International Journal Dental and Medical Sciences Research
Volume 5, Issue 6, Nov-Dec 2023 pp 374-380 www.ijdmsrjournal.com ISSN: 2582-6018

Evaluation of Cell Proliferation Marker MCM-3 in Salivary Adenoid Cystic Carcinoma.

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Date of Submission: 15-12-2023 Date of Acceptance: 25-12-2023

ABSTRACT

Background: Adenoid cystic carcinoma(AdCC) is considered one of the most common types of malignant salivary gland tumor that grows slowly and causes pain.Thisstudy was conducted to access different histological features ofAdCC, and assessment of the immunohistochemical expression of MCM-3.

Methods: Thirty formalin-fixed paraffin incorporated tissue blocks of AdCCwere classified according to the WHO histopathological types. The immunoreactivity of MCM-3 positive area was evaluated according to percentage area as following: Negative = 0% Weak =1%-10% Moderate =11%-49% Strong =50%-100%. The correlations between expression of the marker and different clinico-pathological variables were investigated using Chi-square ($\chi 2$). The P- value $\leq$ 0.05 was considered statistically significant.

Results: The expression of MCM-3 revealed statistical significant difference between the different tumor types ($p \leq 0.05$).

Conclusion: The biological behavior of AdCC can be predicated from the expression of MCM-3.

Keywords: Adenoid cystic carcinoma, Salivary gland tumors, MCM-3 Immunohistochemical, Proliferation marker.

I. INTRODUCTION

Adenoid cystic carcinoma (AdCC) is a malignant neoplasm of head and neck region. It was first described ascylindroma due to its histologic appearance which shows a cribriform pattern formed by tumor cells with cylindrical psudo-spaces [1]. The AdCC is thought to be caused by mucous-secreting glands. It develops primarily from intercalated ducts, and electron imaging revealed that it develops from cells capable of differentiating into epithelial and myoepithelial cells [2,3].

Histologically, it is composed of epithelial (luminal) and myoepithelial (abluminal) cells arranged in heterogeneous morphological growth patterns confirmed by the WHO, including cribriform, tubular and solid structures, the most common type is cribriform pattern [4]. Studies have shown that the solid type of AdCC has a worse prognosis compared with that of the other histological subtypes [5,6].

Mini-chromosome maintenance-3 (MCM-3), member of mini-chromosome maintenance proteins family- expression increases in proliferating cells, whereas intracellular levels decrease significantly in differentiated and growth-arrested cells. MCM-3 protein enters the nucleus at the end of mitosis, remains in the nucleus during most of G1 phase, and becomes predominantly cytoplasmic at the G1/S transition, and disappears at the beginning of S phase.[7]

The protein encoded by this gene is one of the highly conserved mini-chromosome maintenance proteins (MCM) that are involved in the initiation of eukaryotic genome replication. The hexameric protein complex formed by MCM proteins is a key component of the pre-replication complex (pre-RC) and may be involved in the formation of replication forks and in the recruitment of other DNA replication related proteins. This protein is a subunit of the protein complex that consists of MCM2-7. [8]

Because of its vital role in genome duplication in proliferating cells, deregulation of the MCM function results in chromosomal defects that may contribute to tumorigenesis. The MCM proteins are highly expressed in malignant human cancers cells and pre-cancerous cells undergoing malignant transformation. They are not expressed in differentiated somatic cells that have been withdrawn from the cell cycle. Therefore, this protein is ideal diagnostic marker for cancer and promising targets for anticancer drug development.[9]

Genome of the MCM family is necessary for DNA replication and its role has been studied in various diseases and cancers. It is revealed that this family of proteins not only can be considered as a cell proliferation marker but also can out-power previous classic factors of proliferation such as

International Journal Dental and Medical Sciences Research
Volume 5, Issue 6, Nov-Dec 2023 pp 374-380 www.ijdmsrjournal.com ISSN: 2582-6018

Ki67 because MCM expression in all phases of cell cycle. [10]

II. MATERIALS AND METHODS

2.1. Tissues

In this cross-sectional retrospective study, 30 cases of different types of AdCC. These were obtained from thearchives of Pathology Department, Oncology Center, Faculty of Medicine, Mansoura University, Egypt. Ethical approval was obtained prior to the study from the Ethics Committee, via code no (A10060722OP). All cases were thoroughly re-diagnosed histologically blindly by two pathologists. The demographic data including patient's age, sex, site of the tumor and size.

2.2. Immunohistochemistry:

Four-micron tissue sections were provided from paraffin-embedded and formalin-fixed blocks. After deparaffinization and rehydration, antigen retrieval was performed by 10 mm citrate buffer (pH 6.0) for 10-20 minutes. Endogenous peroxidase activity was blocked by hydrogen peroxide for 10-15 min, then washed in phosphate buffer saline (PBS) for 5 minutes. Ultra V Block was applied and incubated for 5 minutes at room temperature to block non-specific background staining, then rinsed in PBS. The sections were incubated by primary ready to use antibody MCM-3, which wasprovided by American cert by Abclonal Technology company with Code No. (A11475)with a 1:200 dilution and all slides were incubated at room temperature for 60 minutes.

The slides were incubated overnight in the humidity chamber at 4^{o}c and then washed in PBS for 5 minutes. After that, Biotinylated Goat Anti-Polyvalent was applied for 10 minutes at room temperature and the slides were washed in PBS for 5 minutes. Streptavidin Peroxidase was applied and incubated for 10 minutes at room temperature and the slides were washed in PBS for 5 minutes. Development of colored reaction product was done by using 3-Diamine benzidine tetrahydrochloride (DAB) as a chromogen: 1 drop (40 ul) DAB plus chromogen was added to 2 ml of DAB plus substrate, mixed by swirling, applied to the tissue, and incubated for 5-15 minutes. Slides counterstained with Mayers hematoxylin bath and were covered using a permanent mounting media (Canada balsam).

Light microscopy was used to analyze the stained tissues, nuclei and/or cytoplasmic areas with brown stains were judged positive. Sections of the cases analyzed were graded based on the percentage area of positive cells staining. Computer-aid digital image analysis was used to examine the specimens. The expression of each case was evaluated according to percentage area as follows:

Negative = 0% Weak = 1% -10% Moderate = 11% - 49 % Strong = 50 % – 100%.

2.3. Statistical analysis

Data were tabulated, coded, and analyzed with the SPSS (Statistical Package for Social Science) program version 26.0 to generate descriptive data in the form of mean, standard deviation (SD), and frequency (Number-Percent).

III. RESULTS

Among the current cases females were the predominant (66.7%), while males represented 33.3%. The patient's ages ranged from 40 to 71 years with a mean age of 49 years (SD±10 years). As for the site, the minor salivary gland was the most affected (36.7%) followed bysubmandibular salivary gland (33.3%), and parotid salivary gland (23.3%). Only two cases occurred in sublingual salivary gland (6.7%). Most of the cases reached the size of T1(50%), while the remaining of the cases reached the size of T2 (16.7%), and (33.3%).Three growth patterns were presented among the cases with different percentages**(Fig. 1).** The cribriform pattern was the mostly observed in 63.3% of the cases. The tubular pattern was the second presented form among the current cases (30%). The solid pattern was the least observed within the cases (6.66%) **(Table1)**.

In normal salivary gland tissue,the MCM-3 was expressed in the cytoplasm of ductal epithelial and few acinar cells. The expression of MCM-3 was positive in all cases of AdCC except one. Moderate expression was represented in 50 % of the cases. Weak expression was observed in (30%). The strong expression was found in 16.6% of the cases. The nuclear reaction only was observed in 86.6%, while both cytoplasmic and nuclear reaction was observed in the remaining (10%)**(Figs 2,3&Table 2).**

IV. DISCUSSION

The present study provoked us to examine the different histological patterns and the role of cell proliferation markerin AdCC. As regard as the age, the current study showed that the patients' ages ranged between the fourth and seventh decade which was in accordance withMeyerset al. [5]. There was a female predilectionamong the studied AdCCs, which was in agreement with Morita et al. [11]. This finding was reported to be related to the hormonal factors such as estrogen receptor beta which upregulatedG protein -coupled receptor gene

that involved in the proliferation of tumor cells Carè er al. [12] in contradiction with our results, Cruz Perez et al. [13] and Elsaid et al. [14] stated that the predominance was among males. Regarding the anatomical site of the tumor, this study showed that the minor salivary glands were mostly affected. Similar finding was reported byBradleyet al. [15]. In contrast to our finding Belulescu et al, reported that parotid salivary gland was the most common site [16].

In the current work, there was no statistically significant difference between clinicopathological characteristics of age, sex, site and size and the histopathological patterns. The solid pattern was the most aggressive type, as all solid cases in this study were T3 size and had perineural invasion, which was supported by Belulescu et al. [16].The current study evaluated the immunohistochemical expression of MCM-3 proliferation marker and their relationship with the clinicopathologic factors. Results showed strong positive expression in ductal and acinar cells in normal salivary gland which was in agreement with Jaafari-Ashkavandiet al. [17]. The MCM-3 showed expression in few acinar cells and positive reactivity in the ductal cells of normal salivary glands, that was in accordance with Elsaidet al.This could be clarified through the fact that the typical salivary glands acinar cells are fully differentiated, whereas the ductal epithelium has the ability to grow[14]. Studies investigatingMCM-3. Regarding the current work, the expression of MCM-3 was mostly nuclear (90%), this was supported in previous study; however few cases revealed both nuclear and cytoplasmic reaction.This observation was explained in an in vivo study inretigted MCM2-7 proteins and reported many interactions between these proteins within the cell cycle. This study reported the importance of this interactions in nuclear accumulation, while only 3 cases were expressed in nuclei and cytoplasm which was in agreement with Elsaid et al. [14]. This is demonstrated by the fact that during the S-phase of the cell cycle, nearly all MCM proteins detach from the chromatin, leaving just a small proportion bound to un-replicated DNA sequences.The MCM proteins then disappeared from chromatin during the G2/M phase and are mostly present in the cytoplasm, as they undergo enzymatic degradation [18].

V. CONCLUSIONS

From the results of this study, it could be concluded that clinical data of patient is not a predicting factor for the biologic behavior of AdCC. The MCM-3 is a prognostic markerforAdCC.

Ethics approval

This research obtained the approval of the Ethical Committee, Faculty of Dentistry, Mansoura University, Egypt (A10060722OP).

CRediT authorship contribution statement

Rawda Elsherbiny: Conceptualization, Formal analysis, Investigation, Visualization, Data Curation, Writing-Original draft preparation. **Mona Hany Emile:** Reviewing and Editing.**Mona Mohsen Abdo Ibrahim:** Formal analysis, Investigation, Visualization, Data Curation, Reviewing and Editing. **Lawahiz M. Ismail:** Conceptualization, Writing-Reviewing and Editing, Project manager.

Conflict of interest

These authors declared no competing interests in this study.

Acknowledgments

Not applicable

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Table 1. The distribution of histopathological patterns among clinical data of the studied cases.

	Histopathological pattern n (%)			**Test of significance**
	Tubular 9 (30%)	Cribriform 19 (63.3%)	Solid 2 (6.66%)	
Age /years				
≤45	4(44.4)	6(31.6)	1(50)	p=0.730
>45-60	3(33.3)	11(57.9)	1(50)	
>60	2(22.2)	2(10.5)	0	
Sex				
Female	5(55.6)	15(78.9)	0	P=0.06
Male	4(44.4)	4(21.1)	2(100)	

Site				
Submandibular	0	8(47.4)	1(50)	
	5(55.6)	3(10.5)	0	p=0.099
Parotid	1(11.1)	1(5.3)	0	
Sublingual	3(33.3)	7(36.8)	1(50)	
Minor segment				
Size				
T1	2(22.2)	11(57.9)	2(100)	
T2	2(22.2)	3(15.8)	0	P=0.247
T3	5(55.6)	5(26.3)	0	

Table 2. The distribution of MCM-3 expressionamong clinical data and histopathological types of the studied cases.

	MCM-3 expression n (%)				**Test of significance**
	Negative 1(3.33%)	Weak 9(30%)	Moderate 15(50%)	Strong 5(16.6%)	
Age /years					
≤45	0	3(33.3)	6(40)	2(40)	
>45-60	1(100)	5(55.6)	6(40)	3(60)	p=0.840
>60	0	1(11.1)	3(20)	0	
Sex					
Female	1(100)	7(77.8)	9(60)	3(60)	p=0.706
Male	0	2(22.2)	6(40)	2(40)	
Site					
Submandibular	0	3(33.3)	6(40)	1(20)	
Parotid	0	2(22.2)	4(26.7)	1(20)	p=0.907
Sublingual	0	1(11.1)	1(6.7)	0	
Minor	1(100)	3(33.3)	4(26.7)	3(60)	
Size					
T1	1(100)	3(33.3)	7(46.7)	4(80)	P=0.659
T2	0	2(22.2)	3(20)	0	
T3	0	4(44.4)	5(33.3)	1(20)	
Histopathology pattern					
Tubular	0	5(55.6)	4(26.7)	0	**P=0.02***
Cribriform	1(100)	4(44.4)	11(73.3)	3(60)	
Solid	0	0	0	2(40)	

International Journal Dental and Medical Sciences Research
Volume 5, Issue 6, Nov-Dec 2023 pp 374-380 www.ijdmsrjournal.com ISSN: 2582-6018

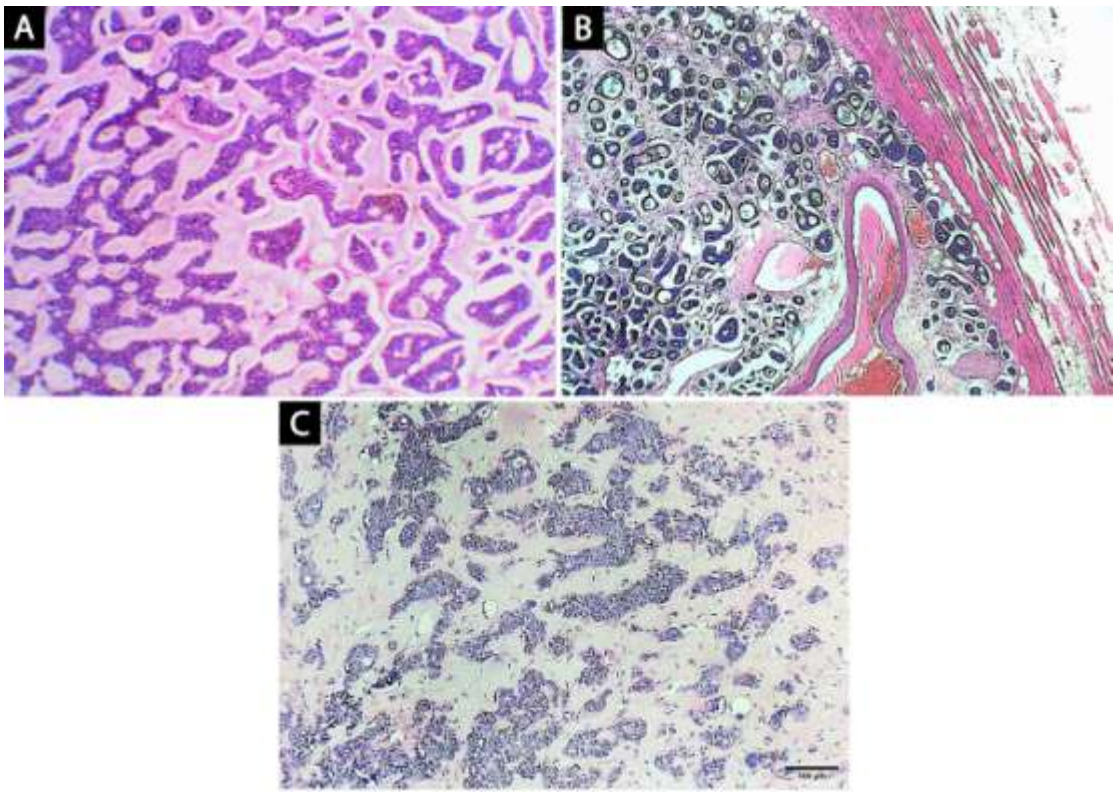

Fig. 1. Photomicrograph showingdifferent patterns of AdCC; (a) tubular, (b) cribriform, and (c) solid.

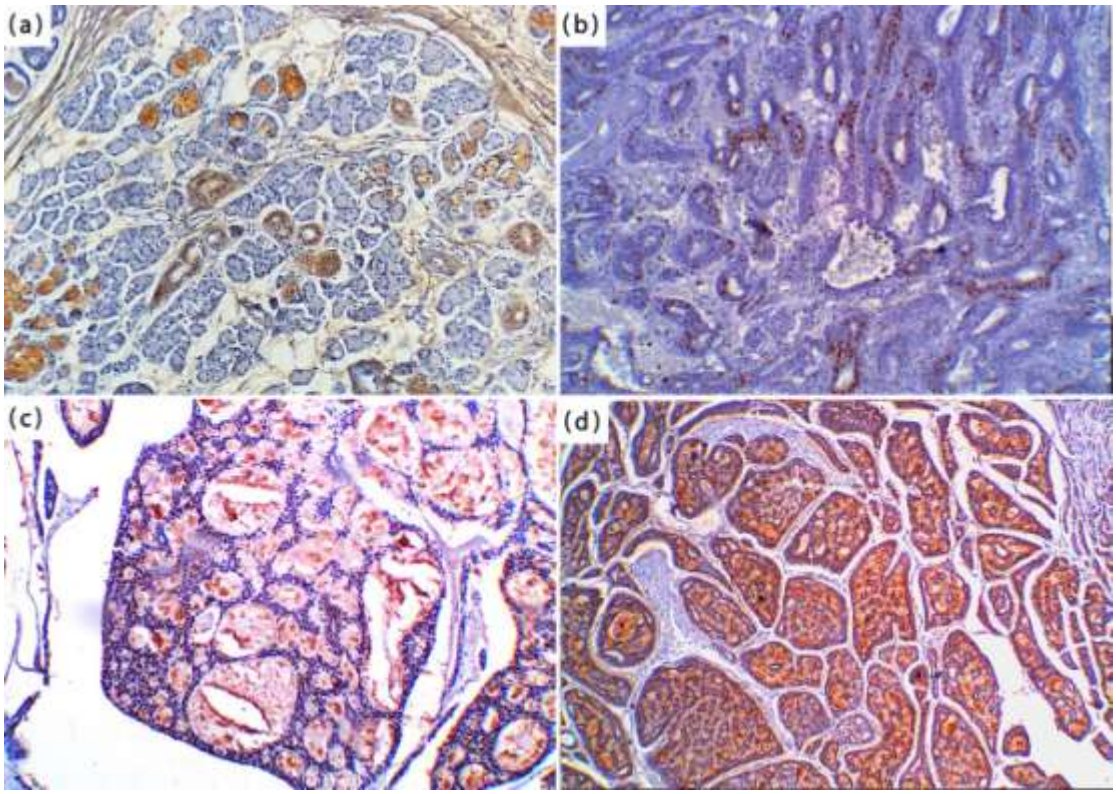

Fig.2. Photomicrograph showing expression of MCM-3; (a) in normal salivary gland, (b) weak expression in AdCC, (c) moderate expression in AdCC, and (d) strong expression in AdCC.

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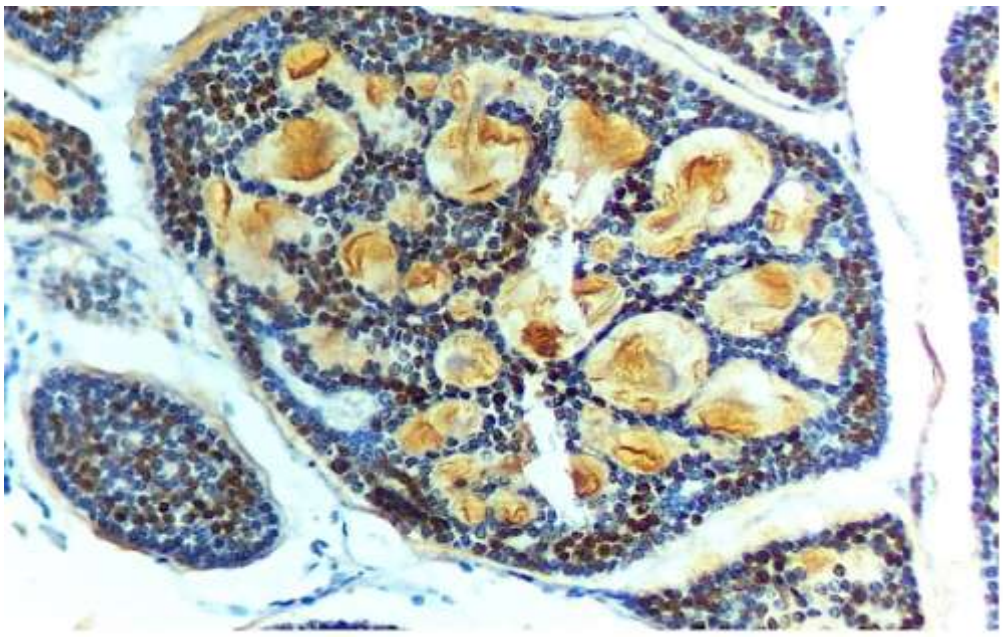

Fig. 3. Photomicrograph showing nuclear cellular localization of MCM-3.