Comparison of cytokeratin expressions among orthokeratinized odontogenic cysts, epidermoid cysts and odontogenic keratocysts: An immunohistochemical study

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INTRODUCTION

Orthokeratinized odontogenic cyst (OOC) was first described by Schultz in 1927.¹ They are more often associated with an impacted tooth (60.8%) and are usually found in a dentigerous cyst association, around

Background: Orthokeratinized odontogenic cysts are keratinizing jaw cysts and due their association with impacted teeth and keratinaceous content, they resemble odontogenic keratocysts but differ in regards to biological behaviour, being less aggressive. To unravel the nature of OOCs, as they resemble epidermoid cysts histologically and due to their developmental resemblances to OKCs, this study was conducted.

Aim and Objective: To compare the cytokeratin expressions of CK 10 and CK 19 among orthokeratinized odontogenic keratocysts, epidermoid cysts and odontogenic keratocysts by immunohistochemical study.

Materials and Methods: 30 cases of all three cysts were collected, 10 cases in each of these cysts (OOCs, EDCs and OKCs) were incubated with CK 10 and CK 19 markers respectively. IHC staining was performed and assessed all layers of epithelium. All the data were analyzed using SPSS software, P values were obtained by the Chi-square test and Fisher’s test.

Results: The expression pattern of CK10 showed 100% positive in both OOCs and EDCs with significant difference in OKCs. CK19 expression, between EDCs and OKCs was significant but between OOCs & EDCs and OOCs & OKCs was found to be statistically insignificant.

Conclusion: CK 10 expressions in both OOCs and EDCs were near identical both in terms of expression and patterns of expression in surface and spinous layers. OOCs may not be distinguished from EDCs both histologically and with CK 10 expression. CK19 expression between OOCs & EDCs and OOCs & OKCs was statistically insignificant. Thus, based upon CK 19 expression, no significant differences were found between OOCs & EDCs and OOCs & OKCs, implying that OOCs resemble both EDCs and OKCs.

Keywords: Cytokeratin expressions, cytokeratins, immunohistochemical, orthokeratinized odontogenic cyst

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the mandibular third molar. These tumors can reach a large size causing cortical expansion and can present as a painful swelling, although in most cases, it can be detected incidentally during a radiographic examination. In 2005, the World Health Organization (WHO) redefined odontogenic keratocysts (OKCs) as neoplasm and redesignated it as keratocystic odontogenic tumor (KCOT), and it became imperative that OOCs had to be separated from KCOT as a distinct entity.[2] Epidermoid cysts (EDCs) are the benign lesions developing from the abnormal epithelial components of ectodermal tissue formed during the fetal period or from implanted epithelium posttrauma or surgery. Nearly 7% occurs in the head and neck areas.[3,4] They are rare in the oral cavity with an incidence of 1.6%. Midline of the floor of the mouth is the most favored site.[3,4] KCOT is a benign, unicystic or multicystic intraosseous tumor of odontogenic origin with potentially aggressive and infiltrative behavior.[7] In 2005, the WHO reclassified OKCs and labeled it as KCOT. Many authors are reluctant to agree this notion. The reason behind this reluctance is that not all OKCs behave aggressively, similar to a neoplasm. There were not enough reports to justify the change in the classification based on the genetic and molecular studies. Hence, the term KCOT is unanimously not accepted.[8,9] OKCs comprise approximately 11% of all cysts of the jaws.[10,11] OKCs arise from the remnants of dental lamina and are the third-most common type of odontogenic cyst in a study from the Indian population.[12] It may present as pain in the jaw, soft-tissue swelling and paresthesia of lip/teeth or may be asymptomatic as it tends to grow in an anteroposterior direction within the medullary cavity of the bone without causing obvious bone expansion. Distinctive clinical features include potential for local destruction and a tendency for multiplicity, especially when the lesion is associated with nevoid basal-cell carcinoma syndrome syndrome.[13] OOCs are keratinizing jaw cysts and due their association with impacted teeth and keratinaceous content, they resemble OKCs but differ from them in regard to biological behavior and being less aggressive. OOC's histological resemblance to EDCs of the skin is perplexing. Whether this OOC is an inclusive or odontogenic in origin, due to its resemblance to EDCs and OKCs is to be elucidated.

Cytokeratins (CKs) are the proteins of keratin-containing intermediate filaments found in the intracytoplasmic cytoskeleton of the epithelial tissue.[13] There are two types of CKs: Basic Type I CK 1–8 and acidic/neutral Type II CK 9–20. In the oral epithelium, CK 4/13, CK 6/16 and CK 1/10 are expressed in nonkeratinized, parakeratinized and orthokeratinized epithelium in the intermediate and superficial layers, respectively. CK 5/14 is expressed by the basal layers of all the three types of the epithelia.[14,15] In odontogenic apparatus, cells of enamel organ express CK 7, 13, 14 and 19. Stellate reticulum and Hertwig Epithelial Root Sheath express CK 7. Preameloblasts and secretory ameloblasts (secretory differentiation) express CK-19 and cell rests of Malassez express CK 5/19.[16] CK-10 is an early marker for mature keratinocytes, and CK-19 is expressed in odontogenic and secretory epithelium. One of the main clinical implication in the study of CK profile by immunohistochemistry techniques is its utility in investigating and identifying the source of origin of a tumor/cyst and its characterization.[17]

To unravel the nature of OOCs, as they resemble EDCs histologically and due to their developmental resemblances to OKCs, immunohistochemical expressions of CK-10 and 19 is evaluated and compared in the lining epithelia of OOCs, EDCs of the skin and OKCs in the present study.

**MATERIALS AND METHODS**

This study was conducted on the archival retrieved formalin fixed, paraffin-embedded tissue sections which were obtained from the Department of Oral and Maxillofacial Pathology, in a Dental Institute at Chennai. The study groups include histological sections of previously diagnosed 10 different cases of OOCs (Group A), 10 different cases of EDCs (Group B) and 10 different cases of OKCs (Group C).

Two subsequent sections, each 4 µ thick, are taken from each case of histologically diagnosed with OOCs, EDCs and OKCs, and the sections were treated with immunohistochemical reagents of anti-CK 10 and anti-CK-19 Tissues samples embedded in paraffin wax were obtained (OOCs [n = 10], EDCS [n = 10] and OKCs [n = 10]). Test slides sections of OOCs for CK-10 and CK-19. Comparative slides sections of EDCs of the skin are taken for CK-10 and CK-19. Sections of OKCs of jaws are taken for CK-10 and CK-19.

Formalin-fixed, paraffin-embedded tissues sectioned at 4 µ thickness were obtained from each block and subjected to the immunohistochemical staining by using the Polymer Horseradish Peroxidase (poly-HRP) detection system. Antigen retrieval was carried out by “heat-induced antigen retrieval method” in which tissue sections were placed in pressure cooker along with 10 mM aqueous citrate buffer (pH 6.0) and pressure cooker operated at 120°C with full pressure. Tissue sections were then immersed in 3% hydrogen peroxidase for 10 min to block endogenous peroxidise and subsequently incubated with antibody to
CK-10 and CK-19 overnight at 4°C. HRP-labeled rabbit anti-mouse antibody was added to the tissue sections at the room temperature for 1 h. Reaction product was developed by adding 3, 3’ diaminobenzidine tetrahydrochloride to the tissue sections. Tissue sections were then counterstained with H and E stain and evaluated under the light microscope at ×10 and ×40. The presence of brown-end product at the site of target antigen indicated positive and absence of staining indicated negative immunoreactivity-staining confined in the scatter areas of the epithelium. Each slide was analyzed, scored and noted.

**Immunoistochemical assessment**

Assessment of antigen expression of cells was performed using the Olympus light microscope at ×10 and ×40. The antigen positive cells stain brown in color. An arbitrary semiquantitative analysis was carried out to determine the immunohistochemical expression pattern and classified according to the presence or absence of staining in the epithelial layers of the cyst.

According to Hoshino *et al.*, the study cases were classified based on the presence or absence of expression of CKs into two groups: Positive (+) and negative (−) in the cytoplasm, and further, the epithelium is sub-classified into surface, spinous and basal layers to analyze the expression of these CKs in various layers. The obtained data are computed in Microsoft Office Excel Worksheet, and statistical analysis was performed using the SPSS software, version 20 (Armonk, NY, USA: IBM Corp.). A *P*-value of <0.05 is considered as statistically significant which was obtained by the Chi-square test and Fisher’s test.

**RESULTS**

The results obtained for CK-10 and CK-19 staining in all three cysts are tabulated in Table 1. Epithelial layers surface and spinal and basal expressions of CK-10 and CK-19 are mentioned in Table 2.

For CK-10 marker, OOCs showed all 10 cases to be positive. On analyzing various layers among 10 positive cases, surface and spinous layers expressed positivity in all 10 cases, but none of the cases showed positivity in the basal layers [Figure 1]. EDCs exhibited all 10 cases to be positive for CK-10. On analyzing various layers among 10 positive cases of EDCs, surface and spinous layers exhibited positive in nine cases and basal layers in three cases [Figure 2]. OKCs showed five cases to be positive for CK-10. On analyzing various layers among five positive cases, surface layers exhibited positivity in all five cases, spinous layers expressed only in one case and nil positivity in basal layers [Figure 3]. On comparing, OKCs showed a significant difference between OOC and EDC (*P* = 0.009).

### Table 1: Intra/inter comparison among orthokeratinized odontogenic cyst, epidermoid cyst, and odontogenic keratocyst using Cytokeratin 10 and 19 markers

| Marker | CK-10 | CK-19 | *P* |
|--------|-------|-------|-----|
|        | Positive, n (%) | Negative, n (%) | Positive, n (%) | Negative, n (%) |   |
| Cyst   |       |       |     |       |     |
| OOC    | 10 (100.0) | 0 (0.0) | 6 (60.0) | 4 (40.0) | 0.011* |
| EDC    | 10 (100.0) | 0 (0.0) | 7 (70.0) | 3 (30.0) | 0.003** |
| OKC    | 5 (50.0)  | 5 (50.0) | 8 (80.0) | 2 (20.0) | 0.350 |
| *Significantly differed at 5% level (*P*<0.05), **Significantly differed at 0.5% level (*P*<0.005), †Significantly differed EDC and OOC (*P*=0.009), ‡Significantly differed EDC (*P*=0.028). OKC: Odontogenic keratocyst, EDC: Epidermoid cyst, OOC: Orthokeratinized odontogenic cyst, CK: Cytokeratin |

### Table 2: Analysis of various layers of orthokeratinized odontogenic cyst, epidermoid cyst and odontogenic keratocyst using cytokeratin 10 marker

| Layers | CK-10 | P |
|--------|-------|---|
|        | Surface | Spinous | Basal |
|        | Positive, n (%) | Negative, n (%) | Positive, n (%) | Negative, n (%) | Positive, n (%) | Negative, n (%) |   |
| Cyst   |       |       |     |       |     |     |     |
| OOC    | 10 (100.0) | 0 (0.0) | 10 (100.0) | 0 (0.0) | 0 (0.0) | 10 (100.0) | <0.001** |
| EDC    | 9 (90.0)  | 1 (10.0) | 9 (90.0) | 1 (10.0) | 3 (30.0) | 7 (70.0) | 0.003* |
| OKC    | 5 (50.0)  | 5 (50.0) | 1 (10.0) | 9 (90.0) | 0 (0.0) | 10 (100.0) | 0.013* |

*Significantly differed at 0.1% level *P*<0.001, †Significantly at 5% level *P*<0.05. OKC: Odontogenic keratocyst, EDC: Epidermoid cyst, OOC: Orthokeratinized odontogenic cyst, CK: Cytokeratin

![Figure 1: Orthokeratinized odontogenic cyst showing cytokeratin-10 expression](image)
For CK 19, out of 10 cases of OOCs, four cases exhibited positivity. On analyzing various layers, among four positive cases, surface layers exhibited positivity in three cases, spinous layer showed positivity in two cases and no cases showed positivity in basal layers [Figure 4]. In 10 cases of EDCs, three cases exhibited positivity for CK-19. Analyzing various layers among three positive cases, no cases exhibited positivity in the surface layers and spinous layers, whereas three cases expressed positivity in the basal layer [Figure 5]. In 10 cases of OKCs, eight cases exhibited positivity for CK-19. Analyzing various layers among 8 positive cases, surface layers exhibited positivity in eight cases, spinous layers in four cases and no cases showed positivity in the basal layers [Figure 6]. On comparing, EDCs significantly differed from between OOC and OKC ($P = 0.028$).

On comparing between the markers CK-10 and CK-19, OOCs showed significant difference with $P = 0.011$ and EDCs showed highly significant difference with $P = 0.003$.

**DISCUSSION**

OOC is keratin-producing jaw cyst, and 75% of these cysts are associated with impacted teeth. Clinical presentation of OOCs resemble OKCs, yet they differ from OKCs with regard to biological behavior. Equally perplexing is its histological resemblance to EDCs. In this study, to clarify the nature of OOCs, keratin profile of OOCs for CK-10 and 19 was evaluated and compared with those of EDCs and OKCs. CK 10, a marker for mature keratinocytes, is specifically expressed in the spinous and surface layers of the orthokeratinized surface of the squamous epithelium and also is an early marker of keratin differentiation. CK-19 is expressed in odontogenic epithelium, secretory epithelium and in basal cells of squamous epithelium.
OKCs [Figure 1]. OOCs, EDCs and OKCs exhibited 100%, 100% and 50% positivity for CK-10, respectively. Strong positive CK-10 expression both in OOCs and EDCs is in accordance with the studies conducted by various authors who reported 100% CK-10 expression both in OOCs and EDCs/dermoid cysts. Contrary to 36% CK 10 expression in OKCs in Hoshino et al.\textsuperscript{[18]} study, there was 50% CK-10 expression in OKCs in our present study. Tsuji \textit{et al.}\textsuperscript{[20]} and Aragaki \textit{et al.}\textsuperscript{[21]} reported 12% and 35% CK 10 expression in OKCs in their respective studies.

Further upon analyzing CK-10 expression among various layers in all 10 cases of OKCs, \textit{Table 2} in the present study, 50% positivity is derived due to its expression entirely in surface layers contrary to 90%–100% expression in both spinous and surface layers in OOCs/EDCs. This is similar to the study conducted by da Silva \textit{et al.}\textsuperscript{[22]} in which OKCs expressed CK-10 only in the superficial layers, whereas CK-10 was intensely expressed both in the spinous and surface layers of OOCs/EDCs. According to the author, the intense expression of CK-10 in OOCs in the spinous and surface layers reveals a constant process of keratinization as seen in the epidermal tissue and EDCs. OOCs/EDCs showed fully differentiated, mature keratinocytes, whereas the expressions of CK-10 only in superficial layers in OKCs indicate a lack of mature keratinocytes and alteration in differentiation process. Thus, the variation in CK-10 expression indicates that OOCs and OKCs are separate entities. OOCs show a pattern of normal differentiation, and CK-10 is uniformly expressed in all the layers of epithelium suprabasally, whereas OKCs show altered keratinization with dysplastic differentiation with focal or superficial layers expressing CK 10.

In this present study, there is 90% CK-10 expression in both spinous and surface layers of all 10 cases of EDCs. CK-10 expression in OOCs and EDCs is almost similar (100% and 90%) both in terms of expression and pattern of expression. Similarly, according to various authors studies on comparing CK profiles of different cystic lesions, CK-10 in OOCs was found to be identical to EDC, both in expression and in the pattern of expression, i.e., positivity in spinous and superficial layers, suggesting that OOC could be considered as an intraosseous counterpart of EDC within the jaw bones. However, the fact that few OOCs are attached to the neck of the tooth is to be answered before naming them as intraosseous counterparts of EDC which are hypothesized to arise due to sequestration of the stomadial ectoderm into the developing jaw during embryogenesis.

CK-19 expression, analyzed in 30 cysts comprising 10 cases of OOCs, 10 cases of EDCs and 10 cases of OKCs, exhibited 40%, 30% and 80% positivity, respectively, in the present study [Figure 2 and Table 1]. Various studies by Hoshino \textit{et al.}\textsuperscript{[18]} Koizumi, Tsuji \textit{et al.}, Pawar \textit{et al.} and Vikas \textit{et al.} expressed 0%–50% CK-19 positivity in OOCs, 0% positivity in EDCs and 75%–100% positivity for OKCs, respectively. In the present study, CK-19 expression in OOCs and OKCs falls well within the reported range of earlier studies. However, 30% expression in basal layers of EDCs \textit{Table 3} in the present study was contrary to 0% expression in earlier studies. Unexpected outcome probably could be attributed to smaller sample size.

CK-19 is associated with immunoreactivity of preameloblasts and secretory ameloblastin embryological odontogenesis.\textsuperscript{[24]} CK-19 is observed exclusively in dental lamina of late stage tooth germ. The utility of CK-19 to identify odontogenic epithelium by numerous studies have been proved to be a useful tool in the diagnosis of cysts of odontogenic origin.\textsuperscript{[21]} This study also supports this concept as there was 80% CK-19 positive expression in OKCs. Moreover, on analyzing each layer, surface and spinous cell layers stained positive with CK-19 \textit{Table 3}, which is in accordance to studies conducted by Hayakawa \textit{et al.}, Okada \textit{et al.}, Pawar \textit{et al.} and Mc donald and Flecher where moderate-to-strong CK-19 positivity was seen in all layers except basal cell layer.

Mild expression of CK-19 may be seen in OOCs, only in superficial layers contrary to the present study where spinous cells too exhibited CK-19 positivity \textit{Table 3}. Pawar \textit{et al.}\textsuperscript{[24]} in their study reported 50% CK-19 positivity in OOCs. This variation could be attributed to small sample size.
Nonetheless, ambiguous cystic lesions in the jaws with sebaceous differentiation are sometimes reported. To call them as intraosseous EDCs or OKCs with sebaceous differentiation has paramount clinical significance due to varied behavior patterns of these two lesions. In such situations, CK-10 could be of certain diagnostic value, as CK-10 is expressed only in the superficial layers in OKCs compared to superficial and spinous layers expressing CK-10 in EDCs. Furthermore, difference in CK-10 expression between OOCs and EDCs is insignificant [Table 1]. Thus, it is difficult to distinguish OOCs and EDCs based on CK-10 expression, so as on histopathological examination. Thus, based upon CK10 expression, OOCs resembles EDCs.

On comparing CK-19 expression between groups, difference in CK-19 expression between EDCs and OKCs is significant. Whereas, difference in CK-19 expression between OOCs and EDCs and OKCs is insignificant, thus implying that CK-19 may not be a distinguishing marker to differentiate OOCs and OKCs and OOCs and EDCs. Application of CK-19 expression to distinguish the origin of OOCs and EDCs and OKCs and OKCs may not be possible according to the present study and based on CK-19 expression OOCs resemble both EDCs and OKCs. Further studies with larger sample size are necessary and other different CK markers can also be utilized to determine confounding origin of OOCs.

### CONCLUSION

Within the limitations of the current study, the following conclusions can be made:

1. Based on CK-10 expression, OOCs could be considered similar to EDCs. CK-10 can be a marker to distinguish OOCs and OKCs and EDCs and OKCs.
2. Based upon CK-19 expression, no significant differences are seen between OOCs and EDCs and OKCs and OKCs, implying that OOCs resemble both EDCs and OKCs.

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### Conflicts of interest

There are no conflicts of interest.

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### Table 3: Analysis of various layers of orthokeratinized odontogenic cyst, epidermoid cyst and odontogenic keratocyst using cytokeratin 19 marker

| Layers | Surface | CK-19 | Basal |
|--------|---------|-------|-------|
|        | Positive, n (%) | Negative, n (%) | Positive, n (%) | Negative, n (%) | Positive, n (%) | Negative, n (%) |
| Cyst   |         |       |       |       |         |       |
| OOC    | 3 (30.0) | 7 (70.0) | 2 (20.0) | 8 (80.0) | 0 (0.0) | 10 (100.0) |
| EDC    | 0 (0.0) | 10 (100.0) | 0 (0.0) | 10 (100.0) | 3 (30.0) | 7 (70.0) |
| OKC    | 8 (80.0) | 2 (20.0) | 4 (40.0) | 6 (60.0) | 0 (0.0) | 10 (100.0) |

**Significant at 0.1% level P < 0.001, *Significant at 5% level P < 0.05. OKC: Odontogenic keratocyst, EDC: Epidermoid cyst, OOC: Orthokeratinized odontogenic cyst, CK: Cytokeratin

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