Journal of American Science

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Clinicopathological significance of cell proliferation marker (MCM3) in malignant salivary gland tumors

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Abstract: Aims: Assessment of the immunohistochemical expression of MCM3 in 54 cases of salivary gland carcinomas to elucidate the possible correlations between its expression and the different clinicopathological variables. **Study design:** Retrospective study. **Place and duration:** Oncology Center Mansoura University (OCMU) and pathology laboratory at OCMU, Egypt, February 2019 to June 2021. **Methodology:** Fifty four formalin fixed paraffin embedded tissue blocks of salivary gland carcinomas (5 low grade mucoepidermoid carcinoma (MEC) cases, 19 high grade MEC cases, 15 adenoid cystic carcinoma (AdCC) cases, 10 carcinoma ex pleomorphic adenoma(CXPA) cases and 5 acinic cell carcinoma (ACC) cases) were examined for MCM3 immunohistochemical expression. The immunoreactivity of MCM3 was evaluated by Computer Assisted digital image analysis (Digital morphometric study). Correlations between the marker's expression and different clinicopathological variables were investigated using Chi-square ($\chi 2$), one way ANOVA test, post hoc tukey test and Spearman's correlation coefficient test. The P-value ≤ 0.05 was considered statistically significant. **Results:** MCM3 expression revealed statistically significant difference among the different tumors ($p \leq 0.05$). On the other hand, significant positive correlation was found between MCM3 expression and MEC grades. Also, the correlation between MCM3 and TNM stages of high grade MEC cases, AdCC cases and CXPA cases was significantly positive but it was statistically non-significant between MCM3 expression and TNM stages of low grade MEC cases and ACC cases. **Conclusion:** The biological behavior of malignant salivary gland tumors can be predicated from the level of MCM3.

[Samar Soliman, Mahmoud El sherbeny, Heba Sheta, Nadia Lotfy **Clinicopathological significance of cell proliferation marker (MCM3) in malignant salivary gland tumors.** *J Am Sci* 2021;17(9):1-10] *ISSN 1545-1003 (print); ISSN 2375-7264 (online). http://www.jofamericanscience.org* 1. doi: 10.7537/marsjas170921.01.

Keywords: Mucoepidermoid carcinoma, adenoid cystic carcinoma, carcinoma ex pleomorphic adenoma, acinic cell carcinoma, MCM3, immunohistochemistry.

1. Introduction

Salivary gland carcinomas (SGCs) represent a diverse group of tumors with different clinical behaviors and morphological patterns that make their classification, diagnosis and treatment of major challenge [1, 2]. Also, SGCs have an unpredictable prognosis so; they constitute an important area in the field of oral and maxillofacial pathology [3, 4]. Mucoepidermoid carcinoma (MEC) and adenoid cystic carcinoma (AdCC) are the most common malignant salivary gland tumors [4].

Mucoepidermoid carcinoma (MEC) is the most common malignant tumor of the major and minor salivary glands. They constitute 12% - 35% of salivary gland carcinomas (5, 6). Microscopically, they are distinguished by a mixed population of cells, including: mucin-producing cells, epidermoid cells with squamoid differentiation, clear cells, and intermediate cells that may prevail in numbers and are thought to be the progenitor of the other types of cells. No myoepithelial cells are present [7].

On the other hand, Brandwein et al,.[8] classified MEC as low, intermediate and high according to the histologic features of the lesion; which are most important in the prediction of the aggressive nature of these tumors. These features are 1) intra cystic component (not more than 25%); 2) tumor necrosis; 3) neural invasion; 4) pronounced nuclear atypia; 5) mitotic activity and 6) tumor front invades in small nests and islands 7) mitosis 8) Lymphatic and/or vascular invasion [7].

Adenoid cystic carcinoma (AdCC) is one of the most common and best-recognized salivary gland malignancies with distinctive histopathological features. It constitutes approximately 10% of all salivary gland tumors [9].

It shows a contradicted history. Firstly, the tumor has a slow growth, but the clinical course is unyielding and progressive. Secondly, the operative intervention is usually possible, but multiple local recurrences are common. Thirdly, regional lymph nodes metastasis is rare, but distant spread to the lungs and bones is common [10].Microscopically, they have a basaloid epithelium clustered in nests in a hyaline stroma. AdCC can be categorized into three growth patterns, cribriform, tubular, and solid patterns. Cribriform pattern is the most common histologic subtype (44%) [11].

Acinic cell carcinoma (ACC) constitutes approximately 17% of primary salivary gland malignancies, representing the third most common epithelial malignant salivary gland tumor in adults, following mucoepidermoid carcinoma and adenoid cystic carcinoma. In the pediatric age group, ACC is the second most common epithelial malignancy following mucoepidermoid carcinoma [12, 13]. Microscopically, ACC is characterized by serous acinar cell differentiation. However, different cell types and growth patterns are known. These incorporate acinar, intercalated ductal, vacuolated, clear, and non-specific glandular and solid-lobular, microcystic, papillary-cystic and follicular growth patterns [13-19].

Carcinoma ex pleomorphic adenoma (CXPA) is defined as a carcinoma arising de novo from a primary or recurrent benign pleomorphic adenoma (PA) [20-22]. CXPA accounts for approximately 3.6% of all salivary gland tumors, 6.2% of all mixed tumors, and 11.6% of all malignant salivary gland neoplasms [23]. Histologically, one small malignant growth within a PA may be present, or the benign tumor may be replaced by malignant lesion with destructive infiltrative growth. Nouraei et al.(21) and Zbaren et al. [24] observed that 25% of their patients and 21% of their patients, respectively, had a previously treated PA.

Cell proliferation is considered as one of the most important biological mechanisms in oncogenesis. Proliferative activity has been shown in several studies to be of high prognostic significance in different types of tumors including salivary gland tumors [25]. Identification of a proliferation fraction within the tumor cell population has been useful in diagnosis and/or prognosis in a range of human cancers. The minichromosome maintenance protein MCM3 is a novel proliferation marker that plays a vital role in determining growth fractions and is used as indicator of cell proliferation [26-28]. Therefore, the present study was carried out to investigate the immunohistochemical expression of MCM3 in salivary gland carcinomas, correlate the expression of MCM3 and the histopathological grade of MEC and identify the correlation between the immunohistochemical expression of MCM3 and TNM stage of salivary gland carcinomas.

2. MATERIAL AND METHODS

2.1 Material

2.1.1 Cases

The present study was carried out on:-

Fifty four formalin fixed, paraffin embedded tissue blocks of salivary gland carcinomas (24 MEC cases, 15 AdCC cases, 10 carcinoma ex pleomorphic adenoma cases and 5 ACC cases). The studied cases were collected from the archival files of pathology laboratory at Oncology Center Mansoura University (OCMU).

Control group, 10 sections of the normal salivary gland present in mucocele cases obtained from the archival files of oral pathology department, Faculty of Dentistry, Mansoura University.

2.1.2 Immunohistochemical marker

Antibody for MCM3**.**

2.2 Methods

2.2.1 Clinical data evaluation

All the available clinical data for the studied cases were collected from patients' registered medical documents in the oncology center regarding the patient age, sex, site of the tumor, tumor size and presence or absence of lymph node metastasis.

Based on the analysis of the previous clinical data, cases were classified into stages according to tumor-node-metastasis (TNM) staging system [29].

2.2.2 Histopathological examination

Serial sections of 4 microns in thickness were prepared for routine hematoxylin and eosin staining of tumors in order to confirm the diagnosis of tumors and reclassify them into their pathological subtypes as follow:

The available mucoepidermoid carcinoma (MEC) cases were classified into 5 cases low grade and 19 cases were high grade (30).

2.2.3 Immunohistochemical examination

Another four micron thickness sections were cut from the paraffin blocks of the tumors and the control groups for immunostaining which was performed using Avidin-Biotin complex according to the manufacturer's instructures [31]. The slides were deparaffinized by immersion in Xylene (15 minutes) then rehydrated in descending grades of alcohol and then washed in water. Blocking the endogenous peroxidase activity by treatment sections with 3% hydrogen peroxide solution for 10 minutes and then washed in phosphate buffer solution (PBS) for 5 minutes. Pretreatment of the tissue sections by immersing in EDTA buffer at 90°C for 20 minutes, cooling to room temperature, and then washing in PBS for 5 minutes. The primary antibody for MCM3

was used at a dilution of 1:200 and the slides were incubated 60 minutes at room temperature, the slides were then incubated with biotinylated secondary antibody for 25 minutes at room temperature, and then they were washed in PBS for 5 minutes. Streptavidin/peroxidase was added to cover sections; tissues were incubated at room temperature for 25 minutes and washed in PBS for 5 minutes. Sections were covered with drops of chromogenic reagent (Diamine benzidine tetrahydrochloride (DAB)); the slides were incubated at room temperature for 10 minutes, and then washed in PBS for 5 minutes. Slides racks were placed in Mayers haematoxyline bath for 3 minutes and then washed in distilled water 3 minutes. Tissues were dehydrated through ascending grades of alcohol (80, 90,100) then xylene for 3minutes for each one and covered with cover slips using DPX. Positive control of the used antibody (MCM3) was performed by staining sections of human prostate cancer under the same conditions. Negative control slides obtained by replacement of the primary antibodies by plain PBS. Also, sections of the normal salivary gland present in mucocele cases were immunostained with MCM3.

2.2.4 Immunostaining evaluation:

Sections of the studied cases were assessed on the basis of the percentage area of positive cells staining in a nuclear and/or cytoplasmic pattern for MCM3. When the tumor cells had a brown cytoplasm/membrane or nuclei, specimens were considered to be positive for staining, and specimens were considered to be negative for staining when tumor cells showed only blue nuclei or cytoplasm/membrane. The specimens were evaluated by Computer Assisted digital image analysis where slides were photographed using Olympus® digital camera installed on Olympus® microscope with 1/2 X photo adaptor, using 40 X objective. The result images were analyzed on Intel® Core I3® based computer using Video Test® Morphology® software (Russia) with specific built-in routine for for stain quantification.

2.2.5 Statistical Analysis

Data were tabulated, coded then analyzed using the computer program SPSS (Statistical package for social science) version 26.0 to obtain.Descriptive statistics were calculated in the form of:

- Mean ±Standard deviation (SD).
- Frequency (Number-percent)

In the statistical comparison between the different groups, the significance of difference was tested using one of the following tests:-

- One way ANOVA (analysis of variance):- Used to compare between more than two groups of numerical (parametric) data followed by post-hoc tukey.
- Inter-group comparison of categorical data was performed by using Pearson's chi square test (X^2-value) for table (2x2) & Monte-carlo for table larger than (2x2).

Spearman's correlation coefficient test was used correlating different parameters. P value <0.05 was considered statistically significant.

3. Results

3.1 Clinico-pathological features of studied cases

3.1.1 Age and sex:

For low grade MEC cases, the mean±SD of patients' ages was 39.4±17.2, while the mean±SD of patients' age in high grade MEC, AdCC, ACC and CXPA cases was 60.5±16.1, 51.3±10.0, 46.5±19.7 and 60.8±15.0 respectively (Table 1). Statistically, there was significant difference between the different tumors regarding the age (table 1).

On the other hand, males were more affected than females in low grade MEC, AdCC, ACC and CXPA cases unlike high grade MEC cases where females were more affected than males (table 1).

3.1.2 Site:

Regarding the site, 3 cases (60%) of low grade MEC were in minor SGs and the remaining 2 cases (40%) were in the parotid. Fifteen cases (78.9%) of high grade MEC were in the parotid and 4 cases (21.1%) were in the submandibular gland. In AdCC; 7 cases (46.7%) were in minor SGs, 4 cases (26.7%) were in submandibular gland, 3 cases (20%) were in the parotid and 1 case (6.7%) was in sublingual gland (table 1).

All cases of ACC (100%) were in the parotid. while in CXPA, 7 cases (70%) were in parotid and 3 cases (30%) were in submandibular gland. Statistically, there was significant difference between the tumors regarding the site (table 1).

3.1.3 Tumor size (T):

The tumor size of all cases of low grade MEC and ACC (100%) was in (T1/T2) group but in high grade MEC; 5 cases (26.31%) were in (T1/T2) group and 14 cases (73.68%) were in (T3/T4) group. Four cases (26.7%) of AdCC were in (T1/T2) group and 7 cases (46.7%) were in (T3/T4) group. 4 cases were presented with missed data (Table 2).

On the other hand, there was an equal distribution in the number of cases between (T1/T2) group and (T3/T4) group (5 cases, 50%) in CXPA (table 2).

3.1.4 Regional lymph node (N):

In the current study, all cases of low grade MEC and ACC yielded score N0, whereas in high grade MEC; 12 cases (63.16%) were found with score N0; 3 cases (15.79%) with score N1 and 4 cases (21.05%) with score N2. In AdCC, 10 cases (90.91%) yielded

score N0 and 1 case yielded N2. Four cases were presented with missed data.

Seven cases (70%) of CXPA was found with score N0 and 3 cases (30%) with score N1 (table 2).

3.1.5 Distant metastasis (M):

No distant metastasis had been registered in all types of the studied tumors (M0).

3.1.6 TNM staging:

All the studied malignant salivary gland tumors were classified according to TNM staging system. It was found that:

Four cases (80%) of low grade MEC were presented with stage I and 1 case (20.0%) was presented with stage II. But, in high grade MEC, most of cases (10 cases) were presented with stage IV (52.6%) followed by stage III (31.6%) and 3 cases were stage I (15.8%) (table 2).

In AdCC; 6 cases (54.5%) were presented with stage IV, 3 cases (27.3%) were presented with stage II, 1 case (9.1%) was presented with stage III and 1 case (9.1%) was presented with stage I. Four cases were presented with missed data. 3 cases (60%) in ACC were presented with stage I and 2 cases (40%) were presented with stage II. (table 2).

Half of cases of CXPA were presented with stage III and the other half was presented with stage II (Table 9), (Chart 5). Statistical significance difference was found between the different tumors regarding to TNM stages (P<0.001) (table 2).

Histologically, the present study included 54 cases of malignant salivary gland tumors, 5 cases were low grade MEC, 19 cases were high grade MEC , 15 cases were AdCC which showed different patterns; cribriform, tubular and solid pattern, 5 cases were ACC which also showed solid and microcystic pattens and 10 cases were CXPA which revealed that 6 cases (60%) were adenocarcinoma NOS ex pleomorphic adenoma, 3 cases (30%) were salivary duct carcinoma ex pleomorphic adenoma and 1 case was myoepithelial carcinoma ex pleomorphic adenoma.

Table (1): Comparison of age, sex & site between different studied groups

		Low MEC No.= 5	High MEC No.= 19	AdCC No.= 15	ACC No.= 5	CXPA No.= 10	Test used	P value
Age		39.4±17.2	60.5±16.1	51.3±10.0	46.5±19.7	60.8±15.0	ANOVA	0.027*
Sex	Male	3(60.0%)	9(47.4%)	10(66.7%)	4(80.0%)	6(60.0%)	MC	0.85
	Female	2(40.0%)	10(52.6%)	5(33.3%)	1(20.0%)	4(40.0%)		
site	Submandibular	0(0%)	4(21.1%)	4(26.7%)	0(0%)	3(30.0%)	MC	<0.001*
	Parotid	2(40.0%)	15(78.9%)	3(20.0%)	5(100.0%)	7(70.0%)		
	Minor SG	3(60.0%)	0(0%)	7(46.7%)	0(0%)	0(0%)		
	sublingual	0(0%)	0(0%)	1(6.7%)	0(0%)	0(0%)		

Data expressed as mean±sd & frequency (no, %)
Sd: standard deviation mc: monte-carlo
P: probability *: significance ≤0.05

Table (2): Comparison of tumor size & regional lymph nodes & TNM stage between different studied groups.

		Low MEC (No.=5)	High MEC (No.=19)	AdCC (No.=11)	ACC (No.=5)	CXPA (No.=10)	Test used	P value
Tumor size	T1/T2	5 (100%)	5 (26.31%)	4 (26.7%)	5 (100%)	5 (50%)		
	T3/T4	0	14 (73.68%)	7 (46.7%)	0	5 (50%)		
Regional lymph nodes	N0	5 (100%)	12 (63.16%)	10 (90.91%)	5 (100%)	7 (70%)		
	N1	0	3 (15.79%)	0	0	3 (30%)		
	N2	0	4 (21.05%)	1 (9.1%)	0	0		
TNM Stage	Stage I	4(80.0%)	3(15.8%)	1(9.1%)	3(60.0%)	0(0%)		
	Stage II	1(20.0%)	0(0%)	3(27.3%)	2(40.0%)	5(50.0%)		
	Stage III	0(0%)	6(31.6%)	1(9.1%)	0(0%)	5(50.0%)	MC	<0.001*
	Stage IV	0(0%)	10(52.6%)	6(54.5%)	0(0%)	0(0%)		

Data expressed as frequency (No, %)
P: Probability *: significance ≤0.05 MC: Monte-Carlo.

3.2 Immunohistochemical findings:

3.2.1 MCM3 expression in the different studied tumors.

In the current study, normal salivary gland showed negative expression in the acinar cells but it showed nuclear and cytoplasmic reaction in the duct epithelium. Among the studied low and high MEC

cases, the reaction was nuclear and cytoplasmic in most of the lesional cells throughout the tumor**.** Also, MCM3 was expressed as membranous reaction in clear cells. The immunoreactivity of MCM3 in AdCC was detected in the nuclei and the cytoplasm of the malignant cells. Also, the expression of MCM3 in ACC was detected in the nuclei and the cytoplasm of the acinar cells. In CXPA, intense diffuse positive nuclear and cytoplasmic reactivity were detected for MCM3 in malignant cells (Fig. 1).

3.2.2 Comparison of MCM3 expression in the different studied tumors

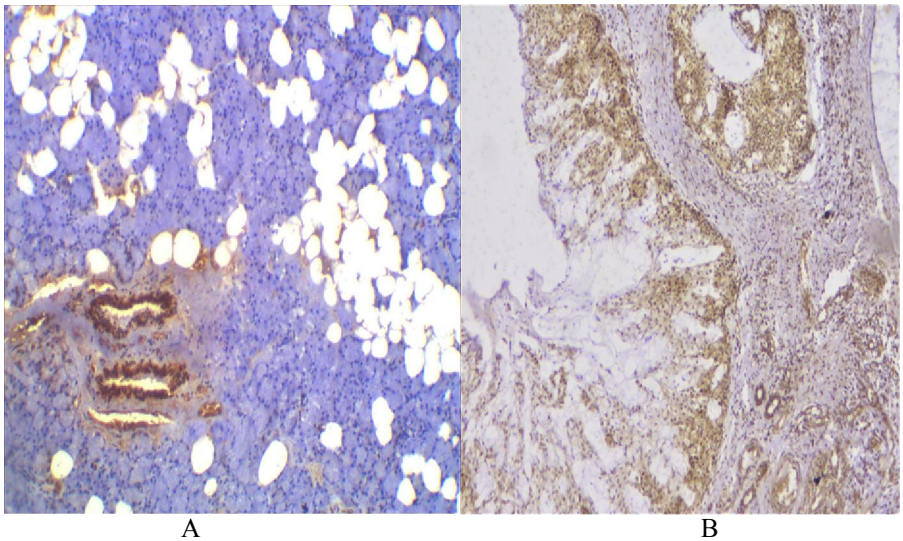
The highest expression of MCM3 was in high grade MEC (405.29±51.72) followed by CXPA (340.91±64.54), AdCC (329.90±89.47), low grade MEC (222.43±69.30) and ACC (112.89±47.84) which was the least expression. Statistically, there was significant difference between all different tumors (table 3).

3.2.3 Correlation between MCM3 expression and MEC grades (low and high MEC).

The correlation between MCM3 expression and MEC grades was significantly positive (r=0.719, P <0.001)**.** MCM3 are highly expressed in high grade MEC cases than low grade ones (table 4).

3.2.4 Correlation between MCM3 expression and TNM stages of the different studied tumors.

Statistically, there were non-significant correlations between MCM3 expression and the increase in TNM stage of low grade MEC and ACC. But, significant positive correlations were found between MCM3 expression and TNM stages of high grade MEC, AdCC and CXPA. As the stage of high grade MEC, AdCC and CXPA cases increases, there is significant increase in MCM3 immunoreactivity (Table 5).



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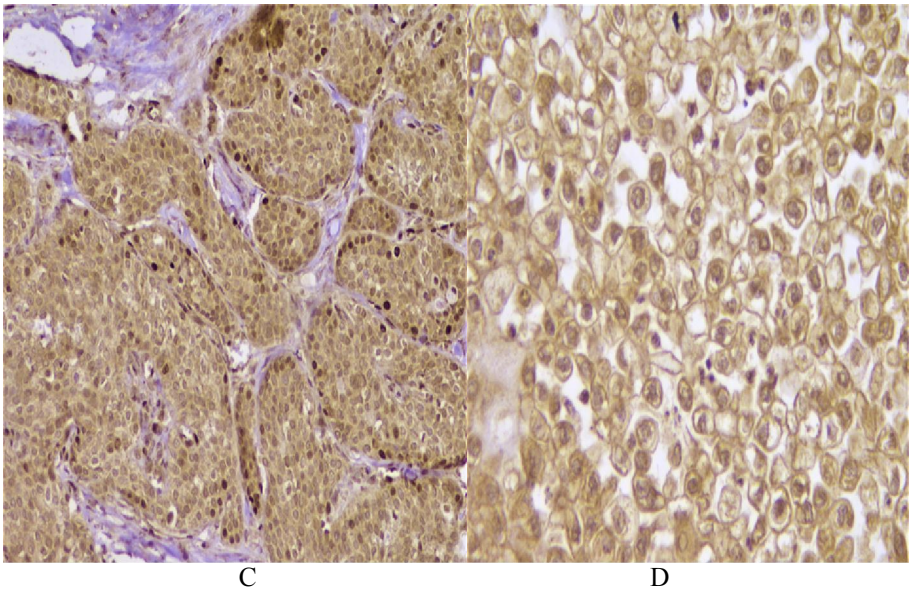

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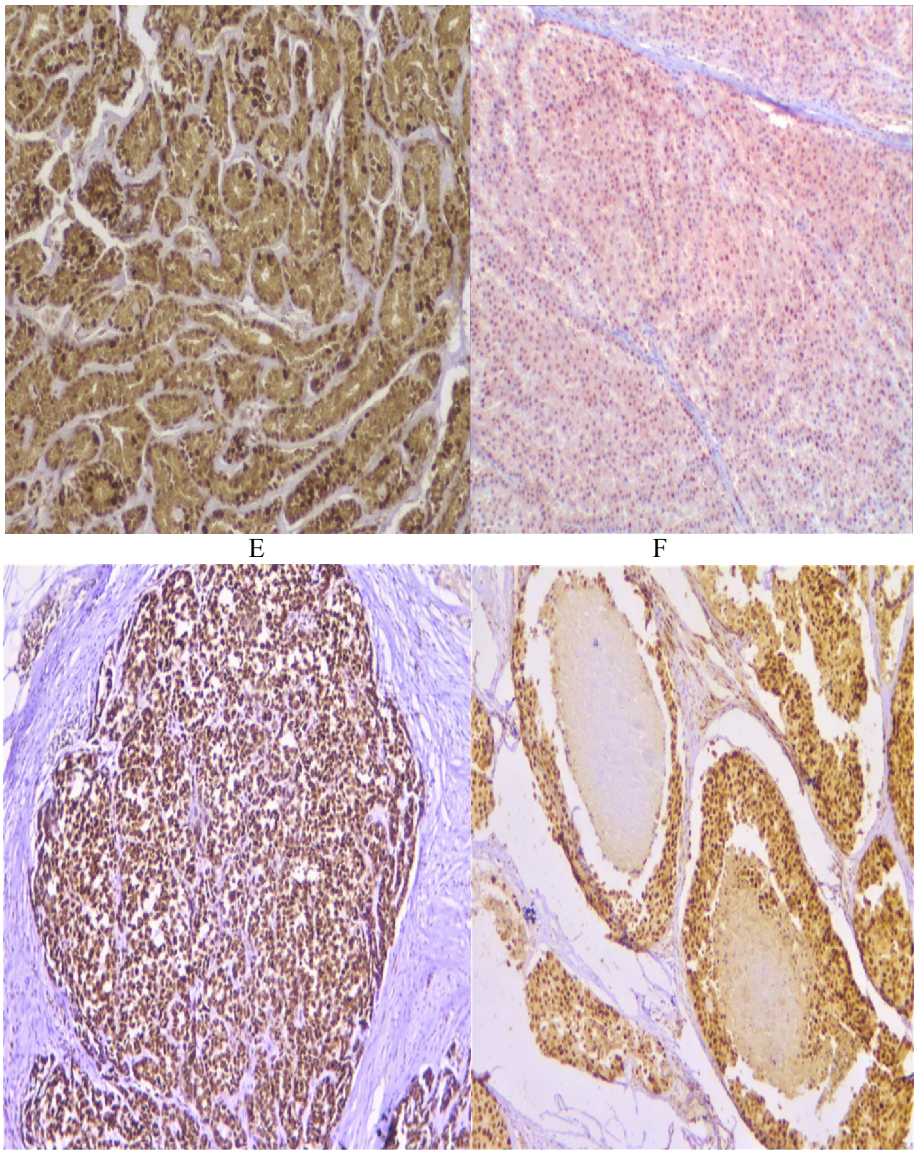

Fig. 1. MCM3 expression; normal salivary gland (A), low grade MEC (B), high grade MEC(nuclear and cytoplasmic) (C), membranous (D), AdCC (E), ACC(F), adenocarcinoma not otherwise specified ex pleomorphic adenoma (G) and salivary duct carcinoma ex pleomorphic adenoma(H)

. **Table (3): Comparison of integrated density of MCM3 in different studied tumors.**

	Low MEC	High MEC	AdCC	ACC	CXPA	ANOVA P value
Mcm3(x10^6)	**222.43±69.30**	**405.29±51.72**	**329.90±89.47**	**112.89±47.84**	**340.91±64.54**	**<0.001***
Post-hoc		**P1=<0.001***	**P1=<0.001* P2=<0.001***	**P1=<0.001* P2=<0.001* P3=<0.001***	**P1=<0.001* P2=<0.001* P3=0.012* P4=<0.001***	

Data expressed as mean±SD, SD: standard deviation, P: Probability *: significance ≤0.05, Test used: One way ANOVA followed by post-hoc tukey, P1: significance vs Low MEC, P2: significance vs High MEC
P3: significance vs AdCC, P4: significance vs ACC

Table 4: Correlation between MCM3 expression and MEC grades (low and high MEC).

	MEC Grades	
	r	P
MCM3	0.719	<0.001*

r: Spearman's correlation coefficient P: Probability *: significance ≤0.05

Table (5): Correlation between MCM3 expression and TNM stages of the different studied tumors.

			TNMstages
Low MEC	MCM3	r	-0.612
		P	0.272
High MEC	MCM3	r	0.772
		P	<0.001*
AdCC	MCM3	r	0.685
		P	0.020*
ACC	MCM3	r	0.167
		P	0.789
CXPA	MCM3	r	0.655
		P	0.040*

r: Spearman's correlation coefficient P: Probability *: significance ≤0.05

4. Discussion:

Interest in malignant salivary gland tumors had arisen as they have unpredictable clinical behavior and prognosis [4]. Therefore, the present study examined the possible role of neoplastic cells proliferation in the clinical behavior of these tumors.

In the current study, MCM3 revealed negative expression in the acinar cells and positive reactivity in the epithelium of the ducts of normal salivary glands (positive control) that was in accordance with Arafa et al. who stated that only ductal epithelium showed positive reactivity to MCM3. While, the acinar cells showed negative reaction in normal salivary glands[32]. Also, these findings were in contrast to Abdalla et al. who found negative reactivity for MCM3 in epithelial and myoepithelial cells [33]. Moreover, Ashkavandi et al. found positive immunoreactivity to MCM3 in the ductal epithelium of normal salivary gland(34) that may be explained by the fact that the acinar cells of normal salivary gland are in fully differentiated. While, the ductal epithelium have proliferative potentiality [32].

In MEC cases, MCM3 reaction was nuclear and cytoplasmic in most of the lesional cells throughout the tumor that was in accordance with Arafa et al. who reported that MCM3 had a preferential nuclear pattern, although some cases showed positive nuclear and cytoplasmic reaction [32]. Also, many studies noted nuclear and cytoplasmic reaction [35-38]. Labib K et al. illustrated this by the fact that in S phase of the cell cycle, nearly the whole amount of MCM proteins dissociate from the chromatin, leaving only a small fraction bound to regions of unreplicated DNA. Subsequently, during G2/M phase, MCM proteins are absent on chromatin and are detectable predominantly in cytoplasm where they later undergo enzymatic degradation[39].

Also in the current work, MCM3 was expressed as membranous reaction in clear cells which was in agreement with Abdalla R et al. who noted membranous reactivity in the mucous secreting cells and clear cells for MCM3 in MEC cases [33].

Furthermore, MCM3 in AdCC was expressed in the nuclei and the cytoplasm of the tumor cells. Abdalla et al. supported the current study result as they stated that AdCC exhibited diffuse total immunoreactivity to MCM3. Ashkavandi et al. noted nuclear reactivity in AdCC cases[34]. ACC revealed nuclear and cytoplasmic reaction for MCM3 which copied with Labib et al. illustration which proved that MCM3 may have nuclear and cytoplasmic reaction(39). On the other hand, CXPA showed intense diffuse positive nuclear and cytoplasmic reactivity for MCM3 which coordinated with Abdalla et al. who reported the same result [33].

On the other hand, Ghazy et al.[40] reported that MCM2 revealed cytoplasmic staining in most of the epidermoid cells in high grade MEC cases, while in low grade cases, little number of epidermoid cells were positive. In AdCC, MCM2 demonstrated cytoplasmic reaction. Furthermore, in malignant pleomorphic adenoma, MCM2 revealed nuclear and cytoplasmic reaction in epithelial and some myoepithelial cells but In ACC, one case of clear cell variant demonstrated membranous MCM2 reaction, while the remaining cases revealed nuclear and cytoplasmic reaction.

The present study revealed that cell proliferation in high grade MEC was the highest, followed by CXPA, AdCC and low grade MEC. ACC was the least in cell proliferation. To the best of our knowledge, few studies compared the proliferative ability between different salivary gland tumors.

some studies agreed with the present study results, Ashkavandi et al. [28] noted that the mean±SD of MCM3 in MEC was higher than that of AdCC. Arafa stated that MCM3 expression increases with increasing the histological grade of MEC, where low‐grade cases showed lower expression, while the high‐grade cases showed higher expression of MCM‐3 which was in consistence with Ghazy et al. and Vargas et al. who noted an increase of MCM‐2 expression from low‐grade MEC to high‐grade cases[37, 40]. Bussari et al.[41] stated that Ki-67 expression in high grade MEC was higher than low grade MEC with statistically significant difference.

On the other hand, Americo et al. found that Ki-67 expression was higher in AdCC than MEC that was in contrast to the current work result. Also, Vargas et al. [37] stated that AdCC had a higher proliferation rate compared to the other salivary gland tumors. Jaafari-Ashkavandi et al.[42] found that there was no significant difference between AdCC and MEC in CDC7 expression (which directly linked with MCM proteins expressions[43]) .

Meanwhile, the present study findings were in accordance with the fact of the cancer that the growth and the spread of differentiated neoplastic cells are at a slower rate than undifferentiated or poorly differentiated cells, which lost the structure and function of normal cells and grow with an uncontrollable manner [44].

Also, a significant positive correlation between the cell proliferation and MEC grades was found in our result that was in consistence with Jaafari-Ashkavandi et al. who reported a positive correlation between CDC7 expression and tumor grades[42]. Bussari et al. [41] suggested also that the Ki-67 correlated very well with histopathological grades of MEC.

In the current work, statistically significant correlation was seen between the proliferative ability and TNM stage of high grade MEC, AdCC and CXPA. While, non-significant correlation was found between the proliferative ability and TNM stage of low grade MEC and ACC that was in accordance with Gul et al. who informed that overexpression of minichromosome maintenance complex 2, 3, 5, 6 and 7 indicated bad prognosis[45]. Also, the poor prognostic effect of MCM3 overexpression was also shown in gliomas, thyroid carcinomas, melanoma, cutaneous T-cell lymphomas and oral squamous cell carcinomas[46].

Peng et al. [47] reported that the abnormally up-regulated MCMs in pancreatic cancer were significantly associated with cancer cell proliferation, disease progression, and poorer outcomes.

Conclusion

The present immunohistochemical study was carried out on fifty four cases which had the diagnosis of malignant salivary gland tumors of varied histologic types. MCM3 expression was investigated in relation to the different tumors, grades of MEC and TNM stage. The biological behavior of malignant salivary gland tumors can be reflected by malignant cells proliferation which showed overexpression of MCM3 in high grade MEC, followed by CXPA, AdCC, low grade MEC and ACC. cell proliferation increase with increasing MEC grade. TNM staging system is a predicting factor for the biological behavior of high grade MEC, AdCC and CXPA.

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9/6/2021