

IMMUNOHISTOCHEMICAL EXPRESSION OF MCM4 AND ITS MAIN REGULATOR IN MOST PREVALENT BENIGN AND MALIGNANT SALIVARY GLAND NEOPLASMS

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ABSTRACT

Review: Salivary gland tumors (SGTs) are considered to be a challenging field in the diagnostic pathology, with many overlapping different histopathological features. PA, WT, MEC and ADCC are common neoplasms arising from the salivary glands. The sonic hedgehog (Hh) signaling pathway has important functions in embryonic development, cell proliferation and differentiation. MCM4 forms complex that binds to DNA and acts in the initiation of DNA replication, regulates cell proliferation and is considered a sensitive proliferation marker for cancer diagnosis.

Aim of study: The current study aimed to examine the immunohistochemical expression of SHH in benign and malignant SGTs and correlate its expression with proliferation of tumor cells through expression of MCM4.

Material and Methods: Immunohistochemical expression of SHH and MCM4 was evaluated in 5 samples of each PA, WT, MEC and ADCC.

Results: The malignant SGTs showed the highest mean value for both SHH (25.69 ± 6.89) and MCM4 expression (27.09 ± 7.35) than benign groups, ADCC showed the higher mean value for SHH and MCM4 expressions while PA showed the lower mean value, with statistically significant positive relation between SHH and MCM4 expression in the benign and malignant SGTs and also in four SGTs studied.

Conclusion: Over expression and strong positive correlation between SHH & MCM4 in benign & malignant SGTs indicates their important role in tumorigenesis of these neoplasms. Also, indicates their crucial role in undifferentiation and progression of cancer cells by increasing their proliferation ability, which makes them promising prognostic factors and new targeted genes therapies in treatment of these tumors.

KEY WORDS, SHH, MCM4, Benign SGTs and SG carcinomas

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INTRODUCTION

Salivary gland tumors (SGTs) are considered to be a challenging field in the diagnostic pathology, with many overlapping different histopathological features. Around 80% of SGTs are benign and the parotid gland is the most common site of their presence while neoplasms in the sublingual gland are most commonly malignant (Lin et al., 2018). Pleomorphic adenoma (PA), Warthin's tumour (WT), mucoepidermoid carcinoma (MEC), and adenoid cystic carcinoma (ADCC) are the most prevalent benign and malignant salivary gland neoplasms (Amit et al., 2015).

Pleomorphic adenoma is a benign SGT which affects major and minor glands accounts for 60% of all SGTs, females are commonly affected than males and reported in all ages (Mashkoo et al., 2014). Clinically, the neoplasm is usually solitary and appears as a slowly growing firm nodular mass which is mobile unless it occurs in the palate (El-Naggar et al., 2017). Microscopically, it is characterized by diverse histological features. Their main components are the capsule which could be variable in its thickness and presence, epithelial, myoepithelial cells arranged in masses or duct-like structures, and different mesenchymal elements such as mucoid, myxoid, cartilaginous or hyalinized (Balachander et al., 2015).

Warthin's tumor is the second common benign SGT and accounts for 10-15 % of total neoplasms. Parotid gland is the exclusive anatomical site for its occurrence with apparent male predilection. Clinically, the neoplasm appears as a painless nodular firm to fluctuant swelling which grows slowly (Martinez-Madrigal et al., 2007). Warthin's tumor is composed of epithelial component as the main neoplastic element and plenty of lymphoid stroma (Raghua et al., 2014). Although both PA and WT are benign neoplasms, the biological behavior and treatment plan are completely different. High

recurrence rate and malignant transformation of PA is very common while WT is slowly growing, rarely recurs and malignant transformation never happens (Yu et al., 2016).

Malignant SGTs represent 6% of all cancers affecting head and neck region. The most common is MEC representing 30%, followed by ADCC with 23.8% of all malignant salivary gland neoplasms (El-Naggar et al., 2017). MEC occurs mainly in parotid glands (Raboh and Hakim 2015). Histologically, MEC is formed of mucous cells, intermediate cells, and epidermoid cells forming masses and duct like structures (Dossani et al., 2016).

Adenoid cystic carcinoma represents 10% of SGTs which occurs more commonly in minor salivary glands and is considered to have a worse prognosis. Pain is usually the main symptom of the tumor, due to its perineural invasion (De Berardinis et al., 2018). ADCC often appears as a slowly growing small tumor so it is usually diagnosed at an advanced stage. Distant metastasis to different organs is more common than local spread to regional lymph node (Dillon et al., 2016). It consists microscopically of islands of basaloid cells surrounded by hyalinized stroma and arranged in three histological patterns; cribriform, tubular, and solid patterns (Abbas et al., 2021).

The sonic hedgehog (Hh) pathway has essential roles in the development of embryos, cell differentiation and proliferation. Sonic hedgehog (SHH) is a ligand protein in the Hh signaling pathway, which, when released, attaches to the Patched (PTCH) receptor, lead to activation of Smoothed (SMO) Trans membrane protein, resulting in activation the Glioma associated oncogene (GLI) which is one of transcription factors family, that control cell differentiation, proliferation and interactions with the extracellular matrix (Amakye et al., 2013). So the activation of the SHH pathway is accompanied by proliferation and

undifferentiation of tumor cells so abnormal activation of the Hh pathway was reported in different tumors (Wan et al., 2014, Hinterseher et al., 2014).

The minichromosome maintenance (MCM) family is composed of 8 members; consist of MCM2-7, MCM8 and MCM10. MCM2-7 form complex which combined with DNA to initiate DNA replication (Liao et al., 2018). Other studies reported that MCM2, MCM4 and MCM7 regulate cell proliferation in non-small cell lung cancer; in addition, MCM4 and MCM7 are high sensitive proliferation markers used in diagnosis of esophageal cancer (Choy et al., 2016).

Distinguishing benign from malignant SGTs becomes more challenging by the fact that benign tumors could be multifocal or absence of capsule or both and can reoccur if not treated properly, By contrast, malignant tumors in the early stage could be well defined, and even encapsulated, so the clinical staging and the grade of the SGTs could function as a prognostic factor in management of these tumors (Paul et al., 2020). MCM 4 gene is used in this study as valuable biomarker for cancer diagnosis and prognosis. Also MCM proteins are considered as an accurate proliferative marker for tumors more than other markers, as the Ki-67 which is unable to identify cells in the early G1 phase or is down-regulated early in the differentiation program (Kikuchi et al., 2011, Juríková et al., 2016).

To our knowledge, few studies were applied to clarify the correlation between SHH and MCM4 in benign and malignant salivary gland tumors (BSGTs, MSGTs) so the current study was performed to examine the immunohistochemical expression of SHH in these tumors and correlate its expression with proliferation of tumor cells by expression of MCM4, suggesting that Hh pathway inhibitor could be a novel targeted gene therapy of SGTs through inhibition of proliferation of tumor cells.

MATERIALS AND METHODS

In the current study, 5 samples of (PA, WT, ADCC and MEC) were selected. All cases were obtained from the archive of Oral Pathology Department, National Cancer Institute, Cairo University. Briefly, Immunohistochemical staining was done as follows: wax blocks were cut at four micrometer thickness. Tissue sections were deparaffinized with xylol and rehydrated in grading concentration of alcohol. Tissue sections were placed in citrate buffer before the immunostaining steps. Peroxidase-anti-peroxidase process utilizing the biotin-streptavidin system was done, 3% hydrogen peroxide was added to the tissue sections to prevent endogenous peroxidase action. The primary antibodies, SHH (Abcam, UK) and MCM 4 (Lab Vision, Fermont CA, USA) were applied and then incubated overnight at room temperature. After washing in phosphate buffer saline (PBS), the link antibody was applied, then streptavidin labeling antibody. After rinsing with PBS, diaminobenzidine chromogen was added to the tissue sections then the counterstain. Tissue sections were dehydrated in grading concentration of alcohol, applied in xylol and mounted. All the steps of immunohistochemical quantitative estimation were carried out on photomicrographs captured at a magnification of X40. The images are captured with a camera linked to the microscope and then the images taken are analyzed with the image software (Image J, 1.41a, NIH, USA).

Data was analyzed using Statistical Package for Social Science software computer program version 26 (SPSS, Inc., Chicago, IL, USA). Data were presented as mean and standard deviation. Student's t-test (unpaired) was used for comparing quantitative parametric data of two different groups while one way analysis of variance (ANOVA) and post-hoc tukey were used to compare quantitative parametric data of more than two different groups. Pearson's correlation coefficient was used to correlate SHH & MCM4 expressions in SGTs. The results were considered statistically significant when P- value less than 0.05.

RESULTS

1- Immunohistochemical Results

A. *Sonic Hedgehog*:

All 5 cases of PA, WT, MEC and ADCC demonstrated positive SHH immunoreactivity. The immune reaction was cytoplasmic in epithelial cells forming masses and lining duct like structures in PA, inner columnar and outer cuboidal epithelial cells of cystic cavities in WT. The immune reaction was cytoplasmic and membranous in epidermoid cells of MEC and epithelial tumor cells of ADCC. (Fig.1. A, B, C, D).

B. *MCM4*:

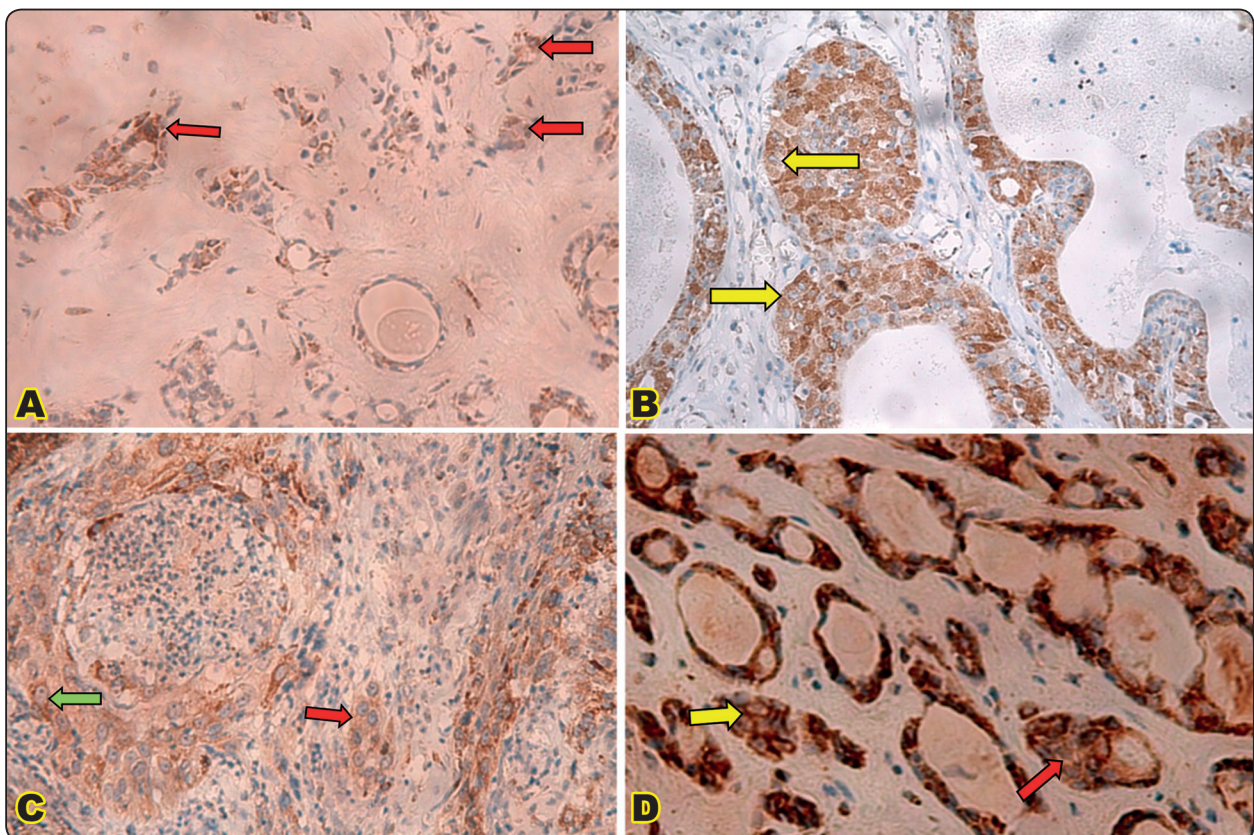
All 5 cases of PA, WT, MEC and ADCC demonstrated positive MCM4 immunoreactivity. The immune reaction was membranous, cytoplasmic and nuclear in ADCC and PA epithelial masses and duct like structures while some cells of PA showed

only cytoplasmic reaction. The immune reaction was cytoplasmic, membranous and nuclear in most of epidermoid cells of MEC while some cells showed cytoplasmic and membranous expression. WT showed only positive cytoplasmic and membranous immune expression in inner columnar and outer cuboidal epithelial cells of cystic cavities. (Fig. 1. E, F, G, H)

2- Statistical results:

A) *Comparison between Benign & Malignant salivary gland tumors.*

The Student's t-test (Unpaired) test revealed that BSGTs expression for SHH and MCM4, was significantly different from that of MSGTs (P-value ≤ 0.05), the malignant tumors showed higher mean value for both SHH (25.69 ± 6.89) and MCM4 expression (27.09 ± 7.35) in comparison with benign group (table1, Fig.2)



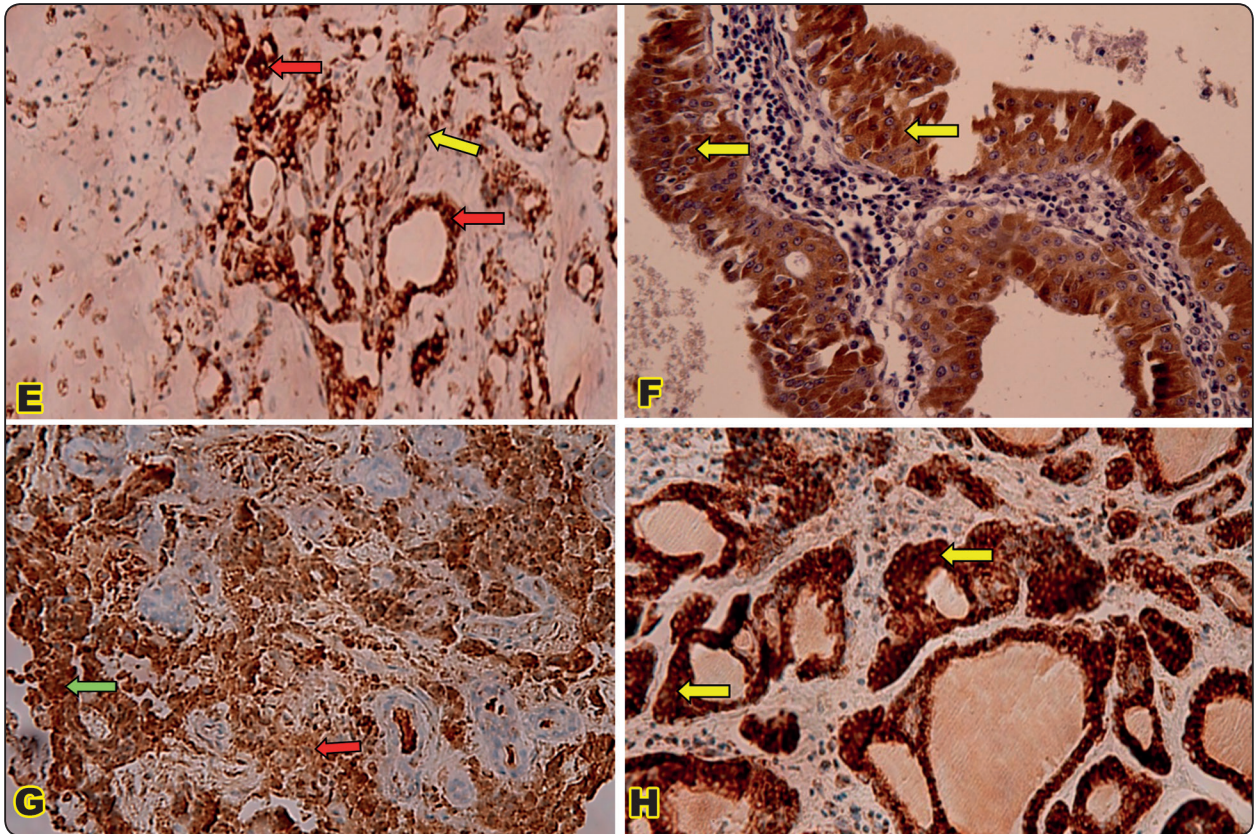


Fig. (1): Photomicrographs of immunohistochemical results of SHH (A, B, C, D) and MCM4 (E, F, G, H) in PA, WT, MEC and ADCC. A: showing the cytoplasmic expression of SHH in epithelial masses and cells lining the duct like structures (red arrows), B: Showing the cytoplasmic reaction of SHH in epithelial cells of WT (yellow arrows), C: Showing cytoplasmic reaction (red arrow) and membranous reaction of SHH (green arrow) in epidermoid cells of MEC, D: Showing cytoplasmic reaction (red arrow) and membranous reaction (yellow arrow) of SHH in epithelial cells of ADCC (Orig. Mag. X40). E: Showing cytoplasmic, membranous and nuclear expression of MCM4 in all of the epithelial cells forming masses and lining duct like structures (red arrows) while some cells showing cytoplasmic expression only of MCM4 in PA (yellow arrow) (Orig. Mag. X20). F: showing the cytoplasmic and membranous reaction of MCM4 in both inner columnar and outer cuboidal epithelial cells of cystic cavities of WT (yellow arrows) (Orig. Mag. X40). G: Showing cytoplasmic, membranous and nuclear MCM4 expression in most of epidermoid cells (green arrow) while some cells showing cytoplasmic and membranous expression in MEC (red arrow) (Orig. Mag. X 40). H: Showing cytoplasmic, membranous and nuclear MCM4 expression in the epithelial cells of ADCC (yellow arrows) (Orig. Mag. X40).

TABLE (1): Comparison of SHH &MCM4 between benign & malignant salivary gland tumors.

	Benign tumors	Malignant tumors	P
SHH in SGTs	14.76±3.94	25.69±6.89	<0.001*
MCM4 in SGTs	16.30±4.13	27.09±7.35	<0.001*

Data expressed as mean±SD, *: significance ≤ 0.05
 Test used: Student's t-test (Unpaired)

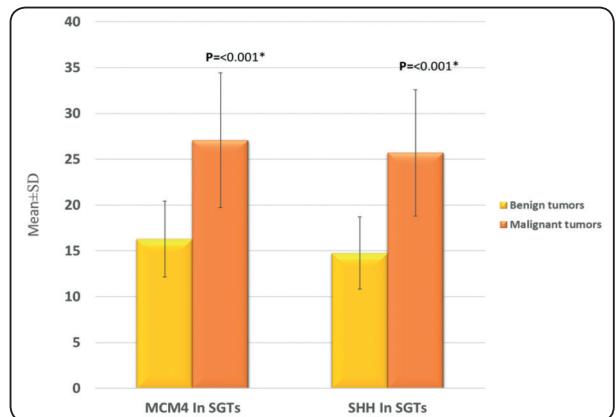


Fig. (2): A bar chart showing the mean values of SHH & MCM4 expression in benign & malignant salivary gland tumors.

B) Comparison between different salivary gland tumors

One way ANOVA test followed by post-hoc Tukey revealed that there was a significant difference in the expression of SHH and MCM4 in the four tumors studied (PA, WT, MEC, ADCC) (P-value ≤ 0.05), ADCC showed the highest mean value for SHH and MCM4 expressions (31.92 \pm 2.83), (33.58 \pm 3.23) while PA showed the least mean value for SHH and MCM4 expressions among them

(12.58 \pm 3.56), (14.14 \pm 3.66), respectively (table2, Fig. 3, 4)

C) Correlation between SHH and MCM4 expression

A statistically significant positive relation between SHH and MCM4 expression (P-value ≤ 0.05) was observed in the benign and malignant SGTs (Table 3, Fig.5) and also was observed in four salivary gland tumors studied (PA, WT, MEC and ADCC.) (P-value ≤ 0.05) (Table 3, Fig. 6)

TABLE (2): Comparison of SHH &MCM4 in different salivary gland tumors.

	PA	WT	MEC	ADCC	P
SHH In SGTs	12.58 \pm 3.56 ^A	16.95 \pm 3.10 ^B	19.46 \pm 2.43 ^B	31.92 \pm 2.83 ^C	<0.001*
MCM4 In SGTs	14.14 \pm 3.66 ^A	18.46 \pm 3.50 ^B	20.60 \pm 3.14 ^B	33.58 \pm 3.23 ^C	<0.001*

Data expressed as mean \pm SD, *: significance ≤ 0.05

Different letters indicate significance in means

Test used: One way ANOVA followed by post-hoc Tukey

TABLE (3): Pearson's correlation coefficient for SHH& MCM4 in different salivary gland tumors.

	PA		WT		MEC		ADCC		All cases	
	r	P	r	P	r	P	r	P	r	P
SHH vs MCM4 In SGTs	.99	<0.001*	.99	<0.001*	.99	<0.001*	.96	<0.001*	.99	<0.001*

r: Pearson's Correlation*: significance ≤ 0.05

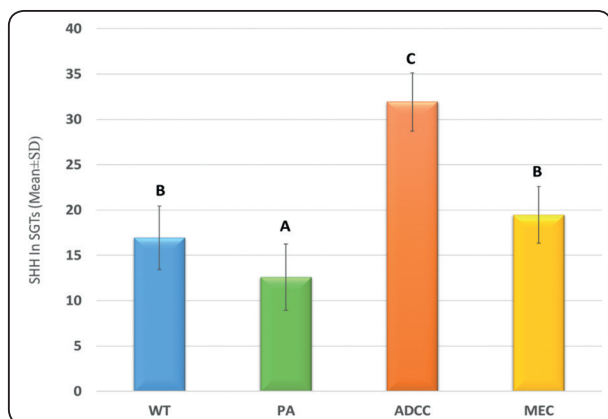


Fig. (3): A bar chart showing the mean values of SHH expression in different salivary gland tumors.

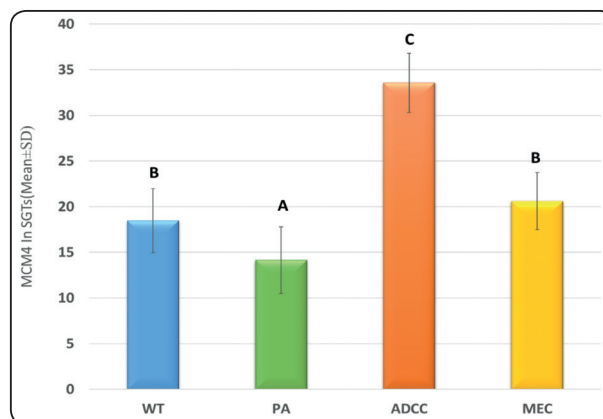


Fig. (4): A bar chart showing the mean values of MCM4 expression in different salivary gland tumors.

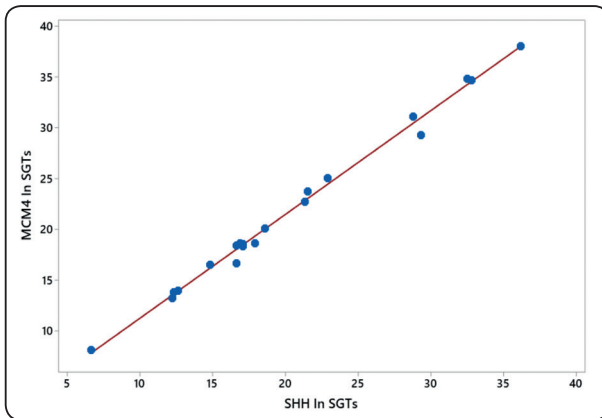


Fig. (5): Scatter diagram representing the correlation between SHH and MCM4 in benign & malignant salivary gland tumors.

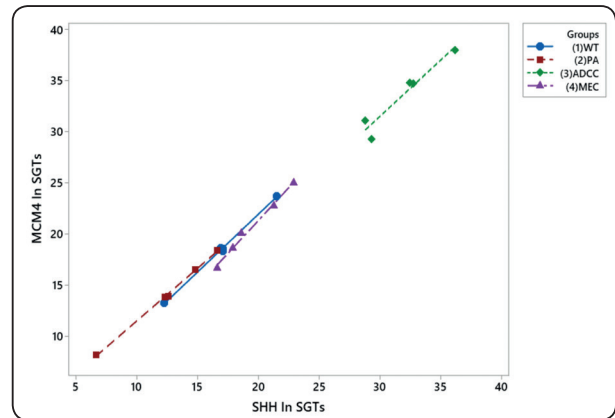


Fig. (6): Scatter diagram representing the correlation between SHH and MCM4 expression in different salivary gland tumors.

DISCUSSION

Salivary gland neoplasms represent a heterogeneous group of tumors with a huge spectrum of biologic characteristics and histopathological overlap. Benign SGTs represent the majority of SGTs and usually found in the parotid gland while malignant neoplasms usually occur in the sublingual gland (Lin et al., 2018). PA, WT, MEC and ADCC are the most common neoplasms originating from the salivary glands (Amit et al., 2015). In many circumstances, a definitive diagnosis might be so difficult without immunohistochemistry (Griffith et al., 2017). So this study was conducted to evaluate the immunohistochemical expression of SHH in benign and malignant SGTs and correlate its expression with proliferation of neoplastic cells by expression of MCM4, also to predict their prognostic value in these tumors and using them as targeted genes therapies in the treatment of these lesions.

Concerning the SHH, the MSGTs showed higher mean value for SHH expression (25.69 ± 6.89) than BSGTs. The expression of SHH in the PA, WT, MEC, ADCC was significantly different (P -value ≤ 0.05), ADCC showed the highest mean value of SHH expression (31.92 ± 2.83) while PA showed the least mean value of SHH (12.58 ± 3.56). All cases of

PA, WT, MEC and ADCC demonstrated positive SHH immunoreactivity. The immunopositivity was cytoplasmic in epithelial cells forming masses and lining duct like structures in PA, inner columnar and outer cuboidal epithelial cells of cystic cavities in WT, while epidermoid cells of MEC and epithelial tumor cells of ADCC showed cytoplasmic and membranous SHH immunoreactivity. These findings were similar to those of Vidal et al. who reported cytoplasmic SHH expression in the masses and strands of epithelium in PA, epidermoid masses in MEC and epithelial cells of cylindroma (2016). Also results of Zedan demonstrated cytoplasmic and membranous immunohistochemical expression of Patched (PTCH) which act as a receptor for SHH in PA, WT, MEC and ADCC (2016), in which SHH ligand protein binds to PTCH receptor in the Hh signaling pathway, inactivating its tumor suppressor function (Vidal et al., 2016). The membranous expression of SHH could be explained by the fact that the whole SHH molecule is divided in the cytoplasm into N- and C- parts, while the C-terminal end is freely released, the N-terminal part is adjusted by lipid hydrophobic alterations and kept in the cell membrane (Noman et al., 2016).

In our results, expression of SHH in MGSTs was higher than BSGTs, this was in accordance with

the study conducted by Wang and his colleagues, who reported that SHH expression was absent in normal gastric mucosa and its expression gradually increased from intestinal metaplasia, gastritis to some neoplastic conditions (2006). In addition, a previous study performed by Vidal et al., showed significant high parenchymal expression of components of Hh pathway in ADCC and MEC cases when comparing them with their expression in the stroma (2016), and SHH ligand released by neoplastic epithelium might induce the activation of the Hh pathway in mesenchymal component of the neoplasm, resulting into a more favorable stroma for tumor development (Yauch et al, 2008, Damhofer et al., 2013). Taking this into consideration, it is suggested that members of Hh pathway act in the epithelial mesenchymal transition in salivary gland neoplasms (Vidal et al., 2016).

High Shh expression might be explained by activation of GLI1 which is the major inducer of Hh pathway (Merchant, 2012, Yan et al., 2103, Che et al., 2012), resulting in activation of SHH (positive feedback loop) (Didiasova et al., 2018), this was reported in thyroid neoplasms (Xu et al., 2012) and in oral squamous cell carcinoma (Wang et al., 2012). In our study, the high expression of SHH in ADCC and low expression in PA might be due to high expression of GLI1 in ADCC which triggered hedgehog interacting protein (HHIP) while HHIP immunoreactivity in the PA cases was low (Vidal et al., 2016).

Aberrant mechanisms of Hh pathway activation was reported in many tumors (Im et al., 2013, Wan et al., 2014, Yang et al., 2012, Hao et al., 2013, Hinterseher et al. 2014, Gurgel et al., 2014) indicating that the Hh signaling pathway could play a role in the existence, tumorigenesis and preservation of the cytological form of SGTs as SHH and GLI1 expression was also seen in areas of typically normal salivary gland tissues (Vidal et al., 2016).

In addition, a study conducted by Yilmaz and Demirkan showed that upregulation of SHH pathway was accompanied by the progression, invasion and poor prognosis (2021) of various types of cancers including retinoblastoma, breast, colorectal and lung cancer (Im et al., 2013, Choe et al., 2015, Al Ghamdi et al., 2015, Bai et al., 2013, Ding et al., 2012)

On the contrary, the Hh pathway could be activated independently without the ligand, which occurs by inductive signaling, down regulation of SMO and is recognized as either the alternative pathway or the SMO-independent pathway. The possible causes for this ligand-independent activation could be irreversible genetic alteration, cytological expression of components of the pathway, and interactions with other independent inductive signaling (Blotta et al., 2012).

In contrast to our result Lee et al., reported that the expression of Hh pathway was high in benign neoplasms as in gastric adenomas (2007) and intestinal adenomas (Oniscu et al., 2004), these results might indicate that the Hh pathway could play a role in the early stages of tumorigenesis (Zedan, 2016). So we recommended further investigations to understand the mechanism by which the Hh pathway induce the development and advancement of SGTs as there is a controversy of its expression in these tumors until now.

MCM protein family members MCM2 to MCM7 have similar molecular structures and biological functions (Nowińska and Dzięgiel, 2010), they function in a dependant manner with each other at the start of DNA formation and produce a stable hetrohexamer with DNA helicase activity which function in the DNA replication of cells (Forsburg, 2004). They also act to initiate and elongate DNA replication and advancement of cell cycle. As it was proved that MCM proteins have a central role in organization of cell- cycle (Alison et al., 2002; Forsburg, 2004), we suggest that MCM4 protein

could be used as a proliferative and diagnostic marker in the most prevalent benign and malignant SGTs.

Regarding MCM4, the MSGTs showed the highest mean value for MCM4 expression (27.09 ± 7.35) than BSGTs. The expression of MCM4 in the PA, WT, MEC and cylindroma was significantly different (P -value ≤ 0.05), ADCC showed the highest mean value of MCM4 expression (33.58 ± 3.23) while PA showed the lowest mean value for MCM4 (14.14 ± 3.66). All cases of PA, WT, MEC and ADCC demonstrated positive MCM4 immunoreactivity. ADCC and PA epithelial masses and duct like structures showed membranous, cytoplasmic and nuclear immunopositivity while some cells of PA showed only cytoplasmic reaction. Most of epidermoid cells of MEC showed cytoplasmic, membranous and nuclear immunoreactivity while some cells showed cytoplasmic and membranous expression. WT showed only positive cytoplasmic and membranous immune expression in inner columnar and outer cuboidal epithelial cells of cystic cavities.

Our results were also similar to those of Arafa et al., who reported MCM-3 expression in epithelial cells arranged in sheets, islands and duct-like structures of PA and epidermoid cells of MEC (2019). In addition, the results of Ashkavandi et al., showed nuclear MCM3 immunoreactivity in the epithelium of ADCC, MEC and PA (2013) and MCM3 expression in cylindroma and MEC was significantly high when compared with PA (2013).

Nuclear and cytoplasmic MCM4 expression in the SGTs may be explained by the fact that most of the MCM proteins segregate from the chromatin in the S phase of the cell cycle with only small amount remain attached to regions of unreplicated DNA. Subsequently, during G2/M phase, MCM proteins could not be seen attached to chromatin and are observed mainly in the cytoplasm where they later become degraded by enzymes (Labib et al., 2001). This finding was also observed by Kodani et al.

(2003), Chatrath et al. (2003), Torres-Rendon et al.(2009) and Vargas et al.(2008).

In the present study, WT showed only positive cytoplasmic and membranous immune expression in inner columnar and outer cuboidal epithelial cells of cystic cavities. This could be explained by the hypothesis stated by Abdalla et al. that the different staining pattern depends on the cell cycle as they reported that un dividing cells had no stain in the nuclei, while premitotic budded cells (single nucleus) represented with stain in their cytoplasm. In contrast, postmitotic large budded cells (two divided nuclei) had either stain in their nuclei or cytoplasm (2015).

In our results, expression of MCM4 in MGSTs was higher than BSGTs, this may be explained by the argument stating that the expression level of MCM proteins is related to the periodicity of the cell cycle, and they are significantly highly expressed in the G1 and S phases in rapidly dividing cells (Tachibana et al., 2005) and gradually decreased, and may even be undetectable, during the G0 phase, in quiescent, aging or differentiated cells (Ritzi and Knippers, 2000). This in accordance with the research done by Gambichler et al., who found that MCM4 could be a valuable marker for differentiating between benign and malignant melanocytic skin lesions (2005). Previous studies showed that MCM4 is a helpful proliferation marker in diagnosing difficult cases of dysplasia (Choy et al., 2017) in which MCM4 expression increased as the lesion progress from squamous epithelium to low-grade dysplasia then high- grade dysplasia and finally into adenocarcinoma (Choy et al., 2016). Also, high expression of MCM4 was significantly associated with higher histologic grade of tumors (Huang et al., 2007) and worse prognosis in lung adenocarcinoma (Li et al, 2019).

In the present study ADCC showed the highest mean value for MCM4 (33.58 ± 3.23) than MEC, WT and PA. This could be explained by the

argument of Vargas et al., (2008) who demonstrated that cylindroma is a salivary gland neoplasm which proliferates with a higher rate than other neoplasms, with high immunopositivity of MCM2 when compared with other SGTs. Another study by Abdelrahman et al., demonstrated that WT has a slower proliferation rate compared to that of ADCC and cell proliferation is related to tumor aggressiveness and prognosis (2019). Our results showed that WT had higher expression of MCM4 than PA, this could be explained by the results of Horri et al., as they have found more Ki-67 positive cells in WT, when compared to PA (1998). Therefore, members of MCM family could be used as markers for cancer screening (Li et al., 2019) and become useful biological markers to diagnose cancer and predict its prognosis (Hua et al., 2014, Peng et al., 2016, Kwok et al., 2014, Liao et al., 2018).

Pearson's correlation showed that the correlation between SHH and MCM4 expression in benign and malignant SGTs was statistically significant positive and also in different SGTs (PA, WT, MEC and ADCC) (P-value ≤ 0.05). This result could be explained by the hypothesis that SHH mediates DNA replication initiation. In which DNA replication is induced by the attachment of origin recognition protein complexes to DNA sequences called replication origins (O'Donnell et al., 2013). This event is followed by binding of MCM2-7 to chromatin (Bell and Kaguni, 2013). In addition, SHH induces expression of pre-replicative complex of MCM2-7, licensing factors, DNA helicase genes and also promotes attachment of MCM2-7 to chromatin (Orrego et al., 2020). So SHH expression could induce cell division through activation of MCM2-7 proliferation markers.

Our study is the first one demonstrating MCM4 expression in SGTs. In conclusion, the present study suggests that MCM4 might be used to indicate the proliferation rate of SGTs and to differentiate between malignant and benign SGTs.

Further studies should be done to target MCM4 proliferation marker in most tumors for possible targeted therapies. More studies should be done to determine whether MCM4 could be a useful marker to predict the behavior of tumors, invasion and its correlation with clinical parameters.

CONCLUSION

The over expression of SHH & MCM4 and positive correlation between them in benign & malignant SGTs indicates their important role in tumorigenesis of these tumors. Also, indicates the crucial role of SHH & MCM4 in undifferentiation and progression of cancer cells by increasing their proliferation ability, which makes them promising prognostic factors and new targeted genes therapies in treatment of these tumors.

RECOMMENDATIONS

- Include clinical data in future studies and correlate this data with the expression of SHH and MCM4.
- Use large sample size and other salivary gland tumors like malignant pleomorphic adenoma, acinic cell carcinoma and epimyoeipithelial carcinoma in future studies.

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