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COMPARATIVE EVALUATION OF AQUAPORIN 1 EXPRESSION IN BENIGN AND MALIGNANT SALIVARY GLAND TUMORS: AN IMMUNOHISTOCHEMICAL STUDY

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ABSTRACT

Aquaporin 1 (AQP1) is a water channel protein located on the cell membranes of several epithelial and endothelial cells, and also significantly in tumor endothelia. Though the role of AQP1 in tumor progression has been substantiated sufficiently in past, very few studies have evaluated its immuno-histochemical expression in salivary gland tumors. The current study comparatively evaluated the AQP1 expression in both benign and malignant salivary gland tumors in human. The study included a total of 26 cases, 13 cases each from benign and malignant categories. The benign tumors included were pleomorphic adenoma (12), myoepithelioma (1) while malignant tumors included mucoepidermoid carcinoma (11), adenoid cystic carcinoma (1) and adenocarcinoma (1). The study demonstrated that AQP1 was immunolocalized to the non-luminal ductal cells in pleomorphic adenoma and adenoid cystic carcinoma. The intermediate and epidermoid cells of muco-epidermoid carcinoma showed a weak expression. Negative expression was noticed in myo-epithelioma and adenocarcinoma. The results showed no significant difference in the expression of AQP1 between benign and malignant salivary gland tumors; however, it hypothesized that AQP1 is significantly expressed by either myoepithelial cells or basal cells considering that the non-luminal region usually comprises these cells in a normal salivary gland.

Keywords: Aquaporin 1, benign salivary gland tumors, malignant salivary gland tumors, immunohistochemistry

INTRODUCTION

Tumors of salivary glands are fairly uncommon. The rare presentation and histopathological perplexity of these tumors makes the diagnosis as well as the prediction of prognosis by assessing its recurrence, malignant potential and metastasis, a challenge. Haematoxylin-eosin staining has been a standard method used for staining a tissue to arrive a diagnosis. However, immunohistochemistry (IHC) holds a considerably significant advantage in the diagnosis of complicated cases with comparatively definite accuracy.

Aquaporins (AQPs) are a group of proteins which are mainly localized in the cell membranes of several tissues in living beings, chiefly related to the function of transportation of water and solutes across the membranes (Wang *et al.*, 2015). Physiological expression of Aquaporin 1 (AQP1) has been observed in red blood cells, astrocytes, adipose cells, choroids plexus, bile duct, gall bladder, eye lens, placenta, neural and renal tissues (Hoque *et al.*, 2006; Tomita *et al.*, 2017). AQP1 has vital role

in tumor pathogenesis. Its expression in cancers of lung, colorectal, liver, brain and breast has been reported (Verkman *et al.*, 2008; Tomita *et al*., 2017). Evidences support its role in tumor cell proliferation and migration, tumor angiogenesis and tumor associated-oedema. In human, 13 members of AQP family have been identified (Wang *et al*., 2015), of which AQP1, AQP3 and AQP5 have been studied in oral squamous cell carcinoma (Ishimoto *et al*., 2012; Dony *et al*., 2019). AQP1 is expressed in vascular endothelium all through the body, except the central nervous system. Its omission decreases endothelial cell migration and limits tumor angiogenesis and growth. Tumor cells expressing this antigen have potential for metastasis and infiltration locally (Verkman *et al.,* 2008). Its marked expression has been reported in cancers of brain, lung, breast, colorectum and ovaries. Tumor cell migration and invasion was inhibited after blocking AQP1 expression in lung adeno-carcinoma and colon cancer, thereby proposing its role as prognostic and therapeutic target for lung cancer (Dajani *et al*., 2018).

The expression of AQP1 in endothelium and myoepithelial cells of normal salivary glands was confirmed in 2006 (Delporte and Steinfield, 2006). Its role in prognosis of salivary gland tumors has been reported by Tan *et al*. (2014) wherein AQP1was identified to be hypomethylated in all of the included cases of adenoid cystic carcinoma. In this study, we attempted to compare and evaluate the expression of AQP1 immunohistochemically in both benign and malignant salivary gland tumors.

MATERIALS AND METHODS

Tissue specimens

A total of 26 formalin-fixed paraffin-embedded archival tissue blocks of histologically diagnosed benign (13) and malignant (13) salivary gland tumors were retrieved from the Department of Oral Pathology & Microbiology, Dr. D.Y. Patil Dental College & Hospital, Pune (India). Paraffin embedded tissue blocks were cut into 4 μm sections and subjected to haematoxylin and eosin staining followed by immunohistochemical staining using the immunohistochemistry kit (Dako Bio-technology) for AQP antibody (monoclonal rabbit antibody, Biorbyt Ltd., Cambridge, UK; dilution 1: 50-100).

Immunohistochemistry

The tissue sections were deparaffinized and rehydrated in a graded series of alcohol. Endogenous peroxidase activity was blocked with 0.3% H_2O_2 (prepared in PBS) for 30 min. The sections were subjected to micro-wave irradiation 3 times for 5 min each in citrate phosphate buffer (pH, 6.0) for antigen retrieval. The sections were then incubated with protein block serum-free medium (0.25% casein in PBS) for 10 min to block non-specific binding. AQP1 antibody was applied as primary antibody and incubated at 4^{o}C for overnight. After washing with phosphate buffered saline, the enzyme-conjugated secondary antibodies were applied to the sections; which were then incubated for 1 h at room temperature. Primary antibody was visualized with diaminobenzidine. Sections were counterstained with Harris's hematoxylin, dehydrated and mount using DPX. Then the slides were examined under a light microscope for immunostaining, staining results and IHC grading.

Grading system of stained slides

The stained slides were interpreted as described by Soini *et al.* (2001).The scoring was based on :a) the immunostaining intensity in the lining epithelial cells (nucleus/cytoplasm/cell membrane/all); where 0 = absent, 1 = weak, 2 = moderate, 3 = strong, and 4 = very strong; b) the percentage of positive cells where 0 = 0% positive cells, 1 = < 25% positive cells, 2 = 25-50% positive cells, 3 = 50-75% positive cells, and 4 = >75% positive cells. The final immunostaining score was the sum of a) + b). The final scores ranged from 0 to 8 where 0 = absent, 1-4 = weak, and 5-8 = strong. The positive immunohistochemical expression present showed brown colour. To check the inter-observer variability, the slides were assessed by 2 observers, followed kappa analysis.

Statistical analysis

The pattern of expression of AQP1 between benign and malignant salivary gland tumors was assessed by using Fischer's exact test.

RESULTS AND DISCUSSION

The present study included 26 cases, 13 each of benign and malignant salivary gland tumors. Benign tumors included were pleomorphic adenoma (12/13; 92.3%) and myoepithelioma (1/13; 7.69%) myoepithelioma. Malignant tumors included mucoepidermoid carcinomas (11/13; 84.61%), adenoid cystic carcinoma (1/13; 7.69%) and adenocarcinoma (1/13; 7.69%). The slides were assessed for the expression of AQP1 as per the grading system proposed by Soini *et al*. (2001). A statistically insignificant difference was noticed in AQP1 immunoreactivity with respect to the grade in each tumor type. Nine (69.23%) of the thirteen benign cases showed strong expression, three (23.07%) cases showed weak expression and one (7.69%) case depicted negative expression. In case of malignant tumors, five (38.46%) cases showed strong expression, six (46.15%) cases showed weak expression and the remaining two (15.38%) cases showed negative expression (Fig. 1).

AQP1 expression in pleomorphic adenoma

The expression of AQP1 was considerably strong in all the 12 cases (100%) of pleomorphic adenoma with 50 to75% cells positive. In 8 out of 12 cases (66.6%) the expression of AQP1 was observed mainly in the non-luminal cells (Fig. 2). Plasmacytoid myoepithelial cells showed positivity for AQP1 in 1 out of 12 cases (8.3%).

AQP1 expression in myoepithelioma

In myoepithelioma, > 25% cells revealed immuno-positive reaction for AQP1 but the intensity of. staining was weak. Overall myoepithelioma expressed mild immuno-reactivity to AQP1.

AQP1 expression in adenoid cystic carcinoma

Out of the 3 cases of ACC, 2 were cribriform variant and 1 was tubular variant. Moderate expression of AQP1 was noted in the periphery of tumor islands in both the variants (Fig. 3).

AQP1 expression in mucoepidermoid carcinoma

There were 9 cases of low grade mucoepidermoid carcinoma. The epidermoid and intermediate cells in the mucoepidermoid carcinoma revealed an expression for AQP1. The intensity of staining was mild and the percentage of positive cells was about 25-50%. No immunoreactivity to AQP1 was observed in mucous cells. (Fig. 4).

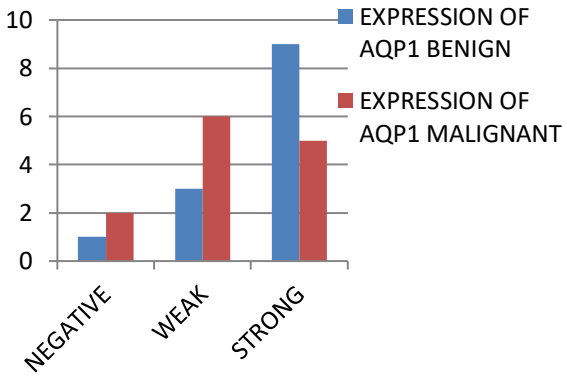

Fig. 1: Intensity of expression of Aquaporin 1 in benign and malignant salivary gland tumors

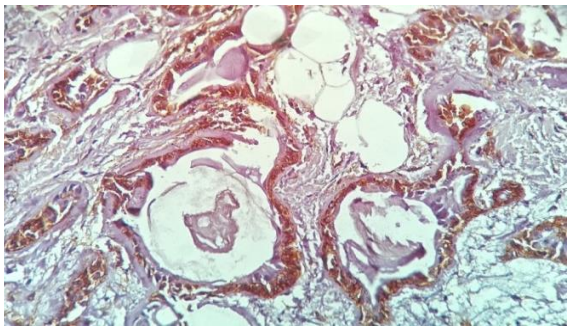

Fig. 2: A photomicrograph showing strong expression of Aquaporin 1 in non-luminal cells (cell membrane and cytoplasm) of duct in lesional tissue of pleomorphic adenoma (Total magnification X100; IHC staining)

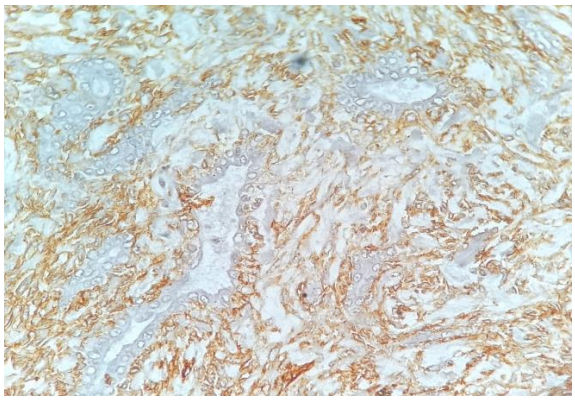

Fig. 3: A photomicrograph showing moderate expression of Aquaporin 1 in non-luminal cells (cell membrane and cytoplasm) in the ducts of adenoid cystic carcinoma (total magnification X100; IHC staining)

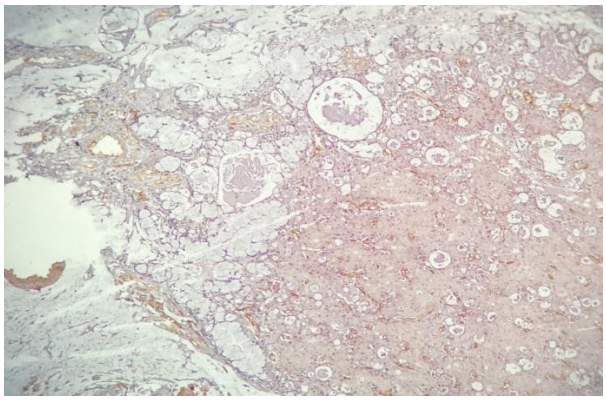

Fig. 4: Photomicrograph showing weak expression of Aquaporin 1 by epidermoid and intermediate cells (cell membrane and cytoplasm) in mucoepidermoid carcinoma (total magnification X100; IHC staining)

AQP1 expression in adenocarcinoma

No expression of AQP1 was noticed in the neoplastic cells, except for the positive reactivity in blood vessels.

The use of immunohistochemistry with regards to salivary gland started about three decades back with several proteins such as various cytokeratins (CK 7, 8, 14, 19), vimentin and desmin being employed as biological markers for their cell of origin (de Araújo *et al*., 2000). Histogenesis of these tumors have major association with the myoepithelial cells which also account to its diverse histopathology (Regezi and Batsakis, 1977). Since then, the various markers to identify these cells have been recognized such as S100, glial fibrillary acidic protein (GFAP), muscle specific actin (MSA) and calponin. Epithelial membrane antigen (EMA) and carcinoembryonic antigen (CEA) have been identified as acinar cells markers. To assess the prognosis of tumors, antibodies like Ki-67 and p53 have also been identified (Wei and Dong, 2015). AQP1, a cell membrane antigen mainly expressed in the vascular channels, have also been reported to show an immunoreactivity to salivary gland myoepithelial cells (Tan *et al.*, 2014). However, there is no substantial evidence available on its functional role. Several tumors, including lung, liver, breast and many others have been reported to express AQP1 significantly with its involvement in proliferation, migration and metastasis of tumor cells (Wang *et al.,* 2015). Based on the existing data, an attempt was made in this study to assess the immunohistochemical role of AQP1 in benign and malignant salivary gland tumors. The study comprised a total of 13 benign and 13 malignant salivary gland tumors. Our results have shown the expression of AQP1 in some benign and malignant tumors with different staining intensities. In pleomorphic adenoma, a strong positive expression of AQP1 was observed in the ductal areas predominantly the non-luminal cells and focally in some plasmacytoid cells. This probably depicts the immuno-localization of neoplastic myoepithelial cells or basal cells in the tumor tissue. These findings are in agreement with those of Delporte and Steinfield (2006), wherein AQP1 has shown similar expression in normal salivary glands too. Moreover, the myoepithelial cells present in pleomorphic adenoma are possibly differentiated neoplastic myoepithelial cells and considering that the strong expression was seen in these cells, a possibility of association of an upregulated AQP1 expression with tumor progression can be suggested.

Contrastingly, in myoepithelioma there was no expression of AQP1. Also, as the study included only one case of myoepithelioma, it was inconclusive to say about the expression in such cases hence necessitating more elaborative study with large number of cases.

In a study by Stamboni *et al.* (2020), AQP1 expression was seen only in the tumor vasculature of MEC and was absent in the tumor parenchyma which is in contrast to the current study which

showed AQP1 expression by epidermoid cells and intermediate cells in MEC, although weakly (Stamboni *et al.*, 2020). Past studies have provided evidences regarding a significant role of AQP1 in proliferation of tumors cells (Shimasaki *et al*., 2018). Nguyen *et al*. (2003) have reported a negative and weak expression of Ki-67, a cellular marker for proliferation, in low grade mucoepidermoid carcinomas and strong expression in high grade mucoepidermoid carcinomas. In present study also a weak expression in low grade mucoepidermoid carcinoma was observed. Our study sample had no cases of high grade mucoepidermoid carcinoma so it was not possible to correlate the expression of AQP1 in the same. However, our findings suggest these proteins as a probable prognostic marker in this malignant tumor.

In 2014, a cohort study investigated the role of AQP1 in salivary adenoid cystic carcinoma and reported significantly hypomethylated in the tumor unlike normal controls, thus proving its prognostic role (Tan *et al*., 2014). In present study, we noted moderate AQP1 expression in the periphery of tumor islands in both cribriform and tubular variants of adenoid cystic carcinoma. In cribriform and tubular variants, the process of duct formation by the luminal cells was induced by the peripherally located myoepithelial cells. This indicated that myoepithelial cells serve important function in histogenesis of this tumor. However, the moderate immunohistochemical expression of AQP1 observed suggested a probable role in the pathogenesis of these tumors.

In case of low grade adenocarcinoma, there was no immunoreactivity observed for AQP1. Considering that AQP1 shows immuno-specificity to myoepithelial cells in salivary glands, its negative expression can be correlated to the absence of myoepithelial cells in these tumors (Turk and Wenig, 2014). Additionally, AQP1 expression was observed in blood vessels distributed in the connective tissue stroma of all the benign as well as malignant tumors included in the study.

The results of this study revealed that the expression of AQP1 in pleomorphic adenoma is confined only to the non-luminal cells; whereas in adenoid cystic carcinoma the expression was seen surrounding the tubular structures so depict non-luminal cells. The non-luminal cells in pleomorphic adenoma and adenoid cystic carcinoma can either be basal cells or myoepithelial cells (Dardick and Burford-Mason, 1993). Therefore, the cells expressing AQP1 are localized to non-luminal region. It appears that AQP1 is perhaps expressed by either in basal or myoepithelial cells. To confirm the specificity of AQP1 expression to myoepithelial or basal cells, further studies with large sample size of benign and malignant tumors is suggested. The current study is the first of its kind to report the expression of AQP1 in tumors of salivary glands. Also, due to the scarcity of available literature, only broad categorization of these tumors into benign and malignant was considered without precise sub-categorization. Thus, the study suggests further studies to be undertaken in this direction and also to investigate the functional role of AQP1 in the pathogenesis of salivary gland tumors.

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Ethical statement: The study was approved by the Ethics Subcommittee of Dr. D.Y Patil Vidyapeeth, Pimpri Pune (India) in a meeting held on 4/10/2017. (Study approved vide letter No. DPU/R &R(D)/112(29)/18 dated 03/02/2018).

Disclosure statement: The authors have no conflicts of interest to declare.

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