ORIGINAL ARTICLE

IMMUNOHISTOCHEMICAL EVALUATION OF OESTROGEN RECEPTORS IN ADENOID CYSTIC CARCINOMA OF SALIVARY GLAND

Hasan Mujtaba, Muhammad Atique*, Iffat Batool, Muhammad Farooq Umer*****

Research Scholar, Institute of Cancer Research, Xi'an Jiaotong University, Xi'an-China, *Department of Pathology, Combined Military Hospital (CMH), Lahore, **Fauji Foundation Hospital Rawalpindi, ***School of Public Health, Xi'an Jiaotong University, Xi'an-China

Background: Oestrogen has a physiological role throughout the body including oral cavity. The effects are mediated by binding to two receptors in nucleus alpha and beta, which are ligand-activated transcription factors. The alpha receptors have a prognostic significance in cancer of breast while in Adenoid cystic carcinoma of salivary glands the results are inconsistent. This study was conducted to determine the oestrogen receptor Alpha staining in adenoid cystic carcinoma of salivary gland. **Methods:** Paraffin blocks of thirty cases of adenoid cystic carcinoma of salivary gland were retrieved and evaluated through immunohistochemistry by anti-oestrogen antibody clone 1D5.The intensity and proportion of nuclear staining was scored using Allred scoring system. **Results:** From total of thirty cases, 5 cases expressed as mild staining of oestrogen receptors using Allred scoring system. Three cases of cribriform and two cases from tubular pattern expressed positivity. In the case series selection of our study cohort there was no association seen in age, gender, site and histological type of tumour with the expression of oestrogen receptor. **Conclusion:** Role of oestrogen is well established in breast cancers, some of salivary gland adenoid cystic carcinoma also express these receptors and could be involved in the pathogenesis. Further studies are recommended to seek possible explanation of variable staining pattern observed in many other studies, and also to determine the possible therapeutic use of tamoxifen in such tumours.

Keywords: Adenoid cystic carcinoma of Salivary Gland; Oestrogen receptor; Immunohistochemistry

J Ayub Med Coll Abbottabad 2017;29(4):535–9

INTRODUCTION

Adenoid cystic carcinoma is a biphasic tumour of exocrine glands and is composed of epithelial and myoepithelial cells arranged in tubular, cribriform and solid pattern.[1] The tumour most commonly arises in salivary gland constituting about 10% of all the salivary gland neoplasm and about 1 percent of the head and neck malignancies.[2–4] Other uncommon sites of the tumour presentation are the seromucinous gland of upper respiratory tract, breast, lacrimal gland, ear canal, tracheobronchial tree, cervix, skin, oesophagus and prostate.[3,4] In salivary gland it is one of the most devastating disease to afflict head and neck, causing morbidity and mortality due to its tendency of local recurrence and late onset of distant metastasis.[2,3] Adenoid cystic carcinoma of salivary gland (ACC-SG) is second most common salivary gland malignancy and the tumour is deceptive to surgical management and radiotherapy used for the treatment.[2–6] The optimal treatment of ACC-SG has not been established. The choice of therapy depends upon site, stage, histological grade and biological behaviour. Surgery is the treatment of choice while radiotherapy is indicated for positive surgical margins or for non resectable tumours.[2–5] Local recurrence, perineural or bone invasion and late onset of distant metastasis are the characteristic features which lead to the frustrating treatment outcome.[2–5] Despite all the improvement in diagnostic and management methods the prognosis of ACC-SG still remains unpredictable.[2–4]

Association in the breast and salivary gland have been observed owing to the presence of tubulo-acinar structure, epithelial and myoepithelial cells while both of these tissues can share histological similar type of lesions.[7,8] Glands like salivary, mammary and prostate express oestrogen, progesterone and androgen showing the role of the sex hormones in these tissues. Moreover ACC-SG have been reported to express oestrogen receptors just like the receptor presentation in the breast and its carcinomas.[8] Oestrogen is believed to be involved in development and/or progression of cancers in breast, ovary, endometrium, colorectal, prostate and diseases like osteoporosis, neurodegenerative, cardiovascular, insulin resistance diabetes mellitus and lupus erythematosus.[9,10] The use of selective oestrogen receptors modulators (SERMs) for the management of oestrogen mediated disease is a focal point for the treatment of such type of diseases and cancers.[11–13]

Tamoxifen a SERM is used for the management of oestrogen receptor positive breast cancers since 1971.[14] In ACC-SG successful use of tamoxifen in three patients claiming stability of disease progression also advocates the use of hormone therapy in this particular cancer.[15,16] The management is based on inhibiting oestrogen stimulated growth of cancer cells by competitively blocking oestrogen receptors which are

specifically of two types, alpha and beta and it is the formerly mentioned which has established prognostic significance. The renaming of receptor was done after the discovery of second oestrogen receptor which was called as beta in 1996, however aptly alpha receptor is still denoted simply as oestrogen receptor.[11]

In ACC-SG all the previous studies to detect this receptor in ACC-SG have been controversial. This aspect led us to evaluate its expression in this particular tumour due to the prognostic significance for this relentless tumour.

MATERIAL AND METHODS

The descriptive study with convenient sampling was conducted after approval from the ethical committee and thirty cases of ACC-SG were recovered from the archives of Armed Forces Institute of Pathology, Rawalpindi. The data on gender, age and site of tumour was extracted from clinical histories in each case. Specimen had previously been fixed in 10% buffered formalin and processed for paraffin wax embedding. Freshly prepared slides from the blocks were re-examined. The tumour was classified into cribriform, tubular and solid types as suggested by Szanto *et al*. in 1984.[17] The sections were subjected to the immunohistochemical procedure by avidin-biotin-peroxidase complex method. Oestrogen receptors were detected using Monoclonal anti oestrogen receptor antibody clone 1D5 by Beckman Coulter which detects 67kDa polypeptide chain of ER alpha located at N-terminal (A/B domain). The anti-oestrogen receptor antibody was diluted as recommended (1:50). Positive controls were obtained from breast cancer known to express oestrogen receptor and the negative controls were prepared by omitting the primary antibody. Positive cases were scored on the basis of Allred scoring system commonly used to determine oestrogen receptors in breast carcinoma which is based on percentage of cell stained and intensity of nuclear staining. The percentage of cell stained is given a range from 0-5 and intensity of nuclear staining is graded from 0–3. A total score of 2 or more by summing up the percentage score and intensity score is considered positive.[18]

RESULTS

In total of 30 patients, 18 were male and 12 were female with male to female ratio of 1.5:1. The age of patients ranged from 19–75 years with the mean age of 49.2 years. The most common site of tumour presentation was maxilla making up 36.7% followed by submandibular gland 16.7%, parotid and palate represented 13.33% each, upper lip 6.66% and floor of the mouth 3.33% respectively. According to histopathological classification, cribriform pattern was observed in 19 patients, 8 patients had tubular type and 3 patients had solid adenoid cystic carcinoma. No patient with solid pattern expressed the positivity.

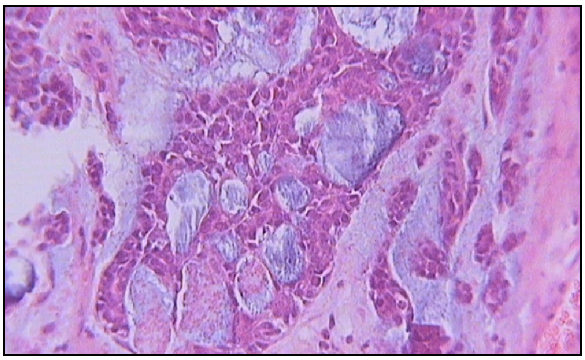

Figure 1a: H&E staining showing solid pattern. Objective magnification x 40

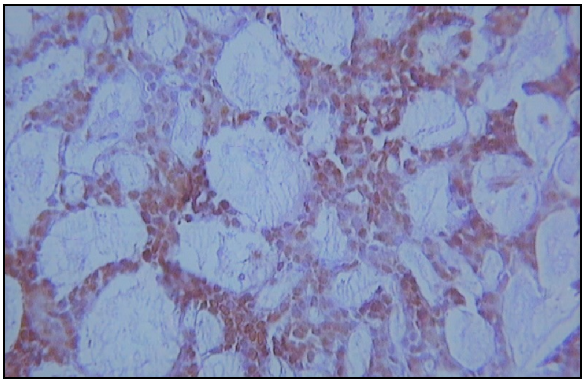

Figure-1b: Immuno-histochemical staining with ERα. Allred score of 3. Objective magnification x 40

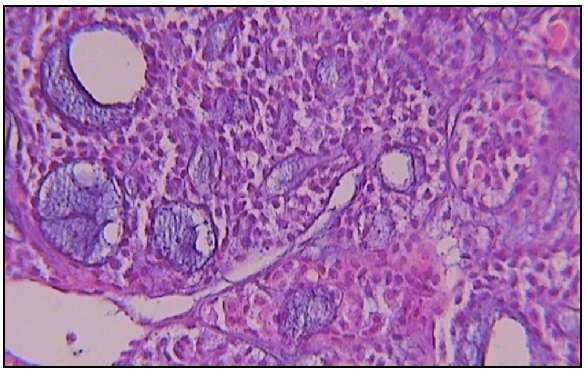

Figure-2a: H&E staining showing cribriform pattern. Objective magnification x 40

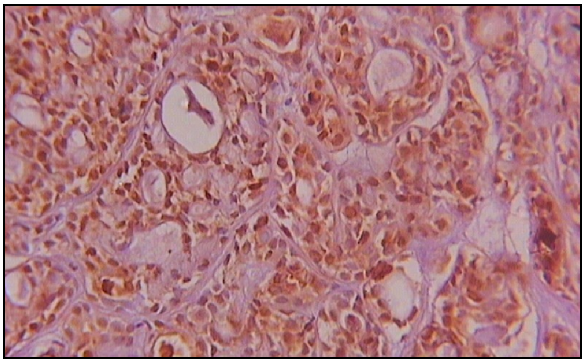

Figure-2b: Immunohistochemical staining with ERα. Allred score of 4. Objective magnification x 40

Table-1: Detail of immunohistochemistry of positive cases

Age	Gender	Site	Type	Nuclear staining of ERα (Score)
35	M	Submandibular gland	Tubular	3
54	F	Maxilla	Cribriform	3
57	M	Maxilla	Tubular	3
43	M	Maxilla	Cribriform	4
58	M	Maxilla	Cribriform	3

Table-2: Comparison of results with previous Studies

Authors	Year	No. of cases	Antibody / clone	ERα
Due *et al*.[21]	1989	8	ER-ICA/ monoclonal	0
Miller *et al.*[22]	1994	5	ER-ICA/ monoclonal	0
Shick *et al.*[23]	1995	12	ER-ICA/ monoclonal	0
Gaffney *et al*.[24]	1995	7	1D5 / Monoclonal	0
Jeannon *et al*.[25]	1999	6	6F11 /monoclonal	0
Dori *et al.*[26]	2000	27	1D5 / monoclonal	0
Nasser *et al.*[27]	2003	10	6F11 / monoclonal	0
Pires *et al.*[28]	2004	70	6F11 / monoclonal	0
Barrera *et al.*[29]	2008	47	N/S / monoclonal	8
Luo *et al*.[6]	2009	12	62A3 / polyclonal	9
Ito *et al*.[30]	2009	30	1D5 / monoclonal	0
Kolude *et al*.[31]	2013	7	N/S / monoclonal	2
Tabatabaei *et al*.[32]	2016	24	1D5 / monoclonal	0
Our study		30	1D5 / monoclonal	5

N/S= Not specified

DISCUSSION

It was the study of Dimery *et al*. in 1987 who were the pioneers to suggest oestrogen receptors in ACC-SG by using filtration assay of oestradiol titration on pulverized fresh frozen tissue and concluded that three of four tumours (75%) were oestrogen positive.[19] However, the technique was objected upon because it used homogenization of stromal components.

Ozono *et al*. in 1992 immunohistochemically demonstrated oestradiol in all 5 cases of ACC-SG suggesting hormonal involvement in the tumour pathogenesis.[20] Afterwards numerous studies have been carried out to determine the expression of oestrogen receptors in this tumour through immunohistochemistry but the results are discrepant. Table-2 summarizes the results of all the previous studies.

The study of Barrera *et al*. in 2008 showed expression of oestrogen receptor in eight out of forty-seven (17%) cases of ACC-SG.[29] This is in accordance with our study in which 5 cases (16.66%) are positive for oestrogen receptors. Luo *et al*. in 2009 used polyclonal antibody to detect oestrogen receptors but in adenoid cystic carcinoma of sinonasal tract and found that 75% of his cases expressed oestrogen receptors.[6] It is also established fact that polyclonal antibody is known to have higher sensitivity but lower specificity than the monoclonal.[31]

Kolude *et al*. in 2013 detected oestrogen receptors in 28% of cases he studied using monoclonal antibody. Although the type of antibody he used was not mentioned. The last study in 2016 by Tabatabaei *et al*. disregarded his one case of oestrogen positivity by claiming that the moderate staining observed was not meaningful in his cohort.[32]

Although the scoring system used in our study is different from all the previous studies but it should not affect the results. It is reported that Allred method has got better sensitivity and specificity as compared to conventional methods to determine the oestrogen receptors status.[33] Generally weak staining pattern was also observed by us in our samples. Other studies used different staining score proposed by Ozono *et al*. in 1992, as no visible staining 0, less than 10 percent +1, 10–50 percent +2 and more than 50 percent +3.[20] Thus Luo *et al*. observed moderate staining in 6 cases and weak in 3 cases while Kolude *et al*. observed moderate staining in his samples. In 2008 Barrera *et al*. observed +2 staining (moderate) in 5 cases and +1 (spotty) in 3 cases out of 8 positive samples.

Immunohistochemistry has become a standard method to determine the oestrogen receptor status and subsequently deciding the hormone therapy for the breast cancer.[34,35] Despite the extensive use of the antibody there are still issues around the methodology, interpretation and quantification.[36] The recommended clone of antibody to determine the status of oestrogen receptors alpha are 1D5, 6F11 and SP1 by American College of Clinical Oncology and College of American Pathologists.[37]

The intrinsic difficulty of achieving standardized testing in IHC specimen is subjected to multiple variables like fixation time, half-life of fixative, types of fixative, solutions in the processor,

temperature of processor components, oven temperature, drying time, reagents and variable interpretation of scoring of the final IHC reaction product by the pathologist are some of the factors which we have to keep into account.[38] Immunohistochemistry is useful assay to identify the cellular and tissue constituents and can also be used to determine the treatment protocol for the patients. Standardization of IHC technique is thus mandatory to avoid false results at molecular level.

It is recommended that further investigation regarding the use of tamoxifen / toremifene in ACC-SG must be carried out owing to reported successful use in recurrent and metastatic ACC-SG.[15,16] As few of ACC-SG do express oestrogen receptor and the tumour development and or progression may be dependent on oestrogen targeted gene transcription just like in the breast cancers. Moreover, tamoxifen has also oestrogen receptor independent mechanism which induces programmed cell death, growth inhibition, impairment of multidrug resistant proteins, inhibition of protein kinase C, inhibition of angiogenesis, reduction of VEGF level and regulation of essential tumour growth factor.[16]

Thus, in detail evaluation by considering and combining all these factors may lead us to better understanding as few of the ACC-SG do express oestrogen receptors and the possible role of tamoxifen cannot be ruled out. In addition, the role of oestrogen receptor beta still is under study and the possible role of tamoxifen through oestrogen receptor beta or through oestrogen independent
mechanism cannot be ruled out.

CONCLUSION

Role of oestrogen is well established in breast cancers, some of salivary gland adenoid cystic carcinoma also express these receptors and could be involved in the pathogenesis. However, some studies show inconsistent result, further studies are recommended with perhaps better technique or procedure / antibody quality to get improved results

AUTHORS' CONTRIBUTION

HM, MA: conceived, designed and did statistical analysis and editing of manuscript. IB: Data collection. MFU: Final review.

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Received: 30 April, 2017	*Revised: 9 August, 2017*	*Accepted: 17 August, 2017*

Address for Correspondence:
Hasan Mujtaba, Institute of Cancer Research, Xi'an Jiaotong University, Xi'an-China
Email: drhasanmujtaba@ymail.com