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A Comparative immunohistochemical analysis of epithelial–mesenchymal transition biomarkers in odontogenic keratocyst, dentigerous cyst, and radicular cyst

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ABSTRACT

Background: Odontogenic keratocyst (OKC) is one of the common odontogenic cysts with aggressive clinical behavior and a high recurrence rate. Epithelial–mesenchymal transition (EMT) is a process, in which the epithelial cell loses its epithelial characteristics and acquires mesenchymal features. Since the evidence for the involvement of EMT in the development of OKC is still limited, the present study aimed to investigate the immunohistochemical expression of EMT-related proteins (E-cadherin and N-cadherin) in OKC and compare them to radicular cyst (RC) and dentigerous cyst (DC).

Materials and Methods: In this descriptive analytical study, 75 paraffin blocks, including 25 DCs, 25 OKC, and 25 RCs, were selected. Immunohistochemical staining was performed to determine the expression and staining intensity of E-cadherin and N-cadherin proteins. The specimens were examined under an optical microscope, and the data were analyzed using the Kruskal–Wallis test in SPSS statistical software (version 23) with a significance level of 5%.

Results: The expression of N-cadherin in OKC was higher than that in other cysts; nonetheless, there was no statistically significant difference (P = 0.331). The staining intensity of N-cadherin was weak in most cases, and this difference was not statistically significant (P = 0.252). E-cadherin expression in OKC was significantly lower than that in radicular and DCs (P = 0.003). In addition, the staining intensity of E-cadherin in OKC was weak and moderate (P = 0.003).

Conclusion: In this study, we observed an increase in the expression of N-cadherin in OKC. In addition, the protein expression levels of E-cadherin in OKC were significantly lower compared to DC and RC. Therefore, it appears that the EMT process likely occurs in OKC and may contribute to its local aggressive behavior.

Key Words: E-cadherin, epithelial–mesenchymal transition, immunohistochemistry, N-cadherin, odontogenic cyst

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INTRODUCTION

Odontogenic keratocyst (OKC), dentigerous cyst (DC), and radicular cyst (RC) are common cystic lesions in the jaw.[1] OKC comprises a significant proportion of odontogenic cysts. This cyst has

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a locally invasive behavior and a high tendency to relapse after tumor removal. In the World Health Organization classification of tumors 2005, it was classified as a keratocystic odontogenic tumor (KCOT).[2-4] The etiology of OKC is probably related to the development of the dental lamina and its remnants; nonetheless, despite extensive studies, the pathogenesis of this lesion has not yet been determined. Due to its different nature, this lesion has been discussed for a long time and classified as a cyst or tumor several times. Moreover, its treatment options have remained limited due to the lack of sufficient explanations about its mechanism and molecular composition.[5-7]

A DC is the most common developmental odontogenic cyst, which constitutes more than 20% of epithelium-lined cysts. The RC is the most common odontogenic cystic lesion of inflammatory origin.[3]

Epithelial–mesenchymal transition (EMT) is a complex process by which epithelial cells lose their cell polarity and cell–cell adhesion and gain migratory and invasive properties to become mesenchymal stem cells.[7] The EMT process plays a key role in embryogenesis and pathological conditions, such as fibrosis and carcinogenesis.[7] The EMT can endow tumoral cells with invasive and lethal behavior, which is associated with poor clinical outcomes and high recurrence in various tumors.[4,8]

One of the main reasons for the invasive capacity of the tumor is the dissociation of tumor cells in tissues due to changes in cell-to-cell adhesion, which can be a possible explanation for the invasive behavior of OKC.[5] Epithelial cell rests of Malassez, which are the origin of many odontogenic tumors, including KCOT, contain a unique cell population that can promote EMT.[4] However, it is still unclear whether EMT plays a role in the pathogenesis and development of KCOT.[4,9] One of the most well-known processes of EMT is the loss of intercellular adhesion between epithelial cells by inhibiting the expression of E-cadherin (epithelial cadherin) and the aberrant expression of high amounts of N-cadherin (mesenchymal-cadherin).[10]

E-cadherin, one of the calcium-dependent cell adhesion molecules belonging to the cadherin family, plays an essential role in regulating intercellular adhesion in epithelial tissues and is considered the main component of epithelial cell–cell adhesion in all organs.[11] N-cadherin is also a calcium-dependent single-chain membrane glycoprotein that is expressed in neural cells, as well as mesenchymal and mesodermal cells.[12] Considering that the evidence of EMT involvement in the development of OKC is still limited,[9] the present study aimed to investigate the expression level of proteins related to the EMT process (E-cadherin and N-cadherin) in OKC, RC, and DC.

MATERIALS AND METHODS

In this descriptive analytical study following approval of the local Ethics Committee (IR.ZAUMS.REC.1398.357), we analyzed 75 samples including 25 DCs, 25 OKCs, and 25 RCs. These samples were obtained between 2008 and 2018. To confirm the diagnosis, all H and E slides were reviewed. Demographic and clinicopathological data were extracted from the patient's records. Cases with incomplete data in their medical records or insufficient tissue in their paraffin blocks for cutting, were excluded from the study.

Immunohistochemistry and scoring

For immunohistochemistry (IHC) staining, paraffin blocks were sectioned into 4 μm sections using a microtome. The samples were then deparaffinized and rehydrated. Antigen retrieval was performed by microwave heating on Tris buffer (pH = 7.6) for 30 min. After that, the sections were incubated at room temperature for 1 h. The applied primary antibodies were monoclonal antihuman antibody E-Cadherin ready-to-use antibody (Novocastra, United Kingdom, Lot: 50115) and N-Cadherin (Novocastra, United Kingdom, Lot: 6044777) at 1:50 dilution (6046011, Novocastra) according to the manufacturer's instructions (Novocastra). Then, the tissue sections were washed with PBS and the secondary antibody was applied at room temperature. Diaminobenzidine was added and followed by counterstaining with Mayer's hematoxylin. The sections were then dehydrated and mounted. In the negative controls, the primary antibody was omitted.

After the IHC staining, the stained tissue sections were examined using a light microscope (Nikon, Type 2, Tokyo, Japan). The percentage of positive cells for E-cadherin (membranous immunostaining) and N-cadherin (membranous and cytoplasmic immunostaining) was assessed in 10 high-power fields at a magnification of 400. The scores were all calculated as absent, <20%, 20%–50%, and >50% for

E-cadherin and as absent, <10%, 10%–20%, 20%–50%, and >50% for N-cadherin.[7] The intensity of staining was assessed at a magnification of 100, and it was graded as negative (no staining), mild (light brown staining of the cells), severe (dark brown staining of the cells), and moderate (between mild and severe staining of the cells).[7]

Statistical analysis

Statistical analysis was performed using SPSS 23 (SPSS Inc., Chicago, IL, USA). Kruskal–Wallis statistical test was applied to analyze the differences in immunostaining scores between groups. $P < 0.05$ was considered statistically significant.

RESULTS

In this study, the expression and staining intensity of N-cadherin and E-cadherin proteins in 75 samples of odontogenic cysts, including 25 DCs, 25 OKCs, and 25 RCs, were investigated. In total, 54 (72%) subjects were male, 21 (28%) cases were female, and the mean age of participants was 29 ± 14 years. Regarding the location distribution of the lesions, 32 (43%) and 43 (57%) cases were located in the maxilla and mandible, respectively. Table 1 shows the frequency of gender, mean age, and location of lesions in the studied groups separately.

The expression of E-cadherin was observed throughout the epithelium (basal and suprabasal layers) in all investigated cysts. Moreover, 72% of OKCs, 40% of DCs, and 32% of RCs showed a decrease in E-cadherin expression (staining in <50% of cells), which was significant according to the Kruskal–Wallis statistical test. ($P = 0.003$) [Table 2]. The intensity of staining of E-cadherin protein in the examined odontogenic cysts was moderate in most cases. According to the Kruskal–Wallis test, this difference was also statistically significant ($P = 0.003$) [Table 3 and Figure 1].

As illustrated in Table 4, the expression of N-cadherin was negative in the epithelium of most cases of RCs (68%) and DCs (76%). Nonetheless, N-cadherin protein expression was positive in 44% of OKC cases in the epithelium, especially in the basal layer. In addition, in 8% of OKC cases and 20% of RC cases, N-cadherin expression was observed in more than 50% of cells. According to the Kruskal–Wallis statistical test, among the odontogenic cysts examined, the expression of N-cadherin was higher in OKC than in other cysts; however, there was no statistically significant difference ($P = 0.331$).

In terms of staining intensity, among the samples of cysts that showed the expression of N-cadherin protein, most cases had a weak staining intensity; nonetheless, according to the Kruskal-Wallis statistical test, this difference was not statistically significant ($P = 0.252$) [Table 3 and Figure 2].

DISCUSSION

Dentigerous and RCs are nonneoplastic odontogenic lesions, whereas OKC is considered a neoplastic odontogenic lesion with locally invasive characteristics and extensive bone destruction.[13] Since the prognosis of various neoplasms, including odontogenic tumors,

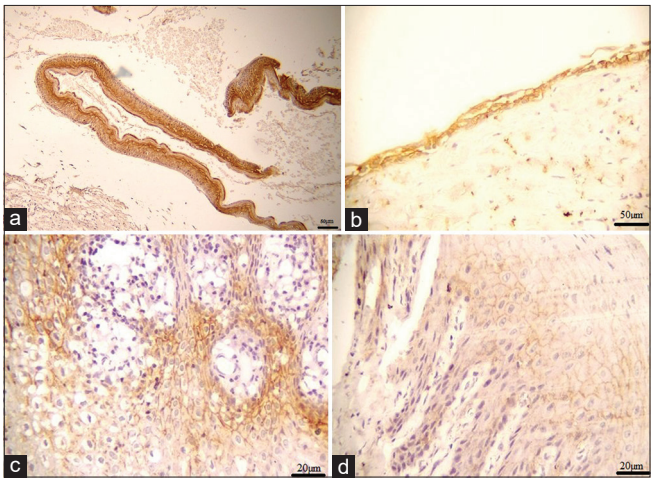

Figure 1: The immunohistochemical staining of E-Cadherin in odontogenic cysts: (a) Severe staining in odontogenic keratocyst (×100). (b) Severe staining in dentigerous cysts (×100). (c) Moderate staining in radicular cyst (RC) (×400). (d) Mild staining in RC (×100).

Table 1: Demographic data of different odontogenic cysts

Odontogenic cysts	Age, mean±SD (year)	Sex		Location	
		Male, *n* (%)	Female, *n* (%)	Maxilla, *n* (%)	Mandible, *n* (%)
OKC	30±11	16 (64)	9 (36)	6 (24)	19 (76)
RC	31±12	18 (72)	7 (28)	15 (60)	10 (40)
DC	24±18	20 (80)	5 (20)	11 (44)	14 (56)

DC: Dentigerous cysts; RC: Radicular cysts; OKC: Odontogenic keratocyst; SD: Standard deviation

cannot be accurately and reliably predicted based on clinical and histopathological features, there is a great desire to find helpful markers in this regard.[14] Epithelial cells are held together by strong cell junctions which are composed of various proteins called junctional adhesion molecules. Cadherins are a family of calcium-dependent binding molecules that act as an invasion suppressor system. When the function of cadherins is blocked by related antibodies, noninvasive cells can turn into an invasive form.[15,16] The present study investigated the expression level of proteins related to the EMT process (E-cadherin and N-cadherin) in OKC, RC, and DC, and their possible role in the aggressive behavior of OKC.

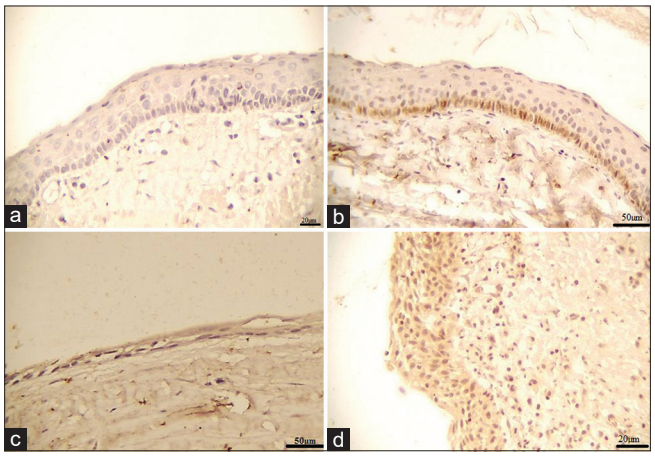

Figure 2: The immunohistochemical staining of N-Cadherin in odontogenic cysts: (a) negative staining in odontogenic keratocyst (OKC) (×100). (b) Severe staining in the basal layer of OKC (×100). (c) Moderate staining in dentigerous cysts (×400). (d) Moderate staining in radicular cyst (×100).

Table 2: Immunoxepression of E-cadherin in different odontogenic cysts

Odontogenic cysts	E-cadherin			
	<20, *n* (%)	20–50, *n* (%)	>50, *n* (%)	*P*
OKC	6 (24)	12 (48)	7 (28)	0.003*
RC	0	8 (32)	17 (68)	
DC	1 (4)	9 (36)	15 (60)	

*Significant, Kruskal–Wallis test. DC: Dentigerous cysts; RC: Radicular cysts; OKC: Odontogenic keratocyst

According to the results of the present study, the expression of N-cadherin protein was higher in OKC compared to that in dentigerous and RCs; however, this difference was not statistically significant. Similarly, in Porto *et al*. study, which investigated the possible role of the EMT process in OKC, increased expression of N-cadherin was reported in OKC and RC compared to normal mucosa; nonetheless, the difference between groups was not statistically significant. This study, due to the high expression of N-cadherin in OKC, pointed to the possible role of this protein in the development of OKC and its probable involvement in EMT signaling through balance with other regulators. It was also indicated that the immune response of RCs to protein is clearly affected by inflammation, and in fact, the presence of inflammation helps to increase the expression of N-cadherin in RCs.[7]

In the present study, the increase in N-cadherin expression in OKC was observed in 44% of cases; nonetheless, the difference was not statistically significant. In the current study, RCs also displayed increased expression of N-cadherin in 32% of cases, and 20% of the samples showed N-cadherin staining in more than 50% of the cells. Considering that

Table 3: Staining intensity of N-cadherin and E-cadherin in different odontogenic cysts

Odontogenic cysts	N-cadherin				*P*	E-cadherin				*P*
	Negative, *n* (%)	Mild, *n* (%)	Moderate, *n* (%)	Severe, *n* (%)		Negative, *n* (%)	Mild, *n* (%)	Moderate, *n* (%)	Severe, *n* (%)	
OKC	14 (56)	6 (24)	3 (12)	2 (8)	0.252	0	9 (36)	13 (52)	3 (12)	0.003*
RC	17 (68)	6 (24)	2 (8)	0		0	1 (4)	15 (60)	9 (36)	
DC	19 (76)	4 (16)	2 (8)	0		0	1 (4)	16 (64)	8 (32)	

*Significant, Kruskal–Wallis test

Table 4: Immunoxepression of N-cadherin in different odontogenic cysts

Odontogenic cysts	N-cadherin					*P*
	Absent, *n* (%)	<10, *n* (%)	10–20, *n* (%)	20–50, *n* (%)	>50, *n* (%)	
OKC	14 (56)	0	6 (24)	3 (12)	2 (8)	0.331
RC	17 (68)	1 (4)	0	2 (8)	5 (20)	
DC	19 (76)	2 (8)	0	4 (16)	0	

DC: Dentigerous cysts; RC: Radicular cysts; OKC: Odontogenic keratocyst

inflammation can cause structural changes in tissues,[7] this increase in the expression of N-cadherin in the RC can be attributed to the effect of inflammation on the expression of this protein. On the other hand, the increased expression of N-cadherin in OKC compared to other cysts points to the possible role of this protein in the behavior of OKC.

In the study by Zhong *et al*., a significant increase was observed in N-cadherin expression in OKC compared to RCs and normal mucosa. Moreover, the results of the study indicated the possible role of EMT in the aggressive behavior of OKC.[4] Similarly, Kusafuka *et al*. reported the positive expression of N-cadherin in 40% of OKC cases, whereas all DC samples were negative.[17]

In the present study, a significant decrease was observed in E-cadherin expression in 72% of OKC cases, compared to RCs and DCs. This finding was also observed in other studies.[4,13,14,16] Zhong *et al*. noted a decrease in E-cadherin expression in OKC compared to that in RCs and normal mucosa.[4] In the same vein, Hakim *et al*. reported a marked decrease in the expression of E-cadherin and β-catenin in 18 sporadic and syndromic OKC samples, especially in the suprabasal layer, whereas DC samples showed preserved staining (staining in more than 50% of cells).[16]

Özcan *et al*. reported that the expression of E-Cadherin in nonneoplastic odontogenic cysts (RC and DC) is higher than in neoplastic odontogenic cysts (OKC and cystic ameloblastoma).[13] A number of researchers have also mentioned that apart from the changes in cell junctions, the reduced expression of E-cadherin in neoplastic odontogenic cysts, such as OKC and cystic ameloblastoma, can reduce the density of Langerhans cells. Therefore, the capacity of dendritic cells to identify tumor antigens is endangered.[11,13] Abdel Samiaa *et al*. also pointed out that the percentage of E-cadherin expression areas in OKC was higher than in solid ameloblastoma; nonetheless, this difference was not statistically significant. Moreover, it was mentioned that the absence of significant differences in the expression of markers, such as E-cadherin, MDM2, and cortactin, in ameloblastoma and OKC can indicate the aggressive and neoplastic nature of OKC.[14]

Nevertheless, unlike the present research and other mentioned studies, Pinheiro *et al*. did not observe a significant difference in the expression of E-cadherin between OKC and RC, and in most cases of OKC, the expression of E-cadherin protein was preserved.[11] Kusafuka *et al*. and Mello *et al*. also reported similar results.[17,18] The results can be affected by some factors, such as the use of different methods to measure the cadherin expression, different cell counting methods, and different sample sizes.

According to the results of the majority of the mentioned studies, E-cadherin plays a key role in mediating cell junctions in odontogenic tumors.[11] The decrease in E-cadherin expression can be related to parameters such as invasion, tumor recurrence, metastasis, and poor prognosis of patients.[15] The present study also investigated the staining intensity of E-cadherin and N-cadherin expression in the odontogenic cysts. For N-cadherin, most cases had a weak staining intensity, and the staining intensity for E-cadherin was moderate in most samples. According to our investigations, no similar study investigated the staining intensity of cadherins in odontogenic cysts.

CONCLUSION

The study of markers that help to better understand the biological behavior of the lesions is important for the better management of the patients. Our present study showed a decrease in E-cadherin expression in DCs, RCs, and OKCs, but the OKC showed a significant reduction compared to other cysts. The expression of N-cadherin was negative in the epithelium of most cases of RCs and DCs. Nonetheless, N-cadherin protein expression was positive in 44% of OKC cases in the epithelium.

Hence, according to the results of this study, the EMT process probably occurs in OKC and can play a role in its local aggressive behavior. However, more studies are still needed to confirm the exact role of the EMT process in OKC and to investigate the correlation between EMT and the clinical behavior of OKC.

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Conflicts of interest

The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial, or non-financial in this article.

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