Cytokeratin and fibronectin expression in orthokeratinized odontogenic cyst: A comparative immunohistochemical study

Kirti Kureel, Aadithya B Urs, Jeyaseelan Augustine
Department of Oral and Maxillofacial Pathology, Maulana Azad Institute of Dental Sciences, New Delhi, India

Abstract

Background: The orthokeratinized odontogenic cyst (OOC) is a rare developmental jaw cyst. It is crucial to distinguish it from keratocystic odontogenic tumor (KCOT) in all parameters including clinical behavior, immunohistochemical (IHC) profile and prognosis. This study aimed to analyze the IHC expression of cytokeratins and fibronectin in OOC, epidermoid cyst and dermoid cyst (EDC/DMC), dentigerous cyst (DC) and KCOT in order to explicate the pathogenesis of OOC.

Materials and Methods: Twenty-five cases of all study groups were incubated with CK10, CK13, CK19 and fibronectin antibodies and staining was assessed in the basal, intermediate and surface layers of epithelium. All the data were analyzed by SPSS software. Nonparametric Chi-square test and Spearman’s correlation test were applied.

Results: The overall expression pattern of CK10, CK13 and CK19 was similar and resembled each other in the study groups of OOC and EDC. The immunoexpression was almost akin to each other in DC and KCOT.

Conclusion: It was inferred from the results that the IHC profile of OOC is different from DC and KCOT and closer to EDC.

Keywords: CK10, CK13, CK19, epidermoid and dermoid cyst, fibronectin, immunohistochemistry, keratocystic odontogenic tumor, orthokeratinized odontogenic cyst, pathogenesis

INTRODUCTION

Orthokeratinized odontogenic cyst (OOC) was initially described under odontogenic keratocysts (OKCs), which is a rare cyst of jaw encompassing approximately 10% of cases of OKCs.

Schultz described it as a dermoid cyst (DMC) in 1927 and later Philipsen termed it as OKC.[1] It was classified as a type of OKC in the WHO classification published in 1992.[2] The WHO 2005 classification christened it keratocystic odontogenic tumor (KCOT) under tumors which has a tendency for recurrence and inherent potential for growth.[3] OOC was then delineated as a distinct entity, which is mostly small, nonexpansile and presents without any signs or symptoms. Radiographically, it is seen as a radiolucent lesion occurring frequently during the fourth and fifth
decades of life with no gender predilection. Common site of occurrence for OOC is posterior mandible. OKC depicts mild predilection for men and occurs mostly in the second and third decades of life. Most often, OKCs occur as single lesions, but when they are associated with nevoid basal cell carcinoma syndrome (NBCCS), multiple lesions can be found. In contrast to OKCs, the OOC does not recur and neither it is associated with the NBCCS.[4]

Epidermoid cyst and DMC (EDC and DMC) are benign developmental cysts, of which about 7% are detected in the head and neck region. Histologically, these cysts exhibit a keratinized stratified squamous epithelial lining.[5] Dentigerous cysts (DCs) constitute approximately 24% of all cysts observed in the jaws and are the most commonly found developmental jaw cyst. It is the second most common odontogenic cyst.[6]

Cytokeratins (CKs) are intermediate filament proteins specific for epithelium, and their expression varies depending on the type of epithelial cells. CKs can be utilized as markers of cell differentiation and malignant transformation.[7]

CK10, CK13 and CK19 are all acidic type of CK expressed in various tissues. CK10 (molecular weight, 56.5 kDa) is explicitly expressed in orthokeratinized surface of the squamous epithelium. Developing enamel organ and cells of dental lamina express CK13, molecular weight – 51 kDa. CK19 with molecular weight 40 kDa is expressed most secretory epithelia and the basal cells of squamous epithelium.[8] Fibronectin comprises a family of closely related, dimeric glycoproteins which are present both as soluble plasma constituents and within connective tissues and play an important role in embryonic development by mediating cell adhesion and migration.[9]

The aim of our investigation was to analyze the immunohistochemical (IHC) expression of CKs and fibronectin in the study groups which comprised OOC, EDC and DMC, KCOT and DC in order to exemplify the pathogenesis of OOC.

MATERIALS AND METHODS

Tissue specimens
The purpose of our retrospective study was to evaluate the IHC expression of CKs (10, 13 and 19) and fibronectin in OOC, EDC, DMC, DC and KCOT. The investigation was in compliance with the ethics committee of our institute, i.e., Maulana Azad Institute of Dental Sciences, New Delhi. Tissue blocks which were formalin fixed and paraffin embedded were retrieved from the archives of the Department of Oral Pathology and Microbiology, Maulana Azad Institute of Dental Sciences, New Delhi. These tissues were diagnosed as OOC, EDC, DMC, DC and KCOT on histopathological examination with hematoxylin and eosin (H&E) staining. The diagnosis was reconfirmed on fresh H&E-stained sections. The study groups were grouped into four groups and were categorized as, Group I – OOC, Group II – EDC and DMC, Group III – 25 cases of DC and Group IV – KCOT. Twenty-five cases were taken in each group.

Immunohistochemistry
Formalin-fixed and paraffin-embedded tissues were studied by immunohistochemistry. Sections of 2.5 µm were procured and mounted on slides coated with poly-L-lysine solution. The sections were dewaxed in xylene followed by hydration in graded ethanol. Antigen retrieval was performed with EDTA-Tris buffer (pH 9) in a pressure-based decloaking chamber (Digital DC2002INTL, Biocare Medical, Concord, California) at 95°C for 9 min and 30 s followed by bench cooling for 45–50 min. Two drops of peroxidase block followed by two drops of protein block were applied to the tissues for 10 min each. The slides were incubated with primary antibodies against CK10 – epithelial marker, Ab-2, mouse monoclonal antibody, Clone DE-K10 (Thermo Scientific Immunohistochemistry Solutions, Germany); CK13 – epithelial marker, rabbit polyclonal antibody (Thermo Scientific Immunohistochemistry Solutions, Germany); CK19 – epithelial marker, Ab-1, mouse monoclonal antibody, Clone A53-B/A2.26 (Thermo Scientific Immunohistochemistry Solutions, Germany) and fibronectin – mesenchymal marker, Ab-11, mouse monoclonal antibody, Clone FBN11 (Thermo Scientific Immunohistochemistry Solutions, Germany). Incubation was done in a hydrated chamber for 1 h followed by washing in TBS. Subsequently, the sections were incubated for 45 min with secondary antibody UltraVision Quanto Detection System, HRP DAB (Thermo Scientific Immunohistochemistry Solutions, Germany) in a closed hydrated chamber. Diaminobenzidine was used as chromogen, and the sections were counterstained with Harris hematoxylin.

Tissue sections of human skin were taken as positive control for expression of CK10, nonkeratinized stratified squamous epithelium of normal oral mucosa was taken as positive control for CK13 and normal salivary gland was taken as control for CK19. Basement membrane of normal oral mucosal epithelium was taken as control for fibronectin. The sections were stained along with the other study cases. By excluding, the primary antibodies served as negative controls for tissues.
Evaluation of immunohistochemical staining

Assessment of cytokeratin staining
The stained slides were scanned at low-power view (×10 objective and ×10 ocular). The epithelium was divided into three layers, i.e., basal layer (B), spinous or intermediate layer (I) and surface layer (S), and the intensity of cytoplasmic staining was observed individually in all the three layers in all the study groups at the high power (×40 objective and ×10 ocular). The scoring system used is as follows: 0 was graded as negative staining, 1 was graded as mild positivity, 2 was graded as moderate positivity and 3 was graded as strong positivity.

Assessment of fibronectin staining
The stained slides were scanned at low-power view (×10 objective and ×10 ocular), and the pattern of positive staining was observed at the high power (×40 objective and ×10 ocular) by three oral pathologists. The observations were graded according to the following pattern: negative staining (N), focal fibrillar (FF), focal nonfibrillar (FN), diffuse fibrillar (DF) and diffuse nonfibrillar (DN).

Statistical analysis
All the data were analyzed by SPSS software, version 20 (Armonk, NY, USA: IBM Corp).

Nonparametric Chi-square test was employed to evaluate the difference between different variables. The correlation between the variables was calculated using Spearman’s correlation.

\[ P \leq 0.05 \] was considered statistically significant. \[ P \leq 0.001 \] was considered highly significant.

RESULTS

The results of this study showed that there was no expression of CK10 in the basal layer of any of the study groups, negative expression of CK10 in all layers of DC, while KCOT expressed CK10 only in the surface layer. The expression of CK10 in OOC and EDC in the intermediate and surface layers was almost similar with respect to the number of cases. Predominantly, mild staining of CK13 was noticed in OOC and EDC in all the layers. Intense staining reaction was evident in KCOT and DC, with maximum number of cases showing intense positivity in the surface layer. Expression of CK19 ranged from negative to mild in OOC. EDC showed negative expression in all the layers, but very few cases showed mild expression in the basal and surface layers. However, CK19 staining was detected in almost all cases of DC and KCOT and intensity of expression varied from moderate to strong and mild to moderate in all the layers, respectively [Figures 1 and 2].

With regard to fibronectin expression, four types of expression patterns were interpreted, most common of which was DN expression [Figure 3]. This type of pattern was observed in maximum number of cases of EDC, followed by DC, KCOT and OOC. Another pattern observed was DF, which was seen in <6 cases in each study group and its expression was negative in KCOT. FF and FN patterns were recognized in very few cases, and FN pattern was not detected in OOC. Comparison of CK10, 13 and 19 expression was done individually in all the layers in all the study groups. In OOC, a significant difference was observed between CK13 and CK19 in the basal layer. Similarly, a stronger expression was noticed with CK10 and it was significantly different with CK13 in the intermediate layer [Table 1]. Assessment of immunoeexpression in EDC revealed significant differences between CK10 and CK19 in the basal layer [Table 2]. A significant difference was observed between CK13 and CK19 in the surface layer in DC [Table 3], and no significant difference was observed among any of the CKs in KCOT [Table 4]. The overall expression patterns between CKs in all the layers were analogous in DC and KCOT.

To better understand the histopathogenesis of OOC, it was compared individually with remaining three study groups by analyzing the expression of CK10, CK13, CK19 and fibronectin in all the layers.

Figure 1: (a) Photomicrograph showing CK10 expression in orthokeratinized odontogenic cyst (×10) inset (×40). (b) Photomicrograph showing CK13 expression in orthokeratinized odontogenic cyst (×10) inset (×40). Score 2 (moderate positivity) in the basal and intermediate and score 1 in the surface layers. (c) Photomicrograph showing CK19 expression in orthokeratinized odontogenic cyst (×10) inset (×40). Score 1 (mild positivity) in the surface layer.
When OOC was compared with EDC, a significant difference in staining was found with CK13 and CK19 in the basal and surface layers, respectively ($P \leq 0.05$). A highly significant difference in staining was found on comparison of CK19 in the basal and intermediate layers ($P \leq 0.001$). On comparison of OOC with DC, a highly significant difference in CK10 and CK13 staining was detected in the intermediate and superficial layers, although CK19 demonstrated a highly significant value in all the layers ($P \leq 0.001$). The results on comparison of OOC with KCOT showed a significant difference with CK13 staining in the intermediate layer ($P \leq 0.05$). A highly significant difference was deduced with CK10 in the intermediate layer, CK13 in the surface layer and CK19 in all the layers ($P \leq 0.001$).

A significant difference in the expression of fibronectin was obtained between OOC and EDC on comparison of fibronectin staining.

**DISCUSSION**

A substantial number of studies have investigated and explained the nature and histopathologic classification of OOC. In the present study, IHC expression of CKs and fibronectin was assessed and compared in OOC, EDC and DMC, KCOT and DC to elucidate the pathogenesis of OOC. Negative expression of CK10 in the basal layer of any of the study groups was found. Similar findings were reported by Hoshino *et al.*[10] and Tsuji *et al.*[11] Expression of CK10 only in the superficial layer in KCOT probably implies an absence or scarcity of mature keratinocytes in this lesion. Aragaki *et al.*[12] also found few cases of KCOT, which showed positive CK10 expression, while Dos Santos *et al.*[13] showed absence of immunostaining for CK10 in all 25 cases of KCOT, which included primary and recurrent tumors and those associated with NBCCS. Tsuji *et al.*[14] detected negative staining for CK13 in both EDC and OOC. Combining data from our study and few other studies, it could be considered that a different keratinization pattern exists in OOC, which is more similar to that of EDC than KCOT. Our findings corroborate...
### Table 1: Correlation coefficient and P values obtained by comparison of qualitative immunoscores of CK10, CK13 and CK19 in the basal, intermediate and superficial layers in orthokeratinized odontogenic cyst

|       | CK10B | CK13B | CK19B | CK10I | CK13I | CK19I | CK10S | CK13S | CK19S |
|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| r     | -     | -     | -     | -     | -     | -     | -     | -     | -     |
| P     | -     | -     | -     | -     | -     | -     | -     | -     | -     |
| CK10B |       |       |       |       |       |       |       |       |       |
| r     | 0.427* | 0.381 | 0.511** | 0.231 | 0.11 | 0.353 | -0.062 |       |       |
| P     | 0.033 | 0.069 | 0.267 | 0.6   | 0.083 | 0.768 |       |       |       |
| CK19B |       |       |       |       |       |       |       |       |       |
| r     | 0.427* | 1     | 0.584** | -0.247 | 0.485* | 0.845* | 0.123 | -0.406* |       |
| P     | 0.033 | 0.226 | 0.439 | 0.002 | 0.234 | 0.761 | 0.231 |       |       |
| CK10I |       |       |       |       |       |       |       |       |       |
| r     | 0.381 | -0.251 | 1     | 0.464* | 0.223 | 0.485* | 0.123 | -0.406* |       |
| P     | 0.06  | 0.226 | 0.02  | 0.284 | 0.014 | 0.557 | 0.044 |       |       |
| CK13I |       |       |       |       |       |       |       |       |       |
| r     | 0.511** | 0.162 | 0.464* | 1     | 0.167 | 0.743 | 0.751** | 0.098 |       |
| P     | 0.069 | 0.439 | 0.02  | 0.424 | 0.241 | 0     |       |       |       |
| CK19I |       |       |       |       |       |       |       |       |       |
| r     | 0.231 | 0.584** | -0.223 | 0.167 | 1     | -0.049 | 0.356 |       |       |
| P     | 0.267 | 0.284 | 0.424 | 0.081 | 0.666 | 0.596 |       |       |       |
| CK10S |       |       |       |       |       |       |       |       |       |
| r     | 0.11  | -0.247 | 0.485* | 0.243 | -0.049 | 0.071 |       |       |       |
| P     | 0.06  | 0.234 | 0.014 | 0.241 | 0.816 | 0.736 | 0.09  |       |       |
| CK13S |       |       |       |       |       |       |       |       |       |
| r     | 0.353 | -0.064 | 0.123 | 0.751** | 0.091 | 0.071 |       |       |       |
| P     | 0.083 | 0.761 | 0.557 | 0.666 | 0.752 |       |       |       |       |
| CK19S |       |       |       |       |       |       |       |       |       |
| r     | -0.062 | 0.248 | -0.406* | 0.098 | 0.111 | -0.346 | 0.352 |       |       |
| P     | 0.768 | 0.231 | 0.044 | 0.64 | 0.596 | 0.09 |       |       |       |

*P < 0.05 was considered statistically significant, **P < 0.001 was considered highly significant. Spearman’s rank correlation coefficient was calculated and staining of CK13 was found to be significantly different from that of CK19 in the basal layer (P = 0.033). Significant difference was observed between CK10 and CK13 in the intermediate layer (P < 0.05). CK: Cytokeratins

### Table 2: Correlation coefficient and P values obtained by comparison of qualitative immunoscores of CK10, CK13 and CK19 in the basal, intermediate and superficial layers in epidermoid cyst

|       | CK10B | CK13B | CK19B | CK10I | CK13I | CK19I | CK10S | CK13S | CK19S |
|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| r     | -     | -     | -     | -     | -     | -     | -     | -     | -     |
| P     | -     | -     | -     | -     | -     | -     | -     | -     | -     |
| CK10B |       |       |       |       |       |       |       |       |       |
| r     | 0.408* | 0.273 | 1     | 0.288 | -0.089 |       | -0.189 | 0.3     | 0.500* |
| P     | 0.043 | 0.186 | 0.163 | 0.672 |       | 0.366 | 0.145 |       | 0.011 |
| CK13B |       |       |       |       |       |       |       |       |       |
| r     | -0.174 | 0.354 | 0.288 | 1     | -0.062 |       | 0.405* | 0.02     | 0.288 |
| P     | 0.404 | 0.083 | 0.163 | 0.767 |       | 0.044 | 0.924 |       | 0.163 |
| CK10I |       |       |       |       |       |       |       |       |       |
| r     | -0.127 | 0.196 | -0.089 | -0.062 | 1     |       | 0.421* | 0.718** | -0.312 |
| P     | 0.544 | 0.347 | 0.672 | 0.767 |       | 0.036 | 0     |       |       |
| CK13I |       |       |       |       |       |       |       |       |       |
| r     |       |       |       |       |       |       |       |       |       |
| P     |       |       |       |       |       |       |       |       |       |
| CK19I |       |       |       |       |       |       |       |       |       |
| r     |       |       |       |       |       |       |       |       |       |
| P     |       |       |       |       |       |       |       |       |       |
| CK10S |       |       |       |       |       |       |       |       |       |
| r     |       |       |       |       |       |       |       |       |       |
| P     |       |       |       |       |       |       |       |       |       |
| CK13S |       |       |       |       |       |       |       |       |       |
| r     |       |       |       |       |       |       |       |       |       |
| P     |       |       |       |       |       |       |       |       |       |
| CK19S |       |       |       |       |       |       |       |       |       |
| r     |       |       |       |       |       |       |       |       |       |
| P     |       |       |       |       |       |       |       |       |       |

*P ≤ 0.05 was considered statistically significant. **P ≤ 0.001 was considered highly significant. Spearman’s rank correlation coefficient was calculated and significant difference was observed between CK10 and CK19 of basal layer (P = 0.043). CK: Cytokeratins

Journal of Oral and Maxillofacial Pathology | Volume 23 | Issue 1 | January-April 2019 69
Kureel, et al.: An IHC study on OOC to deduce pathogenesis

Table 3: Correlation coefficient and \( P \) values obtained by comparison of qualitative immunoscores of CK10, CK13 and CK19 in the basal, intermediate and superficial layers in dentigerous cyst

|        | CK10B | CK13B | CK19B | CK10I | CK13I | CK19I | CK10S | CK13S | CK19S |
|--------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| \( r \) | .     | .     | .     | .     | .     | .     | .     | .     | .     |
| \( P \) | .     | .     | .     | .     | .     | .     | .     | .     | .     |
| CK10B  | .     | 1     | 0.258 | .     | .     | .     | .     | .     | .     |
| \( P \) | .     | 0.214 | 0.006 | 0.909 | .     | .     | .     | .     | .     |
| CK19B  | .     | 0.258 | 1     | .     | .     | .     | .     | .     | .     |
| \( P \) | .     | 0.214 | 0.214 | 0.012 | .     | .     | .     | .     | .     |
| CK10I  | .     | .     | .     | .     | .     | .     | .     | .     | .     |
| \( P \) | .     | .     | .     | .     | .     | .     | .     | .     | .     |
| CK13I  | .     | 0.529 | 0.258 | 1     | 0.117 | .     | 0.736 | 0.115 | .     |
| \( P \) | .     | 0.006 | 0.214 | 0.578 | .     | .     | 0     | 0.583 | .     |
| CK19I  | .     | −0.024| 0.495 | 0.117 | 1     | .     | 0.199 | 0.602 | .     |
| \( P \) | .     | 0.909 | 0.012 | 0.578 | .     | .     | 0.341 | 0.001 | .     |
| CK10S  | .     | .     | .     | .     | .     | .     | .     | .     | .     |
| \( P \) | .     | .     | .     | .     | .     | .     | .     | .     | .     |
| CK13S  | .     | 0.314 | 0.199 | .     | 0.736 | 0.199 | .     | 1     | 0.402 |
| \( P \) | .     | 0.126 | 0.341 | .     | 0     | 0.341 | .     | .     | 0.047 |
| CK19S  | .     | −0.042| 0.039 | 0.115 | 0.602 | .     | 0.402 | .     | 1     |
| \( P \) | .     | 0.843 | 0.853 | 0.583 | 0.001 | .     | 0.047 | .     | .     |

*\( P \leq 0.05 \) was considered statistically significant, **\( P \leq 0.001 \) was considered highly significant. Spearman’s rank correlation coefficient was calculated and significant difference was observed between CK13 and CK19 of surface layer (\( P = 0.047 \)). CK: Cytokeratins

Table 4: Correlation coefficient and \( P \) values obtained by comparison of qualitative immunoscores of CK10, CK13 and CK19 in the basal, intermediate and superficial layers in keratocystic odontogenic tumor

|        | CK10B | CK13B | CK19B | CK10I | CK13I | CK19I | CK10S | CK13S | CK19S |
|--------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| \( r \) | .     | .     | .     | .     | .     | .     | .     | .     | .     |
| \( P \) | .     | .     | .     | .     | .     | .     | .     | .     | .     |
| CK10B  | .     | 1     | −0.034| .     | 0.613 | −0.196| 0.092 | 0.793 | −0.05 |
| \( P \) | .     | 0.001 | 0.871 | .     | 0.001 | 0.347 | 0.66  | 0     | 0.811 |
| CK19B  | .     | −0.034| 1     | .     | −0.049| 0.11  | −0.022| 0.091 | 0.218 |
| \( P \) | .     | 0.871 | .     | .     | 0.817 | 0.602 | 0.918 | 0.664 | 0.295 |
| CK10I  | .     | .     | .     | .     | .     | .     | .     | .     | .     |
| \( P \) | .     | .     | .     | .     | .     | .     | .     | .     | .     |
| CK13I  | .     | 0.613 | −0.049| 1     | −0.197| 0.35  | 0.486 | 0.339 | −0.339|
| \( P \) | .     | 0.001 | 0.817 | .     | 0.346 | 0.087 | 0.014 | 0.098 | .     |
| CK19I  | .     | −0.196| 0.11  | −0.197| 1     | −0.401| −0.295| 0.116 | .     |
| \( P \) | .     | 0.347 | 0.602 | .     | 0.346 | .     | 0.047 | 0.152 | 0.58  |
| CK10S  | .     | 0.092 | −0.022| 0.35  | −0.401| 1     | 0.255 | −0.127| .     |
| \( P \) | .     | 0.66  | 0.918 | .     | 0.087 | 0.047 | .     | 0.218 | 0.545 |
| CK13S  | .     | 0.793 | 0.091 | 0.486 | −0.295| 0.255 | 1     | 0.067 | .     |
| \( P \) | .     | 0     | 0.664 | 0.014 | 0.152 | 0.218 | .     | 0.047 | 0.749 |
| CK19S  | .     | −0.05 | 0.218 | −0.339| 0.116 | −0.127| 0.067 | 1     | .     |
| \( P \) | .     | 0.811 | 0.295 | 0.098 | 0.58  | 0.545 | 0.749 | .     | .     |

*\( P \leq 0.05 \) was considered statistically significant, **\( P \leq 0.001 \) was considered highly significant. Spearman’s rank correlation coefficient was calculated and no significant difference was observed on comparison of CKs within any of the layers. CK: Cytokeratins
with those reported by Kamath et al.\textsuperscript{[14]} and Pawar et al.,\textsuperscript{[15]} regarding CK19 staining, in which difference in staining intensity and number of layers stained was seen between KCOT and DC.

Tsuji et al.\textsuperscript{[11]} perceived in their study that OOC is unlikely to originate from odontogenic epithelium because of the absence of CK19 expression whereas positive expression of CK19 in KCOT and DC indicated odontogenic origin. Further, in most previous studies of CK19, mostly all odontogenic cysts under study tended to be positive.

It was hypothesized by Koizumi (2004)\textsuperscript{[16]} on analyzing negative expression of CK19 in OOC that it might arise from other cell rests such as gingival cells and mucosal cells, and intense expression of CK19 in KCOT reinforces the odontogenic origin as previously studied by other authors in various odontogenic cysts and tumors.\textsuperscript{[17,18]}

Regarding fibronectin expression, our results are somewhat similar to that of de Oliveira et al.\textsuperscript{[19]} and Poomsawat et al.,\textsuperscript{[19]} who ascertained a diffuse distribution of fibronectin in the fibrous capsule of KCOTs and DCs in a fibrillar or reticular pattern with a more profound reaction in KCOTs that may be responsible for its aggressive behavior. The expression of CK19 is found in a broad range of epithelial tissues, including many simple epithelia, diverse stratified epithelia and cultured keratinocytes. CK19 usually does not express in epidermis.\textsuperscript{[19]} CK10 which is a keratinization marker differed significantly from CK13 in the intermediate layer. Increased CK10 expression in the intermediate layer reflects keratinization pattern toward the epidermis as CK10 is a keratinization marker. CK13 is usually absent in the epidermis and adnexal structures and is a marker of mucosal stratified squamous epithelium.\textsuperscript{[20]}

Intense expression of CK10 in all the layers except basal in OOC affirms that a constant process of keratinization is taking place, as seen in the epidermal tissue and orthokeratinized epithelium of oral mucosa and in EDC/DMC. It can also be interpreted that the differentiation of OOC cells is almost complete as in epidermis and, therefore, can be associated with the nonaggressive behavior of OOC. CK10 is produced in the suprabasal cells of the epidermis and is considered as important for postmitotic differentiation in stratified keratinizing and cornifying epithelia. Considerable amount of experiments have indicated that CK10 specifically inhibits proliferation and cell cycle progression of keratinocytes, and loss of CK10 leads to increased keratinocyte turnover. Hence, CK10 can be considered as “keratinization markers” of keratinocyte.\textsuperscript{[7,8]}

From a bird’s eye view, the overall expression of various CKs differed significantly in almost all the epithelial layers when OOC was compared with DC and KCOT.

Our study showed mild expression of CK13 in OOC and EDC while intense expression was detected in KCOT and DC. Previously in many studies, other odontogenic lesions such as RCs and DCs also showed positive staining for CK13.\textsuperscript{[21]} Aragaki et al.\textsuperscript{[12]} interpreted that abundant expression of CK19 may be associated with generation of a polarized, vertically elongated cell shape noticed in KCOT. They recognized cells strongly positive for CK19 in dental lamina of a late stage tooth germ, which is in agreement to the view that KCOT arises from the remnant of dental lamina cells.

The results of many studies suggest that KCOT and DC resemble each other in the cellular differentiation, but the keratinization mode of OOC is different from that of KCOT and DC and a complex maturation process is involved in KCOT.\textsuperscript{[13]}

As there is no other study where statistical comparison has been made regarding fibronectin expression in OOC and EDC, it is difficult to deduce the basis of origin of OOC alone on one connective tissue marker. The reason being that fibronectin is a mesenchymal marker and miscellaneous factors can influence its expression, which has broad spectrum of normal physiological functions.\textsuperscript{[22]}

da Silva et al.\textsuperscript{[14]} found that the expression of fibronectin was that of a nonfibrillar pattern and diffused throughout the extracellular matrix of the capsule. Hence, it can be contemplated that a more intense expression of fibronectin in KCOTs may be related to the polarization of basal cells, as seen in odontoblasts during odontogenesis.\textsuperscript{[23]} Similar hypothesis was put forward by de Oliveira et al.,\textsuperscript{[19]} who hypothesized that the characteristic pattern of the KCOT lining epithelial basal cells might be related to the strong presence of fibronectin in the basement membrane.

These diverse and incongruent findings in fibronectin expression are probably because of the difficulty in interpretation of this protein in odontogenic cysts. Fibronectin was noticed both at the junction and throughout the cystic wall. As ECM can regulate cellular behavior via integrins, it is possible that the pattern of expression of fibronectin at the basement membrane in KCOTs is involved in the aggressive behavior of KCOTs.\textsuperscript{[19,24]}
CONCLUSION

On analysis of overall CK expression, it was deduced that CK expression in OOC was analogous to that of EDC. The expression patterns of DC and KCOT varied considerably from OOC. Similar CK10 expression in OOC and EDC suggests normal orthokeratinization taking place in both. Strong CK19 presence in DC and KCOT affirms the odontogenic origin. CK19 expression in few cases of OOC could be accounted to the fact that OOC is occurring in the jaws. OOC is acquiring CK19 during the development of the lesion the epithelial lining is either getting induced or under the influence of some interactions, it is acquiring this CK.

Fibronectin was found to be statistically different among OOC and EDC. This is the first study where fibronectin has been compared with EDC; the conclusion of this remains ambiguous. It could be due to the difference in growth patterns and characteristic nature of connective tissue wall of OOC and EDC.

On comprehensive evaluation of the results gathered from our study, we hypothesize that OOC shares more common features with EDC, a cyst of epithelial origin, though it is occurring in the jaws. Hence, OOC does not appear to be odontogenic in origin and probably represents the intraosseous counterpart of EDC.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

REFERENCES
1. Vuhahula E, Nikai H, Ijuhin N, Ogawa I, Takata T, Koseki T, et al. Jaw cysts with orthokeratinization: Analysis of 12 cases. J Oral Pathol Med 1993;22:35-40.
2. Kramer IR, Pindborg JJ, Shear M. Histological Typing of Odontogenic Tumours. 2nd ed. Berlin: Springer; 1992.
3. Barnes L, Eveson JW, Reichart P, Sidransky D. World Health Organization Classification of Tumours. Pathology and Genetics of Head and Neck Tumours. 3rd ed. Lyon: IARC Press; 2005.
4. da Silva MJ, de Sousa SO, Corrêa I, Carvalhosa AA, De Araújo VC. Immunohistochemical study of the orthokeratinized odontogenic cyst: A comparison with the odontogenic keratocyst. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2002;94:732-7.
5. Reissis D, Pfaff MJ, Patel A, Steinbacher DM. Craniofacial dermoid cysts: Histological analysis and inter-site comparison. Yale J Biol Med 2014;87:349-57.
6. Shear M. Cysts of Oral Regions. 3rd ed. Bristol: Wright P.S.G; 1992. p. 4-48.
7. Bragulla HH, Homberger DG. Structure and functions of keratin proteins in simple, stratified, keratinized and cornified epithelia. J Anat 2009;214:516-59.
8. Crivelini MM, de Araújo VC, de Sousa SO, de Araújo NS. Cytokeratins in epithelia of odontogenic neoplasms. Oral Dis 2003;9:1-6.
9. de Oliveira MD, de Miranda JL, de Amorim RF, de Souza LB, de Almeida Freitas R. Tenascin and fibronectin expression in odontogenic cysts. J Oral Pathol Med 2004;33:354-9.
10. Hoshino M, Inoue H, Kikuchi K, Miyazaki Y, Yoshino A, Har a H, et al. Comparative study of cytokeratin and langerin expression in keratinized cystic lesions of the oral and maxillofacial regions. J Oral Sci 2015;57:287-94.
11. Tsuji K, Wato M, Hayashi T, Yasuda N, Matsushita T, Ito T, et al. The expression of cytokeratin in keratocystic odontogenic tumor, orthokeratinized odontogenic cyst, dentigerous cyst, radicular cyst and dermoid cyst. Med Mol Morphol 2014;47:156-61.
12. Arakaki T, Michi Y, Katsube K, Uzawa N, Okada N, Akashi T, et al. Comprehensive keratin profiling reveals different histopathogenesis of keratocystic odontogenic tumor and orthokeratinized odontogenic cyst. Hum Pathol 2010;41:1718-25.
13. Dos Santos JN, Oliveira GQ, Gurgel CA, de Souza RO, Sales CB, de Aguiar Pires Valença Neto A, et al. Altered expression of cytokeratins in primary, recurrent and syndrome keratocystic odontogenic tumors. J Mol Histol 2009;40:269-75.
14. Kamath KP, Vidya M. Cytokeratin 19 expression patterns of dentigerous cysts and odontogenic keratocysts. Ann Med Health Sci Res 2015;5:119-23.
15. Pawar VM, Patel S, Pathak J, Swain N, Hosalkar R, Iyer J. Immunohistochemical analysis of calretinin and cytokeratin-19 in odontogenic keratocyst and ameloblastoma: A retrospective study. J Contemp Dent 2015;5:98-103.
16. Koizumi Y. Odontogenic Keratocyst, Orthokeratinized Odontogenic Cyst and Epidermal Cyst: An Immunohistochemical Study Including Markers of Proliferation, Cytokeratin and Apoptosis Related Factors. Int J Oral-Med Sci 2004;2:14-22.
17. Kumamoto H, Yoshida M, Ooya K. Immunohistochemical detection of amelogenin and cytokeratin 19 in epithelial odontogenic tumors. Oral Dis 2001;7:171-6.
18. Chiu PG, Weiss LM. Keratin expression in human tissues and neoplasms. Histopathology 2002;40:403-39.
19. Poonsawat S, Panyakeng J, Weerapradist W. Expression of basement membrane components in odontogenic cysts. Oral Dis 2006;12:299-6.
20. Moll R, Divo M, Langbein L. The human keratins: Biology and pathology. Histochem Cell Biol 2008;129:705-33.
21. Steil C, Stollenwerk C, Riediger D, Mittermayer C, Alfer J. Cytokeratin expression patterns for distinction of odontogenic keratocysts from dentigerous and radicular cysts. J Oral Pathol Med 2005;34:558-64.
22. Boudreau NJ, Jones PL. Extracellular matrix and integrin signalling: The shape of things to come. Biochem J 1999;339 (Pt 3):481-8.
23. Thesleff I, Partanen AM, Vainio S. Epithelial-mesenchymal interactions in tooth morphogenesis: The roles of extracellular matrix, growth factors, and cell surface receptors. J Clin Invest 1991;11:229-37.
24. Hynes RO. Integrins: Versatility, modulation, and signaling in cell adhesion. Cell 1992;69:11-25.