

## Original Article

### Immunohistochemical Study of HER2/neu Overexpression in Adenoid Cystic Carcinoma of Salivary Glands

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#### ABSTRACT

**Background and Objectives:** Adenoid cystic carcinoma (ACC), the most common malignant tumor of submandibular and minor salivary glands, is characterized by a high rate of local recurrence and distant hematogenous metastasis and tendency to invade peripheral nerves. In spite of radiotherapy and surgical treatment, the long-term prognosis is dismal. Today, many studies are being conducted using the immunohistochemical markers to help in the evaluation of ACC prognosis. The present investigation was performed to survey immunohistochemically HER2/neu over expression in adenoid cystic carcinoma of salivary glands.

**Materials and Methods:** The 24 existing samples of formalin-fixed paraffin embedded specimen were stained with HER2/neu markers. Tumors with moderate (2+) to strong (3+) complete membrane staining in at least 10% of the tumor cells were scored as positive for over expression.

**Results:** The overall frequency of over expression for HER2/neu was 45.8% and 11 specimen were positive for HER2/neu expression. A significant relationship was found between HER2/neu over expression and grade of ACC. No significant relationship was detected between immunostaining of HER2/neu and histologic pattern.

**Conclusion:** HER2/neu immunostaining might be reliable and useful for evaluation of ACC prognosis.

**Key words:** Adenoid Cystic Carcinoma, HER2/neu, Immunohistochemistry

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## Introduction

**A**denoid cystic carcinoma (ACC), a typical malignant epithelial tumor of salivary glands, has a rare occurrence and slow growing behavior (1). It is mostly located in minor salivary glands (31%) and comprises 5% to 10% of all salivary gland tumors. Nearly half of all intraoral ACC occurs in the palate with the peak incidence in the fifth and sixth decades (2).

In minor salivary glands, ACC occurs equally in men and women, but it is seen more frequently in women in submandibular glands (3). Perinural invasion is one of the diagnostic clues of ACC (4-7).

Patey and Thackray (8) first reported that ACC with a solid growth pattern was associated with a worse prognosis than that of the cribriform type. Perzin *et al.* (9) described a more differentiated type of ACC, the tubular form, which is associated with a favorable prognosis. ACC is categorized into three histologic subtypes: tubular, cribriform, and solid. The grading scheme that categorizes ACCs into three histologic grades is as follows: Grade 1-tubular and cribriform patterns without solid components, Grade 2-pure cribriform pattern or mixed with less than 30% of solid areas, grade 3-solid pattern in >30% of areas (10). Therefore, solid type of ACC is high grade, while the tubular and cribriform types are lower grade.

Proto-oncogens produce proteins called growth factors, which have important role for normal cells. The HER family have a major role in regulating cellular growth, differentiation, and survival (11).

HER2, a growth factor receptor that belongs to the class I (epidermal growth factor) family of tyrosine kinases, is encoded by the HER2/neu, which is placed on chromosome 17q11.2-q21 (12). Over expression of HER2/neu leads to unregulated cell growth and may drive oncogenic transformation (11). This is a frequent molecular event in multiple human cancers, including breast and ovarian cancer. Patients with cancer that over express HER2/neu are associated with unfavorable prognosis, shorter relapse time, and low survival

rate (13, 14).

In a study (15), a 100% over expression of HER2/neu in adenoid cystic carcinoma was found while Shrestha (16) reported no HER2/neu staining in ACC. These observations suggested that alterations of these protein products are associated with the acquisition of a certain malignant phenotype.

Since there are different reports of HER2/neu expression in salivary glands ACC, the purpose of the present investigation was to study the immunohistochemical overexpression of HER2/neu in salivary gland ACC to evaluate this oncoprotein expression according to tumor grading.

## Material and Methods

All patients with ACC diagnosed within 1345-1384 (30 years) were selected from the archive of Oral Pathology Department of Mashhad Faculty of Dentistry and Ghaem Hospital in Iran. The study proposal was approved by the local Ethics Committee of MUMS.

The patients were comprised of 9 men and 15 women and their mean age at surgery was 47 years. A section from specimen blocks was stained with H&E for histological evaluation and was graded according to three growth patterns: cribriform, tubular, and solid. Grading was done according to histopathology grading system done by Szanto *et al.* (10). Tumors with tubular and cribriform areas but without solid components were grade I, cribriform tumors that were either pure or mixed with less than 30% of solid areas were grade II, tumors with a predominantly solid pattern were considered grade III (10).

Representative blocks were chosen for immunostaining. Four-micrometer-thick sections were dewaxed and processed for immunohistochemistry. IHC was performed with the Hercep Test kit (k5204, Dakocytomation, Denmark) according to manufacture instructions. Known positive breast cancer cases were used as positive control, and for negative control, the primary antibody was omitted. All sections were evaluated by two independent pathologists without knowledge of

the clinical outcome of the patients. Grading of the immunohistochemical staining for HER2/neu was done as shown in Table 1 (17, 18).

Statistical analysis was performed by SPSS

version 13 software. The Kruskal-wallis test, Mann–whitney test, and Fisher exact test were used. The results were considered significant when the *P*-value was <0.05.

**Table 1:** Cell membrane staining intensity criteria for HER2/neu

Staining pattern	Score	HER2/neuprotein overexpression assessment
No staining is observed or membrane staining is observed in less than 10% of the tumor cells	0	Negative
A faint/barely perceptible membrane staining is detected in more than 10% of the tumor cells. The cells are only stained in part of their membrane	1+	Negative
A weak to moderate complete membrane staining is observed in more than 10% of the tumor cells	2+	Weakly positive
A strong complete membrane staining is observed in more than 10% of the tumor cells	3+	strongly positive

## Results

A total of 24 salivary gland ACCs were included in the study. Patients included 9 men (37.5%) and 15 women (62.5%). Female to male ratio was 1.66:1. The average age of patients was 46 years in males and 44.46 years in females.

Histologically, mix pattern was predominant (62.5%) followed by cribriform pattern (16.7%). Three cases had solid pattern (12.5%) and 2 cases (8.3%) had tubular pattern.

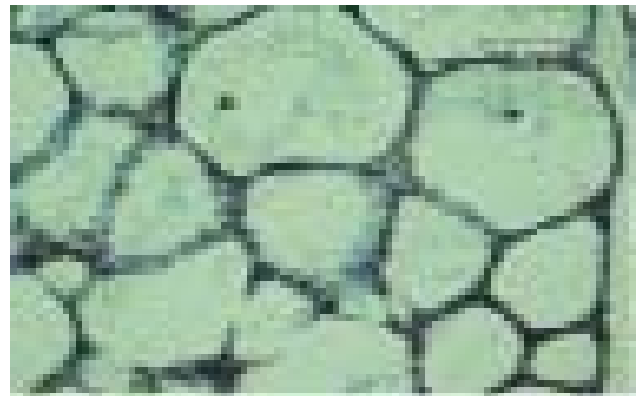
According to grading, 62.5% of the cases were grade II, 25% grade I and 12.5% were grade III.

### HER2/neu staining

There were 11 (45.8%) HER2 positive and 13 (54.2%) HER2 negative cases. Three cases were 3+(12.5%), 8 cases 2+(33.3%), 9 cases 1+(35.5%) and 4 cases were negative (16.7%).

According to histological pattern, 100% tumors with solid pattern showed positive HER2/neu over expression while 46.6% of mix pattern of ACC and 25% of cases with cribriform pattern showed HER2/

neu over expression; but there was no HER2/neu over expression in tubular pattern (Fig. 1, 2). However, the difference between groups was not significant (*P*=0.06).



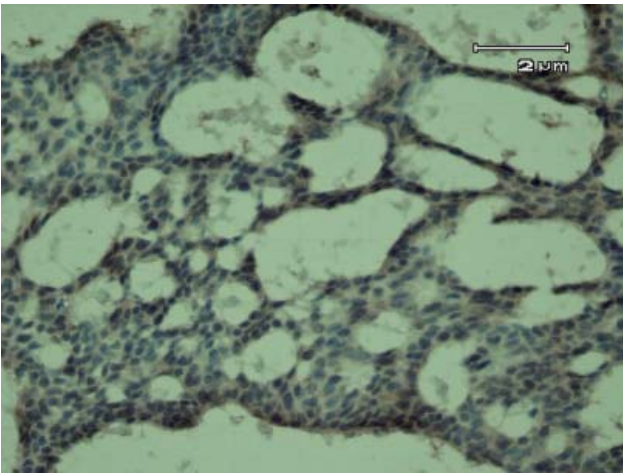
**Fig. 1:** (1+) Weak membrane immunostaining in tubular pattern for HER2/neu protein (score 1+) ( HercepTest; Dako A/S, Glostrup, Denmark) (original magnification ×400)

## Discussion

ACC is a well-known malignant epithelial tumor of salivary gland with rare occurrence (1). In minor salivary glands, this tumor occurs equally in men and women; but when found in submandibular glands; it is seen more frequently in women. (3). In this study, female to male ratio was 1.66:1. In addition, the patient's average age was 47, the same as other reports that mentioned the peak incidence was in the fifth and sixth decades (2).

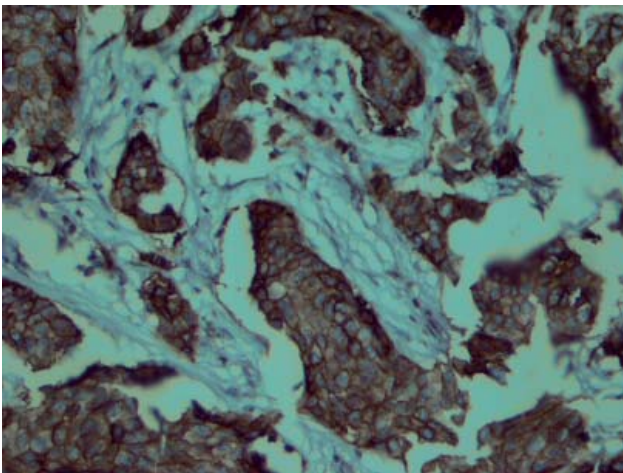
Histologically, mix pattern (62.5% of cases) was the most common pattern, despite the references indicating predominance of cribriform pattern with incidence of 50% (19).

In this study, 11 tumors (45.8%) were immunohistochemically positive for HER2/neu and 13 tumors (54.2%) were negative. In retrospect, the pattern of HER2 positivity suggests a variable frequency in ACC: 0% (16, 20, 21), 5% (22), 17% (23), over 50% (24, 25) and 100% (15, 26). This variety can be due to varying sample size, different immunohistochemistry evaluation and scoring system. For example in Shrestha study, the immunoreactivity of HER2-neu was observed as cytoplasm positive or plasma membrane positive or showing both patterns of staining (16). However, in the present study, only membranous staining was observed and the scoring system was based on membranous staining. Previous studies, have demonstrated that only tumor cell membrane reactivity is related to C-erbB-2 gene amplification (27). Scoring criteria used in other studies is summarized in Table 2.



**Fig.2:-**(2+)Moderate membrane immunostaining in mix pattern for HER2/neu protein (score 2+) ( HercepTest; Dako A/S, Glostrup, Denmark) (original magnification  $\times 400$ )

HER2/neu over expression, regarding to grading, was positive in 100% of tumors with grade III (Fig.3), while 46.6% of tumors with grade II and 16.7% of tumors with grade I showed HER2/neu over expression. The difference between grade I and III was significant ( $P=0.04$ ), although there was an increasing HER2/neu over expression with increasing of the tumor grade, the difference between other groups was not significant.



**Fig. 3:** (3+) Strong membrane immunostaining in solid pattern for HER2/neu protein (score 3+) (HercepTest; Dako A/S, Glostrup, Denmark) (original magnification  $\times 400$ )



**Table 2:** Prevalence of HER2/neu in salivary gland ACC

Studies	No. of cases	HER2/neu antibody	Scoring criteria	No.(%) of cases with positive HER2/neu staining
<b>Kernohan <i>et al.</i> (22)</b>	19	MAB NCL-CB11	Membrane staining(-) or (+)	5%:membrane and cytoplasmic staining
<b>Shrestha <i>et al.</i> (16)</b>	18	PAb (Nichirei,Tokyo)	Membrane or cytoplasm positivity	0% membrane 11%cytoplasm
<b>Sugano <i>et al.</i> (21)</b>	8	PAb (Nichirei,Tokyo)	1+:<1/3 of all tumor cells 2+:1/3-2/3 of all tumor cells 3+:>2/3 of all tumor cells	0%
<b>Karja <i>et al.</i> (24)</b>	26	MAB CB-11 (Triton Bioscience,USA)	Membrane staining (-) or (+)	57.5%
<b>Shintani <i>et al.</i> (15)</b>	16	MAB CB-11 (Triton Bioscience,USA)	-=negative staining +=0-50% positive cells ++=+50% positive cells	100% (50%:+) (50%:++)
<b>Giannoni <i>et al.</i> (25)</b>	16	MAB (oncogen science,USA)	Negative=0 Light=1 Moderate=2 Strong=3 Focal membrane staining in ≥80% of tumor cells	44%:0_1 56%:≥2
<b>Cho <i>et al.</i> (23)</b>	30	(Dako,Denmark)	Membrane staining (-) or (+)	17%
<b>Gibbons <i>et al.</i> (26)</b>	6	MAB(Oncogen Research Products,USA)	Membrane and cytoplasm 0-low 4-high	100%
<b>Present study</b>	24	(Dako,Denmark)	Negative=0 or 1+ Positive=2+ or 3+	54.1%

During the formalin fixation and paraffin embedding procedures, the HER2/neu may not survive. During fixation, loss of antigenic immunoreactivity occurs for almost all proteins, which depends on the duration of fixation. This is particularly significant in neoplasm expressing moderate levels of gene/protein product (28). With the use of antigen retrieval agents for archival, paraffin-embedded tissues, the sensitivity of some antibodies would improve (29). Only Cho *et al.* (23) have used these antigen retrieval agents for ACC, where 17% of HER2/neu positive cases were observed. In the absence of standardized laboratory

procedures, it would be hard to compare these figures with similar studies. So these differences in laboratory procedures, and differences in scoring criteria in different studies can explain variable HER2/neu over expression in ACC.

In the present study, there was a significant difference between HER2/neu overexpression in grade III and grade I, and 100% of grade III of cases showed immunoreactivity for HER2/neu. Although there was an increasing HER2/neu over expression from the lowest grade (grade I) to the highest (grade III), but the difference between grade I and II, and also between grade II and III was not significant.

This finding may indicate that there is a positive relation between ACC aggressiveness and HER2/neu over expression. This shows that administration of drugs like Herceptin in case of high grade ACCs can be beneficial.

Many studies have evaluated this protein over expression regardless of tumor grading and have not reported any HER2/neu over expression according to different grades. Maybe in these studies the samples almost included low grade ACCs. So the overall overexpression was found to be less than that reported in the present study. This shows the advantage of evaluating the HER2/neu overexpression in ACC of salivary gland according to the tumor grade. Moreover, immunohistochemistry evaluation of high grade ACCs can reveal HER2/neu over expression, which is helpful in determination of treatment protocol.

### Conclusion

The overall frequency of HER2/neu over expression was 45.8% and there was a significant relationship between the immunostaining of HER2/neu, and grade of ACC ( $P < 0.05$ ). Based on the results of study, HER2/neu immunostaining might be reliable and useful for evaluation of prognosis for adenoid cystic carcinoma of salivary gland.

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