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THE IMMUNOHISTOCHEMICHAL EXPRESSION OF HER2/NEU IN ODONTOGENIC KERATOCYST (OKC) AND DENTAL FOLLICLE

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Abstract

Introduction: Odontogenic cysts and tumors are an important category of the oral and maxillofacial pathology. Odontogenic keratocyst (OKC) is a developmental cyst with a unique biologic behavior in comparison with other cysts. This study aimed to assess HER2/neu expression in odontogenic keratocyst and dental follicles .**Materials and methods:** 13 odontogenic keratocyst paraffin blocks and 15 dental follicle samples were stained for the HER2/neu immunohistochemically. The results were analyzed statistically and P.value < 0.05 was considered significant. **Results:** Only one of the OKCs showed moderate (+2) and three cases showed weak (+1) immunopositivity for HER2/neu in the epithelial lining and in other cases of OKC and in all cases of dental follicle, staining was negative. The marker expression in OKC and dental follicles were not statistically different. **Conclusion:** Due to the negative staining of HER2/neu in most of the OKC samples, it seems that this protein does not play an essential role in the development and progression of the odontogenic keratocyst.

Keywords: Odontogenic keratocyst, Dental follicle, HER2/neu expression.

Introduction

Odontogenic cysts and tumors include an important part of the Oral and maxillofacial pathology. Although cysts are seen frequently, odontogenic tumors are unlike them rare lesions.(1) Molecular markers are widely examined in different fields of pathology, including odontogenic lesions and it seems that these markers might be helpful in predicting the biological behavior and further in treatment of these lesions.

The odontogenic keratocyst (OKC) is a cystic lesion of odontogenic origin, which is classified as a developmental cyst derived from the dental lamina that must be distinguished from other cysts of the jaw because of its different growth mechanism and biologic behavior. The aggressive biological behavior of OKC and its high recurrence rate might be associated with cell kinetics in the lining epithelium. OKC is now referred to by the World Health Organization (WHO) as Keratocystic odontogenic tumor .This lesion is slightly more common in men (1,2). OKCs are commonly (60-80%) seen in the mandible with the majority occurring in the angle of the mandible and ramus (3). Histopathologically OKC is diagnosed by a thin lining of parakeratinized stratified squamous epithelium with basal cell palisading (4). OKCs might recur after enucleation (5).

Dental follicle is a sac containing the developing tooth and its odontogenic organ and composed of fibrous tissue, remnants of odontogenic epithelium and reduced enamel epithelium (1).

Growth factors and their receptors play an important role in the growth of body's normal tissues and in the initiation and progression of neoplasms. Epithelial growth factor (EGF) is one of the growth factors with protein structure consisting of 53 amino acids involved in the growth and metabolism of many cells, is

considered a cytokine, and is involved in the regulation of proliferation of normal and neoplastic cell. (6,7)

This factor is attached to target cells by epithelial growth factor receptors (EGFR) and subsequently stimulates tyrosine phosphorylation of protein kinase cell that is in fact the first stage of mitosis. EGFR is in category of tyrosine kinase that includes 4 subgroups of HER4, HER3, HER2, and EGFR (7). Several studies have evaluated the expression of these receptors in human tumors such as breast carcinoma (8,9,10), Ovarian carcinoma(11), osteosarcoma (12), lung cancer (13), bladder carcinoma (14), and ameloblastoma (15) and the results were controversial.

HER2/neu or ErB-2 is a known proto-oncogene with a molecular weight of 185 kDa, which is placed in the long arm of human chromosome 17 that is similar to the EGFR (15). This protein initiates and promotes cell proliferation and differentiation by phosphorylation cycle of growth. Previous studies suggest that the oncogene HER2/neu is involved in pathogenesis of many malignancies in humans. In breast cancer cells, high levels of HER2/neu represents poor prognosis of the disease and is therefore used as a marker for prognosis and choosing the therapeutic approach in breast cancer.(16)

According to variable findings reported in previous studies assessing Her2/neu expression in Odontogenic keratocyst (7, 18), the aim of this study was to determine the amount of HER2/neu expression in OKC and dental follicle by Immunohistochemistry.

Materials and Methods

13 OKC paraffin blocks and 15 dental follicle samples were stained for HER2/neu immunohistochemically.

IHC staining of paraffin blocks was follows:

IHC staining was performed by standard Envision methods. After taking slices, samples were placed on slides stained with Poly-L-Lysin deployed for 24 hours at 37°C to dry. The samples were then deparaffinized in Xylene and rehydrated in varying degrees of ethanol. Consequently, in order to stop the inner peroxidase activity, samples were placed in methanol containing peroxide (H_2O_2) 0.3% for 30 minutes at room temperature and then rinsed in Phosphate buffered saline (PBS) solution PH = 7.2. Immunohistochemical staining for HER2 / neu (Dako / Denmark / monoclonal) were performed according to the manufacturer's recommendations. After the incubation with the primary antibody, Envision technique was used. Samples were incubated with Polymer solution (anti-mouse) for 30 minutes and washed with PBS. In the next stage, the 3,3 DiaminobenzidineHydrochloride (DAB) dye that gives brown color to antigen-antibody complex was used. Samples were counterstained with hematoxylin and plates were placed on them. Finally, the immunohistochemical staining status was analyzed by optical microscope by two pathologists. In this study, a breast cancer sample was used as a positive control and an odontogenic sample of keratocysts without adding the primary antibody was used as a negative control.

Evaluation of samples was performed by assessing the intensity and percentage of stained cells. Data were scored according to Dragomir's study (21) as follow:

- 0 : absence of the reaction or membrane reaction, in less than 10% of the cells
- 1+ : weak or incomplete reaction, in more than 10% of the cells
- 2+ : weak or moderate and complete reaction, in more than 10% of the cells
- 3+ : intense and complete reaction, in more than 10% of the cells

The staining results were statistically analyzed and P.value<0.05 was considered as significant.

Results

Only one of the 13 samples of OKC expressed moderate (2+) and three cases expressed weak (+1) staining of HER2/neu in epithelial lining (Figure 1) and in other cases of OKC (Figure2) and in all cases of dental follicle (Figure3), epithelial tissue staining was negative. Based on Mann-Whitney exact test, no statistically significant difference existed in two groups of OKCs and the dental follicle in terms of expression of HER2/neu (P=0/72).

Discussion

Epithelial growth factor (EGF) is considered a mitogenic factor for epithelial cells involved in regulation of the normal and neoplastic cell proliferation. This factor binds to the target cell by specific surface receptors and induces cell proliferation. Epithelial growth factor receptor (ErbB) is the cell membrane receptor of tyrosine kinase category. This category includes four groups; one of them is HER2 / neu that initiates and promotes cell proliferation and differentiation due to phosphorylation of the growth cycle. Increased expression of this receptor occurs human tumors, especially breast cancer.(2)

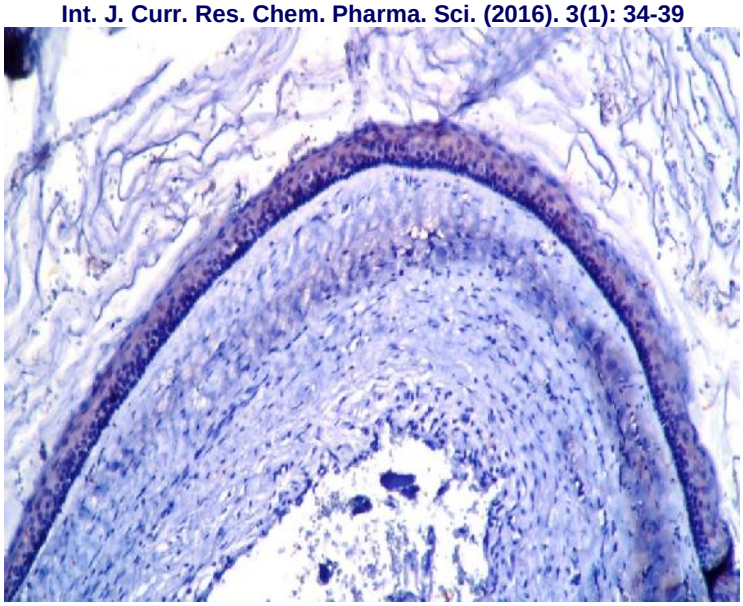

Figure 1: Weak staining of Her2/neu (1+) in OKC

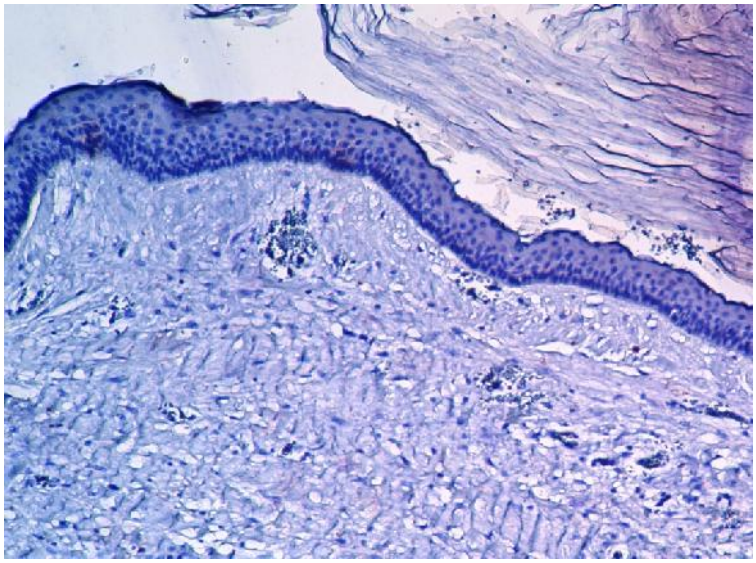

Figure 2: Negative staining of Her2/neu in OKC

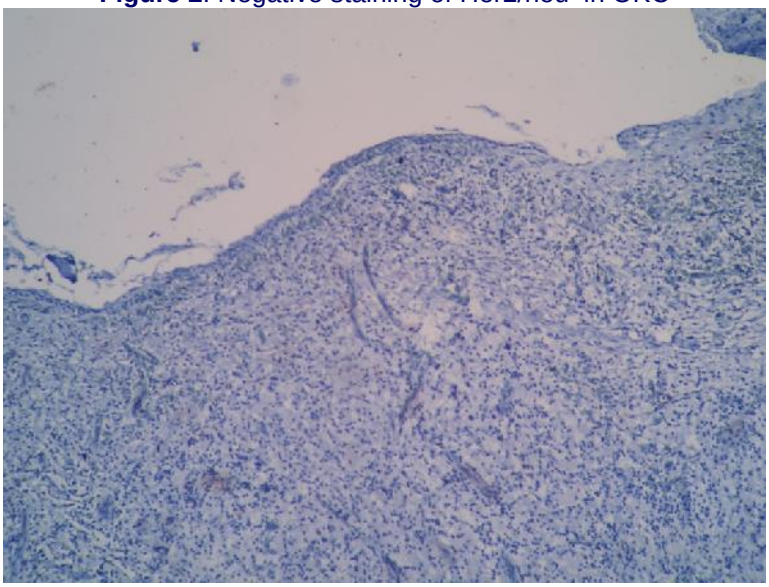

Figure 3: Dental follicle showing no reactivity for HER2/neu.

In this study, two groups of Odontogenic keratocyst and dental follicle were studied. The results of the HER2/neu in all 15 dental follicles (100%) and in 9 OKC samples (70%) were negative, and only in four OKC (30%), were positive; so the difference was not statistically significant.

The only study that investigated the expression of Her2/neu in odontogenic cysts, similar to the present study, was Monsef-Esfahani and colleagues. They reported positive expression of this marker in 87.5% of OKC cases. However, as the marker was present in 100% of radicular cyst samples, they stated that this finding reflects a high level of cell proliferation in radicular cyst epithelium and could explain the high levels of carcinomatosis changes in the peri-apical cyst and the remaining cyst. Inflammation is a major component in the radicular cyst that can lead to cell proliferation.

In the present study, inflammatory OKCs and those characterized by inflammation were excluded from the study to remove the effect of inflammatory factor on cell proliferation and it was in contrast to Monsef-Esfahani's study.(7)

In addition to odontogenic cysts, Her2/neu has been assessed in odontogenic tumors such as ameloblastoma. It is clear that the pathogenesis of tumors is different from cysts and it can be the main reason for the difference in the results of the marker expression, but according to the same odontogenic source in these lesions and the lack of studies on odontogenic cysts, studies conducted on ameloblastoma can also be discussed here.

Oikawa et al reported positive expression of this protein in dental follicle and dentigerous cyst, and in 57% of ameloblastoma cases. However, the expression was lesser than dental follicle and dentigerous cyst. Even in the comparison of unicystic and solid ameloblastomas, the unicystic type had a significantly increased expression. They finally stated that in the Her family, EGFR and HER4 are more involved in the process of proliferation and differentiation of normal and neoplastic odontogenic tissues. (15)

In our previous survey, Her2/neu was positive in just 10% of all cases of ameloblastoma and all dental follicles had negative staining. (17)

Oikawa's study used a polyclonal antibody, while in our present and previous study, the same monoclonal antibody was used. Monoclonal antibodies are generally more specific, while the likelihood of non-specific reaction is more with polyclonal antibodies.

In addition to the Her2/neu, other members of HER family including EGFR (HER1) have been studied in the odontogenic cysts. For example, in studies by Goncalves et al and De-vicente and colleagues, EGFR was positive in OKC, 94% and 73% respectively, and it was stated that this protein can be effective in creating and maintaining the lining of epithelial cysts and these findings can somehow confirm the neoplastic nature of the cyst. (18, 19)

Another member of the family, HER3, has also been considered in previous studies.

Honarmand and colleagues reported this protein in 52.4% of odontogenic keratocysts, 50% of dentigerous cyst and 20% of radicular cyst. The higher incidence of this marker in developmental odontogenic cysts compared to inflammatory cysts can represent the HER3 role in cell proliferation, invasive behavior and reccurance of the lesion. (20)

Although HER3 expression in ameloblastoma has been also reported in Okiawa's study, as mentioned earlier, this study highlights the role of EGFR and HER4 in proliferation and differentiation of normal and neoplastic odontogenic tissues. (15)

In addition to odontogenic tissues, Her2/neu has also been assessed in other oral lesions including a variety of dysplasia and squamous cell carcinoma (SCC) and conflicting results have been reported.

Dragomir and associates reported the expression of this protein in 33% of epithelial dysplasia, and 25% of oral SCC. (21)

Although this study notes the role of EGFR and Her2/neu in the early stages and progression of oral carcinoma, the study by Saifi and colleagues reported no significant difference in Her2/neu expression in normal mucosa, epithelial dysplasia and SCC and the researchers stated that it does not seem that Her2/neu expression be helpful for histopathologic differentiation between dysplastic epithelium from squamous cell carcinoma. (22)

Even salivary levels of HER2/neu was investigated to differentiate premalignant lesions from oral squamous cell carcinoma. Varun and colleagues reported higher salivary levels of HER2/neu in oral SCC compared with pre-malignant lesions, while the serum levels of this protein is not the same. Generally, this study suggests HER2/neu as a marker for distinguishing between pre-malignant and malignant lesions.(23)

Conclusion

Due to the nagetive staining of HER2/neu in most of the OKCs samples, it seems that this marker does not play an essential role in the development and progression of this cyst.

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