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Expression Of Aquaporin 1(AQP1) In Oral Squamous Cell Carcinoma.

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ABSTRACT

Oral Squamous Cell Carcinoma (OSCC),the most common cancer of oral cavity is a multifactorial disease. Various markers have been studied to understand its pathogenesis. Aquaporins (AQPs) are water channel proteins express both physiologically and pathologically in various human tissues. The purpose of the study was to evaluate the expression of AQP1 in OSCCs. 66 OSCC tissues sections were immune his to chemically stained with AQP1 marker. Majority of the cases **(**40.90%) showed mild expression, followed by negative expression (25.75%), moderate expression (21.21%) and severe expression (12.12%). No statistical significant results were found when compared the expression of AQP1 with histological stages and lymph node metastasis. The expression of AQP1 in OSCC indicates its possible role in pathogenesis of OSCC

Keywords: Aquaporins(AQP); aquaporin 1(AQP1);immune his to chemistry; oral squamous cell carcinoma

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INTRODUCTION

Human Oral Squamous Cell Carcinoma (OSCC) is one of the major forms of oral cancer. The conventional treatment for squamous carcinoma is surgery. Despite recent advances made in the field of cancer treatment the survival rate has not made any significant changes in the past years [1]. Various molecules have been studied to understand the pathogenesis of the disease. Aquaporin 1 (AQP1) is one such molecule which was recently studied in SCC. Based on the molecular biology, newer therapeutic strategies can be developed for the better prognosis of the disease.

Aquaporins (AQPs) are water channel proteins seen in variety of human tissues[2]though most of them are distributed in epithelium, endothelium and specialized cells like erythrocytes, astrocytes, adipocytes and skeletal muscles [3].They are known for their role in transportation of small solutes such as glycerol, gas and ions. Their expression is seen mostly in plasma membrane of cells[2].

AQPs are over-expressed in tumors of diverse origins, predominantly in the tumors of aggressive nature. Current understanding of AQP involvement in cell migration and proliferation imply that AQPs play major part in tumor biology[4]. The expression of AQPs has been established in several cancers like lung, colorectal, liver, brain, breast cancers[2].

In humans, AQP family consist of thirteen types [2]. Out of them AQP3 and AQP5 have been studied in oral squamous cell carcinoma [1,5].AQP 1 is physiologically present in erythrocytes and tissues like kidney, choroids plexus, bile duct, gall bladder, eye lens, brain, and placenta[6] but not in vascular endothelial cells of central nervous system. Blockade of AQP1 expression decreases endothelial cell migration, limiting tumor angiogenesis and proliferation. AQP1 demonstrating tumor cells have increased metastatic capacity and local invasiveness[7].AQP1 is strongly expressed in many cancers such as of lungs [5], breast, brain, nasopharynx, colon, cervix, bladder, biliary duct[8-15]. AQP1 was highly expressed in lung adenocarcinoma and masking of AQP1 expression hampered tumor invasion, hence AQP1 expression was anticipated as the prognostic index and therapeutic marker for lung cancer[6].

Therefore, we hypothesized that AQP1, might also express and play an important role in the pathogenesis of OSCCs. The aim of our study is to evaluate the expression of AQP1 in OSCC by immunohistochemistry.

METHODOLOGY

Formalin-fixed, paraffin-embedded 66 tissue blocks which were clinically and histopathologically diagnosed as OSCC with and without lymph node metastasis were included in the studied. The archival tissue blocks were retrieved from the Department of Oral Pathology & Microbiology, Dr. D. Y. Patil Dental College & Hospital, Pune. The tissue sections of 4µm thickness were deparaffinised and rehydrated in a graded series of alcohol and subjected for immunohistochemical(IHC) staining.

IHC staining procedure: The sections were kept in pressure cooker at 90 degree for 10 minutes in citrate phosphate buffer (Ph-6.0) for antigen retrieval and washed with distilled water.Endogenous peroxidase activity was blocked with 0.3% H_2O_2 for 30 minutes. Aquaporin 1 antibody (polyclonal rabbit antibody, Biorbyt Ltd., Cambridge, UK; dilution 1: 50-100) was applied as primary antibody for 45 minutes at room temperature. After two washes with phosphate buffered saline, secondary antibody was applied to the sections; which was then incubated for 30 minutes at room temperature and washed with phosphate buffered saline. Diaminobenzidine was applied for 10 minutes and then the sections were counterstained with Mayer's hematoxylin, dehydrated and mounted. The slide was then observed under a light microscope for IHC grading.

Grading system of IHC stained slides

Aquaporin 1 is a cell membrane antigen. Based on the intensity of the brown stain the slide was graded as mild, moderate and severe. Two observers evaluated the slides and kappa analysis was done for inter-observer variability. Kappa analysis showed 92.5% agreement between the observers.

RESULTS

Clinical characteristics

The present study included 66 OSCC cases. 63.63% were males and 36.36% were females. The age of the patients with OSCC ranged from 30 to 80 years with the mean age 57.51. Most of the cases showed exophytic growth and clinical staging based on tumor size was T1, T2, T3 and T4 had 2, 13, 14, 37 cases respectively.Mandible (87.87%) was most commonly affected than maxilla. Both the jaws were affected in one case (1.51%). Maxilla was affected in five cases (7.57%). In both maxilla and mandible, the most commonly affected region was antero-posterior (54.54%) region. In two (3.03%) cases tongue was affected. (Table 1)

Table 1: Demographic data and patients characteristics of OSCC

Sr. no.	Patient characteristics	Number	Percentage
1	**Total (66)**		
2	**Age (years)**	57.51 (mean)	
	Range	30 -80 years	
3	**Gender**		
	Male	42	**63.63%**
	Female	24	**36.36%**
4	**Site**		
	Mandible	58	**87.87%**
	• Posterior region	26	
	• Antero posterior region	32	
	Maxilla	5	**7.57%**
	• Posterior region	2	
	• Antero posterior region	3	
	Both mandible and maxilla	1	**1.51%**
	Tongue	2	**3.03%**
5	**Clinical staging based on tumor size**		
	T1	2	**3.03%**
	T2	13	**19.69%**
	T3	14	**21.21%**
	T4	37	**56.06%**

Histopathological grading of OSCC and lymph node status

Out of 66 cases, 39 cases (59.09%) were well differentiated OSCC and 27 cases (40.90%) were moderately differentiated OSCC. Lymph node metastasis was present in 29 cases (43.93%) and 37 cases (56.06%) showed no metastasis.

Expression of AQP1 in OSCC

In the present study, AQP1 was expressed mostly as cell membrane stain in the tumor cells, with the intensity as mild, moderate and severe staining. In some cases the AQP1 was expressed even in the cytoplasm of tumor cells. There were 66 cases of OSCC, majority of cases i.e.,27 (40.90%) cases showed mild expression (Fig 1), moderate expression in 14 (21.21%) cases(Fig 2), severe expression in 8 (12.12%) cases(Fig 3) and negative expression in 17 (25.75%) cases (Fig 4).On Chi-square analysis, statistically non significant results were found in AQP1 expression in cases with lymph node metastasis and without lymph node metastasis (Chi-square value =0.3882, P=.942675, $p < .05$) (Table 2).Similarly, non significant results were found when AQP-1 expression was compared among the histopathological stages of OSCC. (Chi- square value =1.5058, P=.680923, $p < .05$) (Table 3).

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Figure 1: Photomicrograph showing mild expression of APQ1 in OSCC [Immuno his to chemistry, total magnification x 400]

Figure 2: Photomicrograph showing moderate expression of APQ1 in OSCC [Immuno his to chemistry, total magnification x 400]

Figure 3: Photomicrograph showing strong expression of APQ1 in OSCC [Immuno his to chemistry, total magnification x 400]

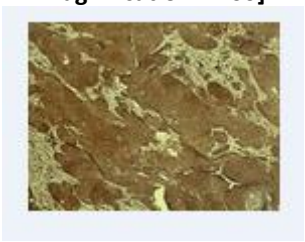

Figure 4: Photomicrograph showing negative expression of APQ1 in OSCC[Immunohistochemistry, total magnification x 400]

Table 2: Correlation of AQP1 expression with lymph node status

Intensity of stain	Lymph node Positive	Lymph node Negative	Total	
Negative	7	10	**17**	**Chi-square value= 0.3882, P=.942675, p < .05**
Mild	13	14	**27**	
Moderate	6	8	**14**	
Severe	3	5	**8**	
Total	**29**	**37**	**66**	

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Table 3: Correlation of AQP1 expression with histopathological grades of OSCC.

Intensity of stain	Well differentiated OSCC	Moderately differentiated OSCC	Total	
Negative	10	7	**17**	**Chi-square value=1.5058, P= .680923, p < .05**
Mild	14	13	**27**	
Moderate	10	4	**14**	
Severe	5	3	**8**	
Total	**39**	**27**	**66**	

DISCUSSION

The AQPs are a family of small membrane transport proteins[16] with thirteen discrete forms in humans[2]. They are organized as tetramers in cell plasma membranes and mainly serve as water-selective pores, helping osmotically driven water move across the membranes[17]. AQPs participate in various physiological roles like transepithelial fluid transport, cell migration, brain oedema, neuroexcitation, fat metabolism[17] and skin moisturization [3] which imply that regulation of AQP expression can have therapeutic associations in oedema, cancer, obesity, brain injury, glaucoma and many other conditions[18].

Aquaporinopathies are diseases that are associated to aquaporins. Nephrogenic diabetes insipidus, caused by inactivating mutations of AQP2 and neuromyelitis optica caused by the occurrence of auto antibodies against AQP4 are two known human aquaporinopathies[18].

Several studies have reported AQP expression in different human tumors including that of brain, breast, colorectal, cervical, laryngeal, lung, nasopharyngeal, ovarian, prostrate, tongue, renal, skin, thyroid, etc. Tumor cells over-express AQPs, including AQPs that are usually present in their cell of origin and also those AQPs which are not seen in the originating cell. Cell membrane and the cytoplasm of tumor cells shows the expression of AQPs mostly[19]. Hypoxia seems to be an inducer of AQP1 up regulation in tumor cells, in association with main down regulating effectors including β-catenin, FAK and the Rho family of GTPases identified for their function in tumorigenesis[3].

AQP1 was the first mammalian AQP discovered and was first observed in red blood cells and renal tubules. It was formerly named as channel-forming integral membrane protein of 28 kDa (CHIP28).AQP1 is an integral transmembrane protein with twofold functions of water and ion transport[3].

Recent studies on transgenic knockout mice deficient of these water channels demonstrated functional significance of AQPs in mammalian patho physiology and sustain its contribution in the development and progression of several cancers through AQP1-dependant tumor migration, invasion and angiogenesis[20]. Saadoun S et al in 2005 observed enhanced survival in AQP1 null mice due to less capability for angiogenesis and extensive tumor necrosis resulting from impaired endothelial cell migration in the cancer cells which was induced by subcutaneous administration of melanoma cells [21].Hu J and Verkman AS in 2006 observed that AQP1 if strongly expressed in cancer cells of mice improved the potential of tumor cell migration, invasion, and metastasis[22].

Out of 13 members of AQPs, the expressions of AQP3 and AQP5 have been studied in OSCC. Their expression in OSCC showed statistically significant results. Kusayama M et al in 2010 observed over expression of AQP3 in primary squamous cell carcinoma of oesophagus and tongue, and lymph node metastasis. Combination of pan- AQP inhibitor with an anti-cancer drug, strongly hindered the development of SCC[1]. Ishimoto S et al in 2012 observed high level of expression of AQP3 and AQP5 in OSCC of tongue. They also found that treatment with pan-AQP inhibitor inhibited cell proliferation in SCC cell lines[5]. These studies suggest the diagnostic and therapeutic role of AQP in treatment of OSCC. This has encouraged us to evaluate the association between AQP1 and OSCC since no studies are done on expression of AQP1 in OSCC.

Our study comprised of 66 cases of OSCC. There was a male predominance with the age group ranging from 30 to 80. This may be due to higher prevalence of tobacco related habits and its easy access in males. These findings were in accordance with the study by Ishimoto S et al[5].

Clinically the cases were classified based on tumor size to T1, T2, T3 and T4. Most of the cases were in T4 stage. In India, it is possibly due to lack of awareness and lack of early diagnosis and intervention of disease among the population. Most common site was gingivobuccal complex. Both the findings are in accordance with a study by MuttagiSS et al[23].

His to pathologically the cases were classified according to Broder's system of classification into well differentiated and moderately differentiated OSCC. In the present study, AQP1 was expressed mostly as cell membrane and cytoplasm stain in the tumor cells, with the intensity of stain graded as mild, moderate and severe.

Few studies showed relevant association between the intensity of AQP1 expression and tumor grade like Saadoun S et al in 2002 observed direct correlation between histological tumor grade and the severity of AQP expression in diffuse astrocytomas[9], Moon C et al in 2003observed that AQP1 was over-expressed even in the initial phase of mild dysplasia in colon cancer development[12] and Oshio K et al in 2005 found that AQP1 is highly expressed in brain tumors and showed direct relationship between the severity and grades of astrocytoma[24]. However, results from our study showed non- significant association of histological tumor grade with the severity of AQP1 expression. Out of 39 cases of well differentiated OSCC, 74.35% showed positive AQP1 expression. However, similar expression was observed in 27 cases of moderately differentiated cases of OSCC, where 74.07% showed positive AQP1 expression. In both the histological grades, majority of positively expressed cases showed mild AQP1 expression followed by moderate and severe expression.

Many authors in their studies suggested the diagnostic and prognostic value of AQP1 in tumors. Mazal PR et al established the diagnostic value of AQP1 in hepatic tumors as they observed AQP1 expression was specifically found in cholangio carcinomas while it was negative in hepatic cellular carcinoma and metastatic colorectal carcinomas. In cases where histopathology alone is not enough for an accurate diagnosis of tumor, the immune his to chemical expression of AQP1 becomes more dependable to distinguish cholangio carcinomas from hepatic cellular carcinomas and metastatic colorectal carcinomas[25]. Yoshida T et al proposed AQP1 as a sole poor prognostic element in colon cancer as they observed that AQP1 expression interrelated with the progression of colon cancer, including lymph node metastasis and lympho-vascular invasion[17]. Machida Y et alfound that the expression of AQP1 was interrelated with increased postoperative metastasis ratios and low survival rates without disease in lung adenocarcinoma cases and hence they suggested that AQP1 can be an important prognostic factor to distinguish tumor stage and histologic differentiation[26]. Zhang B et al observed that AQP1 was chiefly over-expressed in the cytoplasm of breast cancer cells and is associated with poor prognosis in such patients[27]. In our study, out of 29 cases with lymph node metastasis, 75.86% showed positive AQP1 expression and out of 37 cases without lymph node metastasis 72.97% showed positive AQP1 expression. In both the groups, majority of cases showed mild expression of AQP1 followed by moderate and severe expression. Our results were non-significant and were in contrast to the above study findings.

Several studies propose a possible role of AQP1 as an innovative therapeutic goal for the management of cancers. JiangY in 2009 observed that enforced-expression of AQP1 in colon cancer u pregulated the plasma membrane water permeability and migration potential, which could be hindered by AQP1 blockers[28].Xie et al in 2012 found that AQP1-shRNA could control lung cancer cell invasion and migration by inhibiting AQP1 function[29]. Wang et al in 2017 observed that siRNA targeting AQP1 efficiently reduced AQP1 expression, significantly decreased cell viability, migration and invasion and enhanced apoptosis of ovarian cancers cells[30]. Since the role of AQP1 is already known in cell proliferation, angiogenesis and metastasis, further studies should be carried out in different tumors to establish its diagnostic, prognostic and therapeutic utility in tumors.

CONCLUSION

The present study demonstrated the expression of AQP1 in majority of OSCC cases, however we found no statistical significant results in relation to histological grading and lymph node metastasis. Our study was the first one to demonstrate the AQP1 in OSCC. For the better understanding of the pathogenesis of AQP1

in tumor proliferation, angiogenesis and metastasis we suggest to carry out further studies on AQP1 at molecular level so that AQP1 inhibitors can be used as a new therapeutic strategy in anticancer treatment of OSCC

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REFERENCES

[1] Kusayama M, Wada K, Nagata M, Ishimoto S, Takahashi H, Yoneda M et al. Critical role of aquaporin 3 on growth of human eosophageal and oral squamous cell carcinoma. Cancer Sci 2011; 102(6):1128-1136.
[2] Wang J, Feng L, Zhu Z, Zheng M, Wang D, Chen Z et al. Aquaporins as diagnostic and therapeutic targets in cancer: How far we are? J Transl Med 2015; 13:96.
[3] Tomita Y, Dorward H, Yool AJ, Smith E, Townsend AR, Price TJ et al. Role of Aquaporin 1 Signalling in Cancer Development and Progression. Int J Mol Sci 2017; 18(2): 299.
[4] Verkman AS, Hara-Chikuma M, Papadopoulos MC. Aquaporins—new players in cancer biology. J Mol Med (Berl) 2008; 86(5): 523–529.
[5] Ishimoto S, Wada K, Usami Y, Tanaka N, Aikawa T, Okura M et al. Differential expression of aquaporin 5 and aquaporin 3 in squamous cell carcinoma and adenoid cystic carcinoma. Int J Oncol 2012; 41(1):67-75.
[6] Hoque MO, Soria JC, Woo J, Lee T, Lee J, Jang SJ et al. Aquaporin 1 Is Overexpressed in Lung Cancer and Stimulates NIH-3T3 Cell Proliferation and Anchorage-Independent Growth. American J Pathol. 2006; 168(4):1345–53.
[7] Papadopoulos MC, Saadoun S, Verkman AS.Aquaporins and cell migration. Pflugers Arch. 2008; 456(4): 693–700.
[8] Endo M, Jain RK, Witwer B, Brown D. Water channel (aquaporin 1) expression and distribution in mammary carcinomas and glioblastomas. Microvasc. Res. 1999; 58(2): 89–98.
[9] Saadoun S, Papadopoulos MC, Davies DC, Bell BA, Krishna S. Increased aquaporin 1 water channel expression in human brain tumours. Br J Cancer 2002; 87(6): 621–623.
[10] El Hindy N, Bankfalvi A, Herring A, Adamzik M, Lambertz N, Zhu Y et al. Correlation of aquaporin-1 water channel protein expression with tumor angiogenesis in human astrocytoma. Anticancer Res. 2013; 33(2):609–13.
[11] Li Q, Zhang B. Expression of aquaporin-1 in nasopharyngeal cancer tissues. J. Otolaryngol. Head Neck Surg. 2010; 39(5):511–5.
[12] Moon C, Soria JC, Jang SJ, Lee J, Hoque MO, Sibony M et al. Involvement of aquaporins in colorectal carcinogenesis. Oncogene 2003; 22(43): 6699–703.
[13] Chen R, Shi Y, Amiduo R, Tuokan T, Suzuk L. Expression and prognostic value of aquaporin 1, 3 in cervical carcinoma in women of Uygur ethnicity from Xinjiang, China. PLOS ONE 2014; 9(6): e98576.
[14] Liu J, Zhang WY, Ding DG. Expression of aquaporin 1 in bladder uroepithelial cell carcinoma and its relevance to recurrence. Asian Pac J Cancer Prev. 2015; 16(9): 3973–6.
[15] Li C, Li X, Wu L, Jiang Z. Elevated AQP1 expression is associated with unfavorable oncologic outcome in patients with hilar cholangiocarcinoma. Technol Cancer Res Treat. 2017; 16(4): 421-427.
[16] Lehnerdt GF, Bachmann HS, Adamzik M, Panic A, Koksal E, Weller P, Lang S et al. AQP1, AQP5, Bcl-2 and p16 in pharyngeal squamous cell carcinoma.J Laryngol Otol. 2015; 129(6):580-6.
[17] Yoshida T, Hojo S, Sekine S, Sawada S, Okumura T, Nagata T et al. Expression Of Aquaporin-1 Is A Poor Prognostic Factor For Stage ii And iii Colon Cancer. Molecular And Clinical Oncology 2013; 1(6): 953–958.
[18] Verkman AS, Anderson MO, Papadopoulos MC. Aquaporins: important but elusive drug targets.Nat Rev Drug Discov. 2014; 13(4):259-7.
[19] Papadopoulos MC, Saadoun S. Key roles of aquaporins in tumor biology.BiochimBiophys Acta 2015; 1848 (10 Pt B): 2576-2583.
[20] Soveral G, CasinA Aquaporin modulators: a patent review (2010-2015). Expert OpinTherPat 2017; 27(1):49-62.

[21] Saadoun S, Papadopoulos MC, Hara-Chikuma M, Verkman AS. Impairment of angiogenesis and cell migration by targeted aquaporin-1 gene disruption. Nature 2005; 434(7034):786–92.
[22] Hu J, Verkman AS. Increased migration and metastatic potential of tumor cells expressing aquaporin water channels. FASEB J. 2006; 20(11):1892–1894
[23] Muttagi SS, Patil BR, Godhi AS, Arora DK, Hallikerimath SR, Kale AD. Clinico-pathological factors affecting lymph node yield in Indian patients with locally advanced squamous cell carcinoma of mandibular Gingivo-Buccal sulcus. Indian J Cancer 2016; 53:239-43.
[24] Oshio K, Binder DK, Liang Y, Bollen A, Feuerstein B, Berger MS et al. Expression of the aquaporin-1 water channel in human glial tumors. Neurosurgery 2005; 56(2):375–81.
[25] Mazal PR, Susani M, Wrba F, Haitel A. Diagnostic significance of aquaporin-1 in liver tumors. Hum Pathol 2005; 36(11):1226–31.
[26] Machida Y, Ueda Y, Shimasaki M, Sato K, Sagawa M, Katsuda S et al. Relationship of aquaporin 1, 3, and 5 expression in lung cancer cells to cellular differentiation, invasive growth, and metastasis potential. Hum Pathol 2011; 42(5):669–78.
[27] Zhang B, Liu FF, Ma YJ, Gu F. Cytoplasmic expression of aquaporin-1 in breast cancer cells and its relationship with clinicopathological characteristics and prognosis. Zhonghua Zhong Liu Za Zhi 2013; 35(12):904–9.
[28] Jiang Y. Aquaporin-1 activity of plasma membrane affects HT20 colon cancer cell migration. IUBMB Life 2009; 61(10):1001–9.
[29] Xie Y, Wen X, Jiang Z, Fu HQ, Han H, Dai L. Aquaporin 1 and aquaporin 4 are involved in invasion of lung cancer cells. Clin Lab 2012; 58(1-2):75–80.
[30] Wang Y, Fan Y, Zheng C, Zhang X. Knockdown of AQP1 inhibits growth and invasion of human ovarian cancer cells. Mol Med Rep2017; 16(4):5499-5504.