

Immunohistochemical Assessment of HER3 Expression in Odontogenic Cysts

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Background: It has been demonstrated that HER3 plays an important role in some human cancers and the HER3 expression is associated with worse survival in solid tumors.

Objectives: This study was conducted to compare HER3 expression in epithelial lining of radicular cysts (RCs), dentigerous cysts (DCs) and odontogenic keratocysts (OKCs).

Materials and Methods: This was a descriptive-analytical study, which assessed all 57 paraffin blocks of RCs, DCs and OKCs (21 RCs, 16 DCs, 20 OKC) from pathological archive of Dentistry College of Zahedan, Iran. The HER3 expression in cytoplasm and membrane was examined by immunohistochemical method. The data collected was analyzed using SPSS16 by ANOVA and Chi-square. $P < 0.05$ was considered as statistically significant.

Results: The HER3 expression had positive results in 52.4% of OKC, 50% of DC and only 20% of RC samples. There was a significant difference between HER3 expression in OKCs and RCs.

Conclusions: The HER3 expression in developmental odontogenic cysts was higher than that in inflammatory odontogenic cysts. The higher rate of HER3 expression in OKC may justify inherent growth potential, stimulation-independent proliferation capability, invasive growth and high recurrence rate of the cyst accepted today as a tumor.

Keywords: Radicular Cyst; Dentigerous Cyst; Odontogenic Cysts; Human Epidermal Growth Factor Receptor3

1. Background

Jawbone cysts are pathological cavities containing liquid, semi-liquid or gas, which is partially or totally covered by an epithelial tissue. The odontogenic cysts covered by epithelium are divided into two groups of developmental and inflammatory based on their origin. They are resulted from odontogenic tissue (epithelial rests of malassez, dental lamina and rests of enamel organ) (1). Odontogenic cysts are accounted for 14.4% of oral and maxillofacial biopsies (2).

Radicular cyst (RC) is the most common odontogenic cyst originated from epithelial rests of periodontal ligaments resulting from the inflammation of the necrosis pulp. For the cyst to develop, it usually involves the apex of the affected tooth. Dental carries are the most common cause of radicular cyst (3). Dentigerous cyst (DC) is the most common odontogenic cyst next to the radicular cyst involved in about 24% of all real cysts in the jaw (4). The DC is developed from expansion of the dental fol-

licles resulting from the fluid accumulation between the crown of a tooth and its surrounding epithelium (5). The odontogenic keratinized cyst (OKC), first introduced by Philipsen, is an invasive cyst of jaws recurring repeatedly (6). Since 2005, the world health organization (WHO) categorized this cyst as odontogenic tumors due to its neoplastic properties and replaced the term odontogenic keratocyst with keratocystic odontogenic tumor (7).

Epidermal growth factor receptors (EGFR) known as HER1 and erbB1 are in turn recognized as the first group of erbB receptors family as a member of a bigger family called tyrosine kinase receptors (8, 9). Afterwards, other members of this family were introduced including HER2 (erbB2), HER3 (erbB3) and HER4 (erbB4). The activity of such receptors is necessary for normal growth and cell differentiation by providing ATP needed by the cell (10). As an example, HER3 affects the development of tooth germs (11). It has been demonstrated that

HER3 plays an important role in some human cancers such as laryngeal, breast, lung, gastric, ovarian, thyroid carcinoma, melanoma etc.; the HER3 expression is associated with worse survival in solid tumors (12-19). Increased HER3 expression dysplastic epithelium and oral squamous cell carcinomas (SCCs) have been reported in many articles (10, 20-23). Rautava et al. showed lesser expression of HER3 in developing and mature epithelium than dysplastic and malignant oral epithelium (10). Takikita et al. (22) revealed more HER3 overexpression in metastatic head and neck squamous cell carcinoma (HNSCC) compared to primary HNSCC. They also found a correlation between HER3 overexpression and worse overall survival. According to their results, HER3 overexpression, as an independent factor, related to poor prognosis and represented as a potential molecule for targeted therapy.

2. Objectives

Considering limited studies performed to examine the significance of HER3 factor in identifying malignant potential of odontogenic cysts, this investigation evaluated the HER3 marker expression in OKC, DC and RC cysts, which may potentially change to SCC and other malignancies of jaws.

3. Materials and Methods

This descriptive-analytical study was conducted on all 57 samples of odontogenic cyst (fulfilling the inclusion criteria) prepared from pathological archive of Dentistry College in Zahedan, Iran as a referral and governmental center of oral pathology in April 2013. This project was approved as a dentistry dissertation in the Ethics Committee of Zahedan University of Medical Sciences, code 90 - 1000 in July 2011. Thirteen samples did not have inclusion criteria and did not enter the study. Patients' age, gender and location extracted from their files and recorded. The odontogenic cysts slides were reviewed and the diagnosis was confirmed by two Oral and Maxillofacial pathologists. Samples with incomplete information required in their files, inadequate tissue for examination or if contained necrosis and inflammation or not fixed correctly were excluded from the study.

After selecting the samples, their paraffin blocks were cut by 3-micron incisions and mounted on charged glass slides. Then, the incisions were paraffinized in xylene and rehydrated in alcohol. To detect antigens, the samples were placed in a citrate buffer with pH = 6 and incubated in a microwave oven. Afterwards, the samples were stained immunohistochemically based on the manufacturer's instruction with the antibody erbB3 (mouse monoclonal 1 mL, Novocastra, Newcastle, UK; lot No: 6003990).

After staining immunohistochemically, the slides

were assessed separately by two pathologists using a light microscope. The staining patterns of cells were evaluated according to Takikita's et al. (22) study on cytoplasm and membrane. Staining intensity was examined in four grades as 0 (as non-stained), 1 (weakly stained), 2 (moderately stained), 3 (highly stained) and the percentage of stained cells was investigated by counting 1000 cells in five grades as 0 (0%), 1 (1% - 25%), 2 (26% - 50%), 3 (51% - 75%) and 4 (76% - 100%). Total score was calculated through multiplying intensity grade by percentage grade of stained cells. The total score ranged from 0 to 12, where the scores equal to or higher than 4 were considered as positive and below 4 as negative. The positive SCC samples were used as positive control. The comments of the two pathologists were finally compared; those with any difference were rechecked by a third pathologist. All pathologists were blinded to study samples. To measure the inter-observer agreement, the Kappa coefficient was used. The kappa coefficient was 0.78 indicating an acceptable level of agreement between the observers.

Finally, all data was analyzed by SPSS software version 16.0 (SPSS Inc. Chicago, IL). The Kolmogorov-Smirnov test was used to assess the normality of data. The quantitative variables with normal distribution were examined by ANOVA and the Chi-Square test was used to compare qualitative data. $P < 0.05$ was considered statistically significant.

4. Results

16 DCs, 20 RCs and 21 OKCs were studied in this investigation. Data related to their gender and age is shown in Table 1. As seen, 60% of the samples in all the three types of DC, RC and OKC were male. OKC was developed in the fourth decade of life and the two other cysts in the third decade of life. Most cysts were in posterior mandible or anterior maxillary areas.

The data related to average percentage of stained cells is shown in Table 2 as divided by the type of cysts. As seen in Table 3, the staining percentage of cells was higher than 25% in more than 50% of all groups.

Data regarding staining intensity in the three types of cysts is shown in Table 4. As seen, staining intensity in DC and OKC was often weak, while it was strong in RC. Figure 1 shows the samples of cystic cells staining.

The total score was obtained from multiplying intensity grade by percentage grade of stained cells. In 50% (8 cases) of DC samples and 52.4% of OKC samples (11 cases), the staining result was positive and just for 20% (4 cases) of RC samples, positive results were achieved, while chi-square showed no significant difference ($P = 0.07$). However, the difference between OKC and RC was significant ($P = 0.03$), while the difference between DC and RC was close to significant level using chi-square test ($P = 0.06$).

Table 1. Demographic Data of Odontogenic Cysts

Cyst	Gender			Age, y		
	Male	Female	Total	Mean (95% CI) ^a	Median	Interquartile Range (IQR)
Dentigerous cyst	10 (62.5)	6 (37.5)	16 (100)	20.19 (13.75 - 26.5)	20.5	16
Radicular cyst	12 (60)	8 (40)	20 (100)	23.35 (18.06 - 28.88)	21	19
Odontogenic Keratocysts	15 (71.4)	6 (28.6)	21 (100)	36.1 (30.45 - 42.5)	33.5	24
P value	0.725 ^b	0.725 ^b	0.725 ^b	0.001 ^c	0.001 ^c	0.001 ^c

^a 95% confidence interval calculated with bootstrapping.^b Chi-square.^c ANOVA.**Table 2.** Average Percentage of Stained Cells Divided by the Types of Cysts Under Study

Cyst	Minimum	Maximum	Mean (95% CI) ^a	Median	Interquartile Range (IQR)
Dentigerous cyst	0	95	43.75 (25.94 - 61.25)	42.50	78
Radicular cyst	0	80	31.6 (20.8 - 43.1)	35	45
Odontogenic keratocysts	0	95	42.9 (30 - 56.09)	40	52
P value	0.406 ^b	0.406 ^b	0.406 ^b	0.406 ^b	0.406 ^b

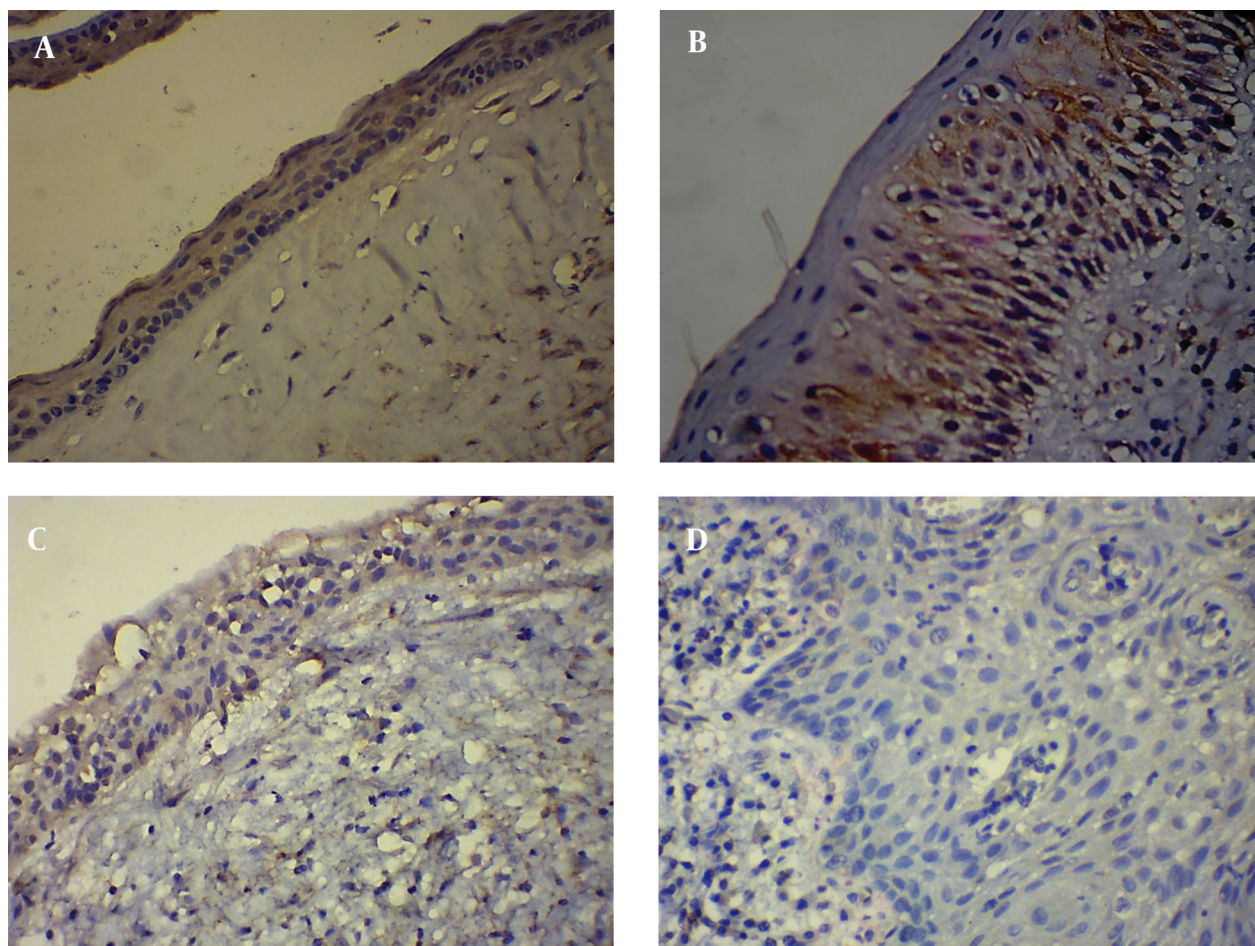
^a 95% confidence interval calculated with bootstrapping.^b ANOVA.**Table 3.** Frequency Distribution of the Stained Cells Divided by the Type of Cysts Under Study^a

Staining Percentage of Cells Group	Frequency					Total
	0	1 - 25	26 - 50	51 - 75	76 - 100	
Dentigerous cyst	4 (25)	3 (18.75)	2 (12.5)	3 (18.75)	4 (25)	16 (100)
Radicular cyst	4 (20)	5 (25)	7 (35)	3 (15)	1 (5)	20 (100)
Odontogenic keratocysts	1 (4.8)	5 (23.8)	9 (42.9)	2 (9.5)	4 (19)	21 (100)

^a The values are presented as No. (%).**Table 4.** Frequency Distribution of Staining Intensity Cells Divided by the Type of Cysts Under Study^a

Staining Intensity Group	Non-Stained	Weak	Moderate	Strong	Total
Dentigerous cyst	4 (25)	7 (43.8)	1 (6.3)	4 (25)	16 (100)
Radicular cyst	4 (20)	4 (20)	0 (0)	12 (60)	20 (100)
Odontogenic keratocysts	1 (4.8)	11 (52.4)	1 (4.8)	8 (38.1)	21 (100)

^a Values are presented as No. (%).

Figure 1. Immunohistochemical Staining With HER3 in Odontogenic Cyst

A, Staining pattern with high intensity of epithelial covered cells of OKC. B, Staining pattern with moderate intensity of epithelial covered cells of OKC in basal and supra-basal layers. C, Staining pattern with weak intensity of epithelial covered cells of dentigerous cyst. D, Non-stained epithelial cover cells of radicular cyst (Magnification $\times 400$).

5. Discussion

The present study was performed to evaluate HER3 expression in epithelial lining of RCs, DCs and OKCs. The results obtained based on the total score indicated the positive HER3 expression in 52.4% of OKC, 50% of DC and 20% of RC samples. The staining procedure of cells was the same in cytoplasm and membrane in basal and supra-basal layers.

HER3 is one of the four members of epidermal growth factor receptors as ERBB, which is activated by connecting to Neuregulin-1 and Neuregulin-2 ligands. Since HER3 lacks intrinsic kinase activity, induction of signal occurs through formation of Heterodimers with EGFR, HER2 and HER4 (24).

Most studies examining the HER3 expression in oral lesions were about dysplastic and cancerous lesions of oral mucosa (10, 20-23), in which some significant results were obtained with respect to increased expression and lymph node metastases, prognosis and invasion (2, 20, 22). It

was recently found that HER3 plays an important role in response to radiotherapy of head and neck carcinomas and blocking its activity along with radiotherapy may be beneficial for the treatment of human tumors (25). Shintani et al. (26) studied HER3 expression in adenoid cystic carcinoma of salivary glands; HER3 expression was found in all samples and staining intensity of tumor cells for this marker in invasive areas was more intense. The results of another study revealed that systematic conditions such as diabetes would increase HER3 expression in certain stages of oral oncogenesis, which is likely resulted by increased cellular proliferation and apoptosis inhibition (27).

No similar study was found examining the effect of HER3 marker on these three types of cysts. Accordingly, the results of studies examining other members of erbB family in odontogenic cysts and tumors are suggested. In an investigation by Shrestha et al. (28) EGFR expression was positive in 60% of OKC, 47.4% of DC and 35% of RC

samples. On the other hand, in the study performed by Li et al. (29) the higher expression of EGFR was reported as the first member of Tyrosine Kinase receptors family in developmental cysts such as OKC and DC compared to RC. Furthermore, EGFR expression was known to be related to inflammation. Inflammation was identified as a factor for decreased expression of this receptor in RC. In the present study, RC showed the lowest rate of HER3 expression based on the total score. In an investigation by de-Vicente, EGFR expression reported as 100% of cases of ameloblastoma, 73% of OKCs, 40% of DC and 30% of RCs, where EGFR expression was found significantly more in ameloblastoma (30). Moreover, it is suggested in some studies to consider anti-EGFR factors to reduce the size and treat inoperable tumors as close to vital structures (31, 32).

In the investigation conducted by de Oliveira et al. (33) cytoplasm and membrane expression of EGFR marker in basal and supra-basal layers of DC and OKC were examined; cytoplasmic expression of EGFR was significantly higher in OKC basal, supra-basal layers and cytoplasm and membrane expression of EGFR in supra-basal layer of DC. Furthermore, they stated that EGFR might indicate high capability of cells in response to stimulation, which may be considered as a cause of odontogenic lesions. In addition, Goncalves reported EGFR expression in 100% of RC and DC cases as well as 94% of OKC cases, while he considered membrane and cytoplasm placement of EGFR in cells as an important factor in response to stimulating proliferation (34); this may be generalized to cytoplasm and membrane expression of HER3 as well.

Kolar et al. (35) compared sporadic and syndromic OKC and reported a high expression of Bcl2, p27kip1 and c-erbB2 in syndromic keratocysts, and lower proliferative activity in basal cells of sporadic OKC. Furthermore, they suggested the difference between DC and RC compared to sporadic and syndromic OKC in higher proliferation in basal cells layer, and by contrast, lower proliferation in supra-basal cells layer.

Heikinheimo examined HER3 immunohistochemical expression in 12 sporadic OKC samples; their results showed that HER3 has a high incidence in epithelium supra-basal layers and the epidermal growth factor receptor pathway was known as a part of OKC pathogenesis (7). This result differs slightly from the present study, so that HER3 expression was observed as monotonous and even a case was found as non-stained (4.8%).

In some studies like Shintani et al. (20) and Rautava et al. (10) ones, researchers discussed merely on the percentage of stained cells while in other studies like ours, both percentage and intensity were used to evaluate HER3 expression (18, 21, 22). Assessment of staining only based on intensity is not an appropriate criterion, because staining intensity may be differently reported by different pathologists, especially in weak and moderate levels of intensity. Evaluation of HER3 expression in microscopic examination of staining slides immunohistochemicalized based on staining intensity and percentage stated

as numbers would provide researchers with a full and more comprehensive result with lower error compared to HER3 expression as separately in accordance with intensity or percentage of stained cells (22).

In general, the results of this study showed that the average percentage of stained cells was high in DC, OKC and RC, respectively. For total score of staining, OKC, DC and RC were high, respectively. However, the results were quite different based on moderate and high staining intensity, and it was high in RC, OKC and DC, respectively. It may be concluded that HER3 has a higher expression in developmental cysts including OKC and DC compared to inflammatory cysts of RC. Meanwhile, the higher rate of HER3 marker expression in OKC may justify inherent growth potential, stimulation-independent proliferation capability, invasive growth and high recurrence rate of the cyst accepted today as a tumor. Lack of research in the field of HER3 expression in odontogenic cysts necessitates further investigations in this respect.

Finally, it is suggested to conduct further studies about HER3 expression in relation to the behavior of odontogenic cysts (e.g. recurrence after treatment, transformation to malignancy) and/or in comparison with tumors such as ameloblastoma and SCC.

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Authors' Contributions

Study concept and design: Marieh Honarmand and Shirin Saravani. Analysis and interpretation of data: Marieh Honarmand, Shirin Saravani, Nazanin Kamyab and Mehdi Jahantigh. Drafting of the manuscript: Marieh Honarmand, Shirin Saravani and Nazanin Kamyab. Critical revision of the manuscript for important intellectual content: Marieh Honarmand, Shirin Saravani, Nazanin Kamyab, Mehdi Jahantigh and Molouk Torabi Parizi. Statistical analysis: Marieh Honarmand, Shirin Saravani and Nazanin Kamyab. Administrative, technical and material supports: Shirin Saravani, Mehdi Jahantigh and Molouk Torabi Parizi. Study supervision: Saravani and Mehdi Jahantigh.

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