Immunohistochemical Expression of EZH2 in Mucoepidermoid Carcinoma of Salivary Glands

Amira A. Mansour[1], Mona M. Ibrahim [2], Ahmed E. Ahmed[3], Azza A. El Sissi[4]

Abstract:

Objective: This study aimed to disclose the variation in EZH2 expression among the different grades of MEC and to analyze the relation of the marker's Immunohistochemical (IHC) expression to the clinicopathologic data of all cases. **Materials and Methods:** The present study was conducted on 15 blocks diagnosed as MEC including equal numbers of different grades. These blocks were selected from the archives of the Oncology Center, Mansoura University. The specimens embedded in the retrieved paraffin blocks were all fixed in 10% formalin. Sections of 4 microns' thickness intervals were serially cut and prepared for the EZH2 staining. Chi-square, Monte Carlo, and Fisher Exact tests were used for statistical analysis. **Results:** The parotid gland was the most affected site (66.7%). The age of patients ranged from 20 to 80 years with a mean of 58.3 years and slight female predominance. Stage III was the most frequent stage (40%) followed by stage IV (26.7%). Most MEC cases (73.3%) showed positivity to the EZH2 reaction. There was a statistically significant difference between histopathological grades regarding patients' ages ($p< 0.05$). There was a statistically significant difference between age groups regarding expression scores ($p< 0.05$). No statistically significant difference was observed among the different histopathological grades of MEC regarding EZH2 expression .**Conclusions:** EZH2 was expressed in most of the studied MEC cases, particularly the high-grade cases. Overexpression of EZH2 in MEC cases with nodal metastasis and histological invasions could predict a poor prognosis of the tumor.

Introduction:

Mucoepidermoid carcinoma (MEC) is the most common malignant epithelial salivary gland tumor (SGT) according to the WHO classification (2017) accounting for 35% of all salivary gland cancers[1] and 10% of all salivary gland tumors.[2,3] It usually appears in the fifth decade of life with an average age of 47 years and slight female predominance. It usually develops as a solitary painless mass. High-grade tumors are characterized by the presence of pain, rapid growth, ulceration, fixation to the deeper structures, and facial nerve paralysis.[4,5] On gross examination, the neoplasm is not well encapsulated. It usually has ill-defined borders with an invasion of the surrounding structures.[4] Low-grade tumors are well circumscribed enclosing greyish white mucin-filled cysts. It is believed that MEC originates from the excretory duct reserve cells.[3,6] Microscopically, a mixed pattern of cells has been manifested to constitute the tumor; mucous secreting cells, squamous (epidermoid) cells, and intermediate cells.[7]

Mucous secreting cells are large cells that secrete mucin into the lumen of cystic spaces. Epidermoid cells are polygonal in shape and contain eosinophilic cytoplasm, vesicular nuclei, and prominent nucleoli. Intermediate cells are smaller in size than both types of cells.[4] The most commonly used grading systems are: the modified Healey grading,[8] the Armed Forces Institute of Pathology (AFIP) grading,[9] the Brandwein grading[10] and Katabi grading system.[11]Enhancer of zeste homolog 2 (EZH2) is a widely studied histone-lysine N-methyl transferase enzyme encoded by EZH2 gene that participates in histone tri-methylation.[12] It has a principal role in tumorigenesis, malignancy, and poor prognosis.[13] It is overexpressed in various human carcinomas and associated with adverse clinicopathologic characteristics and biological behavior.[12,14] In SGTs, EZH2 has been recently reported to be sensitive in discriminating between benign and malignant entities regardless of the tumor type.[15] Additionally, EZH2 high expression has been reported to predict poor survival in patients with adenoid cystic carcinoma of salivary glands. [16]

Material and methods:

The sample size calculation was based on 1.02 % benign lympho-epithelioma reported in (Ghartimagar et al., 2020) and Using Daniel's equation (1999),

$$n = \frac{Z^2 p(1-p)}{d^2}$$

Where: With a 95% Confidence interval and acceptable margin error of 5%; the calculated sample size in the study was at least 15 cases. Z= 1.96 at 95% confidence

[1]*Post Graduate Master Student, Department of Oral Pathology, Faculty of Dentistry, Mansoura University* 35516, *Mansoura, Egypt. E-mail: amiraabdelhamid92@gmail.com*
[2] *Lecturer, Department of Oral Pathology, Faculty of Dentistry, Mansoura University 35516, Mansoura, Egypt.*
[3] *Lecturer, Department of Oral Pathology, Faculty of medicine, Mansoura University 35516, Mansoura, Egypt.*
[4]*Professor of oral pathology, Faculty of dentistry, Mansoura University 35516, Mansoura, Egypt.*

DOI: 10.21608/mjd.2022.151293.1064

level P= expected prevalence (0.0102) d= precision (margin of error) = 0.05.

The present study was conducted on a total of 15 blocks diagnosed as MEC following the criteria of the WHO classification (2017). These blocks were selected from the archives of the Oncology Center, Mansoura University. The MECs were intentionally selected to equally include the three grades; low (n= 5), intermediate (n= 5) and high grade (n= 5).[17] Following the instructions of the manufacturer, blocks of colon cancer also were retrieved to be employed as the positive control group. The concentrated monoclonal EZH2/KMT6 (clone 6G4F4) obtained from Medaysis (USA) was employed at a dilution of 1:50 in EDTA for the IHC staining. The specimens embedded in the retrieved paraffin blocks were all fixed in 10% formalin. Two Sections of 4 microns' thickness intervals from each of the retrieved blocks were serially cut and prepared for staining with H&E and EZH2 antibodies. One paraffin section from each block was stained with routine H&E staining. IHC staining for EZH2 antibody was performed on all sections according to the manufacturer's instructions. One paraffin section from each block was prepared and mounted on a positively charged slide for immunohistochemistry. Immunostaining was conducted in an automated immunostainer using a standard avidin-biotin-peroxidase technique.[18,19] IHC was assessed by light microscopy among different cells of the investigated sections. The mean percentage of positive cells in each case was recorded and accordingly scored semi-quantitatively as following: Negative: (score = 0 where positive cells < 5%), Mild: (score = 1 where positive cells 5-10%), Moderate: (score = 2 where positive cells 11-50%), Strong: (score = 3 where positive cells > 50%).The obtained data were tabulated, coded then fed to the computer and analyzed using IBM SPSS Corp. Released 2013. The significance of the obtained results was judged at the (0.05) level. Qualitative data were analyzed using Chi-square, Monte Carlo, and Fisher Exact tests

Results:

Clinical findings: The age in MECs ranged from 20 to 80 years with an average of 58.3 years being most frequent among the age period of 30 to 60 years. There was a slight female predominance with a male-to-female ratio of 1: 1.1. The parotid was the most frequently involved (66.7%) site for the occurrence of MEC. T2 was the most frequent presentation (46.66%) of the tumor size in MEC group followed by T3 (26.7%). Forty percent of the studied MEC showed nodal involvement was equally distributed among N1 (20%) and N2 (20%). Most of the presently studied MEC groups were of stage III (40%) followed by stage IV (26.7%).

Histopathological findings: The 15 studied MEC were equally distributed according to the criteria set by Brandwein et al (2002)[10] into low, intermediate, and high grades**.** Low-grade MEC showed multiple cystic spaces and occasional solid follicles surrounded by fibrous stroma. Mucous secreting cells appeared as large cells containing pale, foamy abundant eosinophilic cytoplasm with peripherally located compressed nuclei. The epidermoid cells appeared as large polygonal cells with eosinophilic cytoplasm and ovoid closed face nuclei. Intermediate cells varied in appearance from small basaloid cells to slightly large ovoid cells with a small centrally located nucleus. Intermediate-grade MEC showed less prominent cystic spaces than the low-grade MEC. Tumor cells exhibited a moderate degree of atypia and pleomorphism. High-grade MEC showed solid nests with epidermoid cells predominating throughout the tumor. Mostly, cases of this group revealed a high degree of atypia, frequent mitotic figures, pleomorphism, and necrosis (Figure, A).

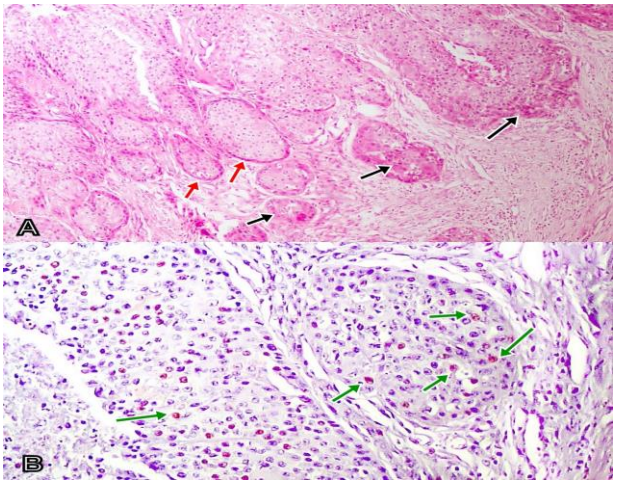

Figure: A- Photomicrograph showing tumor cells in high-grade MEC growing in solid nests with occasional cystic spaces. The tumor cell population is composed mainly of epidermoid cells (black arrows) and intermediate cells (red arrows) (H&E, 100x). B- Photomicrograph of High-grade MEC showing the scattered nuclear reaction of EZH2 (green arrows) (DAB, 200x).

Immunohistochemical findings: In the current study, the reaction was encountered in 11 out of 15 cases representing 73.3% of all MEC groups. While most of the positive MEC showed focal nuclear expression, only one case showed diffuse immunoreactivity to EZH2. Immuno-reactivity of EZH2 was more extensive in epidermoid cells. Among the positive MEC group, two were low-grade MEC, four were intermediate grade and all high-grade cases were positive (Figure, B).

Statistical results: -The age group between 30 to 60 years showed a significantly higher score of expression of EZH2 than the other age groups (p= 0.039*), Table 1. No significant difference was found in EZH2 expression (positivity, score of expression) among different histopathological grades of the studied MEC cases, Table 2.

Table 1: Association between immunohistochemical expression Score and clinical data among MEC group

Mucoepidermoid carcinoma (MEC)	Score of expression			P
Clinical data	Negative n (%)	Mild n (%)	Moderate & Strong n (%)	
Age/years				
<30	2 (50)	0	1 (11.1)	P= 0.039*
30-60	1 (25)	0	7 (77.8)	
>60	1 (25)	2 (100)	1 (11.1)	
Sex				
Male	1 (25)	2 (100)	4 (44.4)	P= 0.217
Female	3 (75)	0	5 (55.6)	
Site				
Parotid	3 (75)	1 (50)	6 (66.7)	P= 0.866
Submandibular	1 (25)	1 (50)	2 (22.2)	
palate	0	0	1 (11.1)	
Tumor Size (T)				
T1	0	1 (50)	2 (22.2)	P= 0.662
T2	3 (75)	1 (50)	3 (33.3)	
T3	1 (25)	0	3 (33.3)	
T4	0	0	1 (11.1)	
Regional lymph node (N)				
N0	2 (50)	1 (50)	6 (66.7)	P= 0.746
N1	1 (25)	0	2 (22.2)	
N2	1 (25)	1 (50)	1 (11.1)	
Clinical stage				
Stage I	0	1 (50)	1 (11.1)	P= 0.612
Stage II	1 (25)	0	2 (22.2)	
Stage III	2 (50)	0	4 (44.4)	
Stage IV	1 (25)	1 (50)	2 (22.2)	

Monte Carlo test, p: probability, statistically significant if p <0.05

Table 2: Association between immunohistochemical expression (positivity and score) and histopathological grade among MEC group

H/P grade of MEC	Positivity of expression		P	Score			P
	negative	positive		Negative n (%)	Mild n (%)	Moderate&Strong n (%)	
Low	2 (50)	2 (11.2)	P= 0.216	2 (36.7)	1 (50)	1 (11.2)	P= 0.278
Intermediate	2 (50)	4 (44.4)		1 (33.3)	0	4 (44.4)	
high	0	5 (45.5)		0	1 (50)	4 (44.4)	

Monte Carlo test, p: probability, statistically significant if p <0.05

Discussion:

Mucoepidermoid carcinoma is the most common epithelial salivary gland malignancy accounting for 35% of all salivary gland cancers. Among MEC of the current work, gender showed slight female predominance similar to Ozawa et al.[20], Qureshi et al.[21], and Esmail et al.[22] It was probably attributed to the effect of female sex hormones in the pathogenesis Of salivary gland tumors.[23] The parotid gland was the most commonly affected site by MEC (66.7%). This agrees with Ozawa et al.[20], Qureshi et al.[21], and El-Sherbiny et al.[24] and contradicts the few studies conducted by Cipriani et al.[25] and Tian et al.[26] reporting a higher incidence of MEC in minor salivary glands. Regarding the tumor size, most (46.7%) of the current MEC cases were categorized as T2 (> 2, ≤ 4 cm); the

same tumor size was reported by Esmail et al.[22] and others.[20,27] However, a higher score of the tumor size (T4) was reported in other series as the dominant presentation of the reported MEC.[28] Nodal metastasis was reported in nearly 40% of the current MEC group equally distributed among N1 and N2 grades and this was in partial agreement with Esmail et al.[22] Stage III was the most frequent stage (40%) among the current MEC group followed by stage IV (26.7%). This was similar to the study made by El-Sherbiny et al.[24] and Esmail et al.[22] This differed from other studies showing that most MEC cases were of stage IV.[29]Statistically, consistence with Qureshi et al.[21], no significant difference was found between histopathological grades and clinical data of the studied MEC cases except for age. This might reflect the absence of any clinical impact on the histopathological grading of MEC except age which may affect the histopathological grade of the tumor.[30] Similar findings were reported by others who showed no significant correlation with clinical data except for nodal involvement.[20,22] Following Hajósi-Kalcakosz et al.[15], EZH2 was expressed as a focal nuclear reaction in most of the studied MEC cases (73.3%) with more extension in the epidermoid cells. This was similar to most studies that reported nuclear reaction of EZH2 in several cancers [16,31,32] except Anwar et al. [33] which demonstrated cytoplasmic localization of EZH2 in invasive breast carcinoma.It was observed that most of the positive cases (45.5%) were of the high-grade category and that might suggest its role in detecting the biological behavior of the tumor. However, no statistically significant difference was observed among histopathological grades regarding EZH2 expression and this was in agreement with Hajósi-Kalcakosz et al.[15] Also, no statistically significant correlation was detected in the score of expression among clinical data of the present cases except for the age. This could be interpreted as the progression and aggressiveness of cancer increases with increasing age.[34]

Conclusions:

EZH2 was expressed in most of the studied MEC cases, particularly the high-grade cases. Overexpression of EZH2 in MEC cases with nodal metastasis and histological invasions could indicate their aggressive behavior and predict the poor prognosis of the tumor.

References:

1- Rousseau A, Badoual C. Head, neck: Salivary gland tumors: an overview. Atlas Genet Cytogenet Oncol Haematol.2011;15(6):533-541.
2- Devaraju R, Gantala R, Aitha H, Gotoor SG. Case Report: Mucoepidermoid carcinoma. BMJ Case Rep.2014;1-5.
3- Neville BW, Allen CM, Damm DD, Chi AC. Oral and Maxillofacial Pathology. 4th edition. St. Louis: Elsevier;2015.
4- Shafer, Hine, Levy. Shafer's text book of oral pathology. 8th edition. India: Elsevier; 2016.
5- Thompson LDR, Bishop JA. Head and neck pathology.3rd edition. A volume in the series: Foundations in diagnostic pathology. Elsevier; 2018.
6- Luna MA. Salivary mucoepidermoid carcinoma: Revisited. Adv Anat Pathol.2006; 13(6):293-307.
7- Odell EW. Cawson's Essentials of Oral Pathology and Oral Medicine. 9th edition. London: Elsevier;2017.
8- Batsakis JG, Luna MA. Histopathologic grading of salivary gland neoplasms: I. Mucoepidermoid carcinomas. Ann Otol Rhinol Laryngol.1990; 99 (10 Pt 1):835-838.
9- 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; 82:1217-1224.
10- Brandwein MS, Ferlito A, Bradley BJ, Hille J, Rinaldo A. Diagnosis and classification of salivary neoplasms: pathologic challenges and relevance to clinical outcomes. Acta Otolaryngol.2002;122(7):758-764.
11- Katabi N, Ghossein R, Ali S, Dogan S, Klimstra D, Ganly I. Prognostic features in mucoepidermoid carcinoma of major salivary glands with emphasis on tumor histologic grading. Histopathology.2014;*65*(6):793–804.
12- Viré E, Brenner C, Deplus R, Blanchon L, Fraga M, Didelot C, et al. "The Polycomb group protein EZH2 directly controls DNA methylation". Nature .2006;439 (7078):871–874.
13- Gan L, Yang Y, Li Q, Fing Y, LIU T, Guo W. Epigenetic regulation of cancer progression by EZH2: from biological insights to therapeutic potential. Biomark Res.2018; 6:10.
14- Xu B, Abourbih S, Sirkar K, Kassouf W, Mansure J, Aprikian A, et al. EZH2 expression is associated with metastasis and adverse clinical outcome in clear cell renal cell carcinoma: A comparative study and review of the literature. Arch Pathol Lab Med.2013;137(10):1326-1336.
15- Hajósi-Kalcakosz S, Vincze E, Dezso K, Paku S, Rókusz A, Sápi Z, et al. EZH2 is a Sensitive marker of malignancy in salivary gland tumors. Diagn Pathol.2015;10:163.
16- Vékony H, Raaphorst FM, Otte AP, Van Lohuizen M, Leemans CR, Van Der Waal I, et al. High expression of polycomb group protein EZH2 predicts poor survival in salivary gland adenoid cystic carcinoma. J Clin Pathol.2008; 61:744-749.
17- El-Naggar AK, Chan JK, Grandis JR, Takata T, Slootweg PJ. WHO Classification of Head and Neck Tumors. 4th edition. IARC.2017.
18- Wagener N, Macher-Goeppinger S, Pritsch M, Hüsing J, Hoppe-Seyler K, Schirmacher P, et al. Enhancer of zeste homolog 2 (EZH2) expression is an independent prognostic factor in renal cell carcinoma. BMC Cancer. 2010;10:524.

19-Huet S, Xerri L, Tesson B, Mareschal S, Taix S, Mescam-Mancini L, et al. EZH2 alterations in follicular lymphoma: Biological and clinical correlations. Blood Cancer J.2017;7(4).
20-Ozawa H, Tomita T, Sakamoto K. Mucoepidermoid Carcinoma of the Head and Neck: Clinical Analysis of 43 Patients. Jpn J Clin Oncol.2008; 38 (6): 414–418.
21-Qureshi SM, Janjua OS, Janjua SM. Mucoepidermoid carcinoma: a clinico-pathological review of 75 cases. Int J Oral Maxillofac Pathol.2012; 3:5–9.
22-Esmail DA, Gaballa ET, Mourad MI, Shahin RA. Immunohistochemical Evaluation of Ki-67, CD-44 and MDR-1 Proteins in Salivary Mucoepidermoid Carcinoma. A doctoral thesis, Mansoura University.2017.
23-Aquino G, Collina F, Sabatino R, Cerrone M, Longo F, Ionna F, et al. Sex Hormone Receptors in Benign and Malignant Salivary Gland Tumors: Prognostic and Predictive Role. Int J Mol Sci. 2018;19(2):399.
24-Elsherbiny MR, Farag D A, El-Nagdy SY, Khater AA. Expression of mammary serine protease inhibitor (maspin) in salivary glands mucoepidermoid carcinoma and its clinicopathologic significance. A master thesis, Mansoura University.2016.
25-Cipriani NA, Lusardi JJ, Mcelherne J, Pearson AT, Olivas AD, Fitzpatrick C, et al. Mucoepidermoid Carcinoma: A Comparison of Histologic Grading Systems and Relationship to MAML2 Rearrangement and Prognosis. Am J Surg Pathol.2019;43(7), 885–897.
26-Tian Z, Li L, Wang L, Hu Y, Li J. Salivary gland neoplasms in oral and maxillofacial regions: a 23-year retrospective study of 6,982 cases in an eastern Chinese population. Int J Oral Maxillofac Surg.2010*;* 39:235-242.
27- Pires FR, Almeida OP, Arau´jo VC, Kowalski LP. Prognostic Factors in Head and Neck Mucoepidermoid Carcinoma. Arch Otolaryngol Head Neck Surg. 2004; 130:174-180.
28- Chen AM, Lau VH, Farwell DG, Luu Q, Donald PJ. Mucoepidermoid carcinoma of the parotid gland treated by surgery and postoperative radiation therapy: Clinicopathologic correlates of outcome. Laryngoscope.2013;123(12):3049–3055.
29- Salama A, Magdy N, Salem M, Ramzy N, Mahmoud M. Mucoepidermoid carcinoma: a reappraisal of the influence of tumor grading on prognosis. Egypt J Pathol. 2012;32(1):82–90.
30- Nance MA, Seethala RR, Wang Y, Chiosea S, Myers E, Johnson J, et al. Treatment and survival outcomes based on histologic grading in patients with head and neck mucoepidermoid carcinoma. Cancer. 2008; 113:2082–2089.
31- Kleer CG, Cao Q, Varambally S, Shen R, Ota I, Tomlins SA, et al. EZH2 is a marker of aggressive breast cancer and promotes neoplastic transformation of breast epithelial cells. Proc Natl Acad Sci U S A, 2003;100(20):11606-11611.
32- Melling N, Thomsen E, Tsourlakis MC, Kluth M, Hube-Magg C, Minner S, et al. Overexpression of enhancer of zeste homologue 2 (EZH2) characterizes an aggressive subset of prostate cancers and predicts patient prognosis independently from pre- and postoperatively assessed clinicopathological parameters. Carcinogenesis.2015;36(11):1333-1340.
33- Anwar T, Arellano-Garcia C, Ropa J, Chen YC, Kim HS, Yoon E, et al. p38-mediated phosphorylation at T367 induces EZH2 cytoplasmic localization to promote breast cancer metastasis. Nat Commun.2018;9(1):2801.
34- White MC, Holman DM, Boehm JE, Peipins LA, Grossman M, Jane Henley S. Age and cancer risk: A potentially modifiable relationship. Am J Prev Med.2014; 46 (3 SUPPL. 1): S7–S15.