Immunohistochemical Analysis of ErbB2 in Odontogenic Lesions: A Pilot Study

Surendra Lakshminarayana¹, Roopa S Rao², Samudrala V Sowmya³, Dominic Augustine⁴, Shankargouda Patil⁵, Vanishri C Haragannavar⁶

ABSTRACT

Aim and objective: ErbB2 also referred to as CD340, HER2/neu belongs to the human epidermal growth factor receptor (EGFR) family. It is a cell surface receptor kinase involved in the regulation of important events like cell proliferation and differentiation. Phosphorylation of ErbB2 by their ligands causes activation of downstream signaling pathways like phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K)-Akt pathways. To evaluate the role of ErbB2 in odontogenic keratocysts (OKCs), dentigerous cyst (DC), and ameloblastoma by correlating the expression levels of ErbB2 with the biological behavior of the disease.

Materials and methods: The present study comprises 10 histopathologically diagnosed FFPE samples, of OKC, DC, and ameloblastoma among odontogenic lesions. These cases were subjected to immunohistochemical staining with ErbB2 antibody. ASCO/CAP scoring criteria 2013 was employed for interpretation. Membranous reactivity was considered positive. Mann–Whitney U test was used for statistical analysis.

Results: The ErbB2 expression levels in nine cases of OKC tissue showed significantly high expression, followed by eight cases of ameloblastoma, and five cases of a DC. All these odontogenic lesions showed positive for cell membrane staining.

Conclusion: In the present study, HER2 is highly expressed in the majority of cases of OKC, and ameloblastoma. However, further studies with a larger sample size have to be carried out along with other members of the ErbB2 family for definitive inference.

Clinical significance: Increased expression of ErbB2 indicates neoplastic transformation.

Keywords: Ameloblastoma, Dentigerous cysts, ErbB2/neu, Immunohistochemistry, Odontogenic keratocyst.

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INTRODUCTION

Odontogenic cysts are the group of cystic lesions derived from the odontogenic apparatus, which can be grouped into different categories depending on the origin as developmental and inflammatory. The commonest developmental cyst being dentigerous cyst (DC) and odontogenic keratocyst (OKC).¹

Dentigerous cyst is the most commonly occurring odontogenic cysts of the oral cavity, which accounts for 20% of developmental cysts of the jaws. It is usually associated with the crown of the tooth attached at the cementoenamel junction, supernumerary teeth, or odontomas.¹² It is formed by the accumulation of fluid between the reduced enamel epithelium and the crown of the teeth. This results in an increase in the size of the follicle beyond 3 mm. These cysts tend to transform into neoplasms. The ameloblastic transformation is more common in the DC compared to any other odontogenic cyst. There are scientific evidence for the transformation of the lining DC into ameloblastoma. There are various proliferative studies on markers of the DC and the ameloblastoma.¹ To prevent recurrence of the odontogenic cystic lesion, complete removal of the cyst with the impacted tooth is essential.² These studies have suggested that an increase in expression levels of markers/cells could be due to the perturbation in the cell cycle, mutations in the tumor suppressor genes, or the oncogenes.

Odontogenic keratocyst is considered developmental in origin. Arising from the derivative of the embryonic dental lamina. Odontogenic keratocyst accounts for 11% of all jaw cysts and it is known for its aggressiveness and high recurrence.⁴ Histologically, cyst shows uniform thin epithelial lining, consists of 8–10 cell layers of thickness. It is often parakeratinized, basal cell layer shows a characteristic palisading arrangement of epithelial cells with uniformly placed nuclei. Budding of basal cell layer into the connective tissue surrounding it and the formation of the microcysts are also seen. The fibrous cyst wall appears thinner and usually inflammatory response will be absent. Odontogenic keratocyst is found to be associated with nevoid basal cell carcinoma syndrome (NBCCS) also known as Gorlin syndrome.Histologically, OKC is unique compared to other odontogenic cysts with rare marginal transformation.³⁶
Ameloblastoma is the most commonly encountered odontogenic epithelial tumor originating within the jawbones. It occurs more in the mandible usually associated with the impacted teeth. It occurs as 1% among all odontogenic lesions, which is more common in the mandible (80%) than the maxilla (20%). In India, it accounts for 60.3% of odontogenic tumors. It is characterized as benign, painless, slow-growing, locally aggressive with a high recurrence rate. This tumor is either solid or unicystic. Histologically, a solid variant of ameloblastoma appears as follicular and plexiform patterns with or without granular cell and acanthomatous changes. The unicystic ameloblastoma appears as luminal, intraluminal, and mural variants. All these odontogenic lesions mentioned tend to be more aggressive or have high chances of recurring if left untreated or incomplete excision.

ErbB-2 is commonly known as human epidermal growth factor receptor (HER)-2, c-Neu, or ErbB-2. It has a molecular weight of 185-kDa transmembrane protein which is encoded by ErbB2 oncogene present on the chromosome 17q21-22. The extracellular ligand binds with the homodimer of HER2, the HER2 is activated by binding with desired coreceptors to form a heterodimer with HER1, HER3, or HER4. Among these heterodimers, intracellular signaling is essentially brought about by HER2-HER3. Homo or heterodimerization causes tyrosine residues autophosphorylation within the cytoplasmic domain of the receptors. The HER2 signaling is essential for the normal development of the mammary gland, glia, heart, and neurons mainly through protein kinase C (PKC), phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K), and mitogen-activated protein kinase (MAPK) pathways.

The heterodimers produced signals are stronger signals than homodimers. The HER2-HER3 heterodimer cause activation of downstream pathways mainly PI3K/Akt, which is very much essential for cell growth and survival. However, HER2 also brings about mislocalization and rapid degradation of the G1 cell cycle inhibitors like p27Kip1 protein which will progress to cell cycle progression. This HER2 also gets activated by binding with other membrane receptors like insulin-like growth factor receptors. The important transduction pathways are regulated by HER family like EGFR, HER2, HER3, and HER4. The increase in expression of the HER2 gene is predominantly seen in breast and ovarian cancers (30 and 15%, respectively). In most of the studies, HER2 overexpression is in association with aggressive behavior of the disease, but the underlying mechanism is not clear. Overexpression and amplification of HER2 oncogene are specifically seen in aggressive metastatic breast cancer. Overall prognosis in breast carcinoma is found to be poor prognosis. Similarly, various studies showed the role of HER2 in various types of human cancers. Some of the HER2–expressed human cancers are breast carcinoma, ovarian carcinoma, lung cancer, bladder carcinoma, osteosarcoma, and ameloblastoma. Increase in HER2 expression is associated with increased cellular proliferation and invasion. Therefore, in the present study, we investigated the expression levels of HER2 in odontogenic lesions like OKCs, DC, and ameloblastoma.

**Materials and Methods**

The archival FFPE tissues were retrieved from the Department of Oral Pathology and were confirmed histopathologically under H&E stain. The study group consisted of 10 each—OKC, DCs, and ameloblastoma (5 unicystic and 5 multicystic ameloblastoma) among odontogenic lesions.

**Immunohistochemical Analysis**

The streptavadin-biotin method was employed in immunohistochemistry. Four-micrometer thick tissue was mounted on a charged slide and deparaffinized using xylene and rehydration was done through graded alcohol. For antigen retrieval, sections were further incubated at 4°C using rabbit polyclonal ErbB2 primary antibody, unconjugated in liquid form. Slides were then incubated at 4°C using rabbit polyclonal ErbB2 primary antibody, unconjugated in liquid form. The secondary antibody was conjugated through incubation at 37°C for a period of 1 hour and rinsed thrice in PBS for 10 minutes. The sections were then immersed in diaminobenzidine (DAB) chromogen and counterstained using hematoxylin. Microscopic examination showed a dark brown cell membrane region in contrast to the cytoplasmic region.

**Immunohistochemical Slide Evaluation**

Interpretation of the IHC slide was done by three observers. Two parameters for staining were considered (1) intensity and (2) percentage of cells stained. IHC score was calculated using a percentage of stained cells and the intensity of the stain taken by the tissue. HER2 membranous staining intensity (negative = 0 score) and positive scores were considered as 0, +1, +2, +3. The score for percentage of cells stained were (0 = negative, 1 = 1–25% cells, 2 = 26–50% cells, 3 = 51–75% of cells, and 4 = 76–100%) of cells. The overall expression was calculated by multiplying the intensity and percentage of stained cells. The value ranges between 0 and 12. A score <3 was considered as negative and >4 as positive.

**Results**

In a total of 10 cases of ameloblastoma 2 showed lower expression and the remaining 8 showed higher expression (80%) (Table 1, Figs 1 and 2). Among the 10 subjects, 5 subjects were unicystic, and the remaining 5 of them were a solid variant of ameloblastoma (Table 2). In five unicystic ameloblastoma, two subjects showed lower expression and the remaining three subjects showed higher expression (Table 2). Similarly, among the 5-solid variant of ameloblastoma 4 of them showed higher expression and the remaining were negative (Table 2). Among 10 cases of OKC, 9 (90%) showed positive expression.

| Table 1: Tabulation of immunoeexpression of HER2 between the groups |
|------------------------|--------|--------|
| Ameloblastoma | OKC | DC |
| Positive cases | 8 | 9 | 5 |
| Negative cases | 2 | 1 | 5 |
| Total | 10 | 10 | 10 |
of them were positively expressed and the remaining subjects were (10%) negative (Table 1, Figs 1 and 2). Lastly, among 10 subjects of DCs, 5 (50%) subjects were positively expressed and the rest 5 (50%) subjects were negatively expressed (Table 1, Figs 1 and 2). In comparison, the statistically insignificant result was obtained between ameloblastoma and OKC ($p$ value = 0.19) and also between ameloblastoma and DC ($p$ value = 0.08). A statistically significant difference was obtained between the DC and OKC ($p$ value = 0.04). This indicates HER2 expression was more positive in OKC followed by ameloblastoma compared to a DC.

**Discussion**

Odontogenic lesions like ameloblastoma, DCs, and OKCs occur in jawbones, it shows locally aggressive behavior and a high recurrence rate. The ErbB2 expression was found to be involved in the aggressive behavior of odontogenic lesions. The ErbB2 protein expression correlates with the disease progression and aggressiveness, especially with lymph node metastasis. Also, known to predict the poor outcome in carcinomas of lung, breast, colorectum, stomach, as well as in head and neck. Recently, ErbB2 has gained attention in the therapy of breast carcinoma. However, very few studies have evaluated ErbB2 receptors in odontogenic lesions. In the present study, we aimed to assess and correlate the ErbB2 expression levels among the OKC, DC, and ameloblastoma.

Epithelial growth factor (EGF) is an essential mitogenic factor for the control of multiplication/division of normal epithelial and neoplastic. This EGF component binds to the specific cell by particular surface receptors and instigates cell expansion. Epithelial development figure receptor (ErbB) is the cell membrane receptor of the tyrosine kinase family. It consists of four groups; one among them is HER2/neu. HER2 induces initiation, promotion of cellular differentiation, and proliferation. Higher expression of HER2 receptors occurs especially in human breast cancer, because of phosphorylation of the growth cycle. Any mutation in this gene could lead to the development of cancer.

In the present study, among 10 cases of ameloblastoma, 8 (80%) cases showed positive expression for HER2. Similar to the present study, Oikawa et al. showed 57% of ameloblastoma were positive for HER2 expression. On contrary, Fateme and Leila showed 90% of ameloblastoma were negative and 10% of ameloblastoma were positive for HER2. According to Monsef Esfahani and Irani’s study, HER2 plays little role in the development and progression of ameloblastoma. Moreover, in a study conducted by Abdel-Aziz and Amin, EGFR was positive in all cases of ameloblastoma.

According to the present study results, 9 out of 10 OKC (90%) specimens showed positive for HER2 expression. Similar to the present study, Monsef Esfahani and Irani reported 87.5% of cases of OKC showed positive for HER2 expression. In contrary, 30% of OKC in Fatemeh Asareh study showed HER2 positivity. Similarly,
Monsef Esfahani and Irani’s study showed 41.7% of OKC expression was positive for HER2/neu expression. Based on the specimen selection concept, the present study is similar to Fatemeh Asareh study since inflammatory OKCs cases were excluded and also inflammatory ameloblastoma, as well as a DC, were excluded from the study, since the inflammatory component will bring about the cellular proliferation and it was contrasted to Monsef Esfahani and Irani’s study.5,19

In the present study, 5 out of 10 cases of (50%) DCs showed positive for HER2 expression. Monsef Esfahani and Irani reported 3.48% of the DC were positive for HER2 expression which is contrary to the present study.19 Oikawa et al.’s study showed 100% HER2 positive expression in DCs which is contrary to the present study on the other extreme value.7

In the present study, HER2 positive expression in ameloblastoma and OKCs were found to be almost similar to each other, i.e., 80 and 90%, respectively, which indicates a statistically insignificant result (p value = 0.19). In comparison to a DC, ameloblastoma also showed insignificant results (p value = 0.08), but between OKC and DC we found statistically significant results (p value = 0.04).

According to Oikawa et al., HER4 showed a major role in the progress and development of ameloblastoma than HER1, HER2, and HER3. HER1 showed ahead among the EGFR group in expressing in the epithelial component including the odontogenic epithelium. The HER2 showed a very minimal role in the development and progression of ameloblastoma.7

The pathogenesis of odontogenic cysts varies from tumors, even though the source for these two remains the same. So, this could be one of the reasons for varied HER2 expression in these lesions. By correlating the present study results and the previous studies on a similar background, it could be suggested that HER2 involvement in the pathogenesis of these cysts could be very minimal. Studies with larger samples and advanced techniques could make us understand its role in the pathogenesis of cysts.

**Conclusion**

In the present study, HER2 is highly expressed in the majority of cases of odontogenic lesions like DCs, OKCs, and ameloblastoma. Odontogenic keratocyst and ameloblastoma showed comparatively higher HER2 expression than DCs. This indicates that OKC and ameloblastoma have a higher potential for tumor growth than DCs. Therefore, HER2 protein functions similar to its other HER family members and influences the biological behavior of odontogenic cysts and tumors. However, further studies with a larger sample size have to be carried out for a definitive inference.

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