their study 42% of patients over 56 years showed high grade mucoepidermoid carcinomasin comparison to 32% of patients below 55 years who showed high grade lesions.

In the current study, 73.34% of MEC were from major salivary glands, with majority occurring in parotid and 26.66% of MEC involved minor salivary glands with most of the lesions occurring in palate. Similarly, Kolude *et al*[21]. (2001) in a study of 34 MEC cases reported 75% of cases from major salivary glands and 25% from minor salivary glands, with parotid and palate being the most common sites respectively.

Cyclins program the cyclin-dependent kinase activity. Synthesis and degradation of cyclins regulates the sequence of cell cycle. Among cyclins, D-type are known to play important role from G1- S phase. Cyclin D1 forms complex with cdk thus initiate cell proliferation through G1 phase. Disorder in this process can lead to the pathogenesis of various tumors. According to a few studies over-expression of this protein is found in the early stages of development of tumor so is considered as an "early marker of cell proliferation" while some suggest its late over-expression[13].

Studies determining the expression of Cyclin D1 in various MEC grades are scarce. However, some studies have been done to evaluate the expression of Cyclin D1 in salivary gland tumors which show significant results. For instance, Perez-Ordonez *et al*[22]. in their study on polymorphous low-grade adenocarcinomas observed weak expression of Cyclin D1. In another study by Shintani *et al*[23] on the expression of this protein in 22 cases of adenoid cysticcarcinoma, they found over-expression in 4 cases. 3 of them found to be of solid pattern. The over-expression suggested high proliferative activity of this tumor.

In this study Cyclin D1 was applied to 30 cases of MEC. Out of these 6 showed a positive while 24 depicted altered expression. Among the 14 cases of low grade carcinomas none (0%) showed positive results. Three (50%) cases of intermediate grade carcinoma were positive and three showed altered expression. On the other hand, out of the 10 high grade tumors 3 (30%) were positive and 7 showed altered results. As per these results Cyclin D1 has no expression in low grade tumors and it showed over-expression in some cases of intermediate and high-grade tumors. Cyclin D1 positivity indicates high proliferation of tumor cells with increasing

grades. Difference of results as compared to the available literature worldwide may be ascribed to the sample size and difference of antibody.

Expression of cyclin D1 is observed on various body tumors. In a study done by Hass *et al*[16] they proved that over-expression of this protein was frequent in carcinomas of tongue and rare in carcinomas of tonsils. This imparts that over-expression of this protein depends on distinct tumor sites. Thus, the authors concluded that various molecular changes occur at distinct locations in head and neck tumors.

In contrast to the present study, Miguel *et al*[13] in a study on 40 MEC cases showed that 3 samples showed over-expression of this marker, 2 were low grade and 1 intermediate grade but there was no labeling evident in any high grade. They concluded from this study that Cyclin D1 does not participate in the etio-pathogenesis of MEC and that other genes may be involved.

Etges *et al*[24] found positive Cyclin D1 expression in 3 cases of MEC, although histological grades of these cases were not mentioned. Moreover, 6 cases of normal tissue were used for comparison but no immune- labeling was observed in these cases. Difference of results of immune-histochemical markers as compared to the available literature worldwide might be ascribed to the sample size.

LIMITATION OF STUDY

In order to decrease the study bias and to generalize the results to entire population, a larger sample size should be studied. However, owing to a relatively lower prevalence of salivary gland tumors in the local population, a large sample size could not be selected.

CONCLUSION

High grade MEC showed positive expression of Cyclin D1, with an increase in expression with increasing tumor grade. A statistically significant association was seen between Cyclin D1 expression and MEC grades (p<0.02). Cyclin D1 can serve as a potential marker for grading of MEC.

CONFLICT OF INTEREST

This study has no conflict of interest to be declared by any author.

REFERENCES

1. Swathi G, Ramesh T, Kishore M, Kumar GR. A case report of unusual presentation of mucoepidermoid carcinoma with review of literature. Int J Oral Health Sci 2018; 8(2): 120-3.
2. Siddique S, Din H, Hashmi SN, Rehman A, Khadim T, Akhtar F. Clinico-Pathological Profile of Mucoepidermoid Carcinoma. J Rawal Med Coll 2015; 19(1): 64-6.
3. Chen MM, Roman SA, Sosa JA, Judson BL. Histologic grade as prognostic indicator for mucoepidermoid carcinoma: A populationlevel analysis of 2400 patients. Head Neck 2014; 36(2): 158-163.
4. Sharma S, Bargotra R, Kaul KK. Morphological patterns of salivary gland tumors. JK Science 2017; 19(1): 48-52.
5. Zaman S, Majid S, Chugtai O, Hussain M, Nasir M. Salivary gland tumours: A review of 91 cases. J Ayub Med Coll Abbottabad 2014; 26(3): 361-3.
6. Granic M, Suton P, Mueller D, Cvrljevic I, Luksic I. Prognostic factors in head and neck mucoepidermoid carcinoma: Experience at a single institution based on 64 consecutive patients over a 28-year period. Int J Oral Maxfac Surg 2018; 47(3): 283-8.
7. deAraujo VC, Passador-Santos F, Turssi C, Soares AB, de Araujo NS. Polymorphous low-grade adenocarcinoma: An analysis of epidemiological studies and hints for pathologists. Diagn Pathol 2014; 8(1): 6-9.
8. Siddique S. Role of Ki-67 in the grading of mucoepidermoid carcinomas. J Rawalpindi Med Coll 2015; 19(2): 120-3.
9. Coca-Pelaz A, Rodrigo JP, Triantafyllou A, Hunt JL, Rinaldo A, Strojan P, et al. Salivary mucoepidermoid carcinoma revisited. Eur Arch Oto Rhino Laryngol 2015; 272(4): 799-819.
10. Simpson RH, Skálová A, Di Palma S, Leivo I. Recent advances in the diagnostic pathology of salivary carcinomas. Virchows Archiv 2014; 465(4): 371-84.
11. Namboodiripad PA. A review: Immunological markers for malignant salivary gland tumors. J Oral Biol Craniofac Res 2014; 4(2): 127-34.
12. Feitelson MA, Arzumanyan A, Kulathinal RJ, Blain SW, Holcombe RF, Mahajna J, Marino M, et al. Sustained proliferation in cancer: Mechanisms and novel therapeutic targets. Semin Cancer Biol 2015; 35(1): S25-S54.
13. Miguel MC, Oliveira MC, Seabra FRG, Queiroz LMG, Freitas RA, Batista de Souza L. Catenin and Cyclin D1 in Mucoepidermoid Carcinoma of Variable Histologic Grades. Arch OtolaryngolHead Neck Surg 2005; 131(8): 701-06.
14. Shiratsuchi H, Nakashima T, Hirakawa N, Toh S, Nakagawa T, Saito T et al. B-Catenin nuclear accumulation in head and neckmucoepidermoid carcinoma: Its role in Cyclin d1 overexpression and tumor progression. Head Neck 2007; 29(6): 577-84.
15. Shieh YS, Chang LC, Chiu KC, Wu CW, Lee HS. Cadherin and catenin expression in mucoepidermoid carcinoma: Correlation with histopathologic grade, clinical stage and patient outcome. J Oral Pathol Med 2003; 32(5): 297-304.
16. Auclair PL, Goode RK, Ellis GL. Mucoepidermoid carcinoma of intraoral salivary glands: evaluation and application of grading criteria in 143 cases. Cancer 1992; 69(8): 2021-30.
17. Goode RK, Auclair PL, Ellis GL. Mucoepidermoid carcinoma of the major salivary glands: clinical and histopathologic analysis

of 234 cases with evaluation of grading criteria. Cancer 1998; 82(7): 1217-24.
18. Tekade SA, Chaudhary MS, Gawande MN, Bagri K. Correlation between mucoepidermoid carcinoma grade and AgNOR count. J Oral Sci 2010; 52(2): 275-79.
19. Haas S, Hormann K, Bosch FX. Expresion of cell cycle proteins in head and neck cancer correlates with tumor site rather than tobacco use. Oral Oncol 2002; 38(6): 618-23.
20. Rapidis AD, Givalos N, Gakiopoulou H, Stavrianos SD, Faratzis G, Lagogiannis GA. et al. Mucoepidermoid carcinoma of the salivary glands. Review of the literature and clinicopathological analysis of 18 patients. Oral Oncol 2007; 43(2): 130-6.
21. Kolude B, Lawoyin JO, Akang EEU. Mucoepidermoid carcinoma of oral cavity. J Natl Med Assoc 2001; 93(5): 178-84.
22. Perez-Ordonez B, Linkov I, Hooves AV. Polymorphous low grade adenocarcinoma of minor salivary gland: A study of 17 cases with emphasis on cell differentiation. Histopathol 1998; 32(6): 521-29.
23. Shintani S, Ohyame H. p12 (DOC-1) is a novel cyclin- dependent kinase. 2-associated protein. Mol Cell Biol 2000; 20(17): 6300-307.
24. Etges A, Nunes FD, Ribeiro KC, Araujo VC. Immunohisto-chemical expression of retinoblastoma pathway proteins in normal salivary glands and in salivary gland tumors. Oral Oncol 2004; 40(3): 326-31.