Expression of stem cell marker cytokeratin 19 in reduced enamel epithelium, dentigerous cyst and unicystic ameloblastoma – A comparative analysis

C N V Akhila1, G Sreenath1, A Ravi Prakash1, M Rajini Kanth1, A Vikram Simha Reddy1, S Naveen Kumar2

1Department of Oral Pathology and Microbiology, G.Pullareddy Dental College and Hospital, Kurnool, Dr. NTR University of Health Sciences, Vijayawada, 2Department of Prosthodontics, CKS Teja Institute of Dental Sciences, Dr.NTR University of Health Sciences, Vijayawada, Andhra Pradesh, India

INTRODUCTION

Odontogenic cysts and tumors represent a group of neoplasms which originate from tooth-forming apparatus.

Background: The process of odontogenesis is complex involving epithelial–mesenchymal interactions, along with the molecular signaling pathways triggering the initiating process. The triggering factors and cells precisely involved in the pathogenesis of odontogenic cysts and tumors are unknown. There is a vast array of biomarkers used to stain different sites, thereby helpful in diagnosing and evaluating the prognosis of these cysts and tumors. Cytokeratins are the intermediate filament proteins which maintain cell integrity and alter their properties in cysts and tumors. In the following study, cytokeratin 19 expression patterns are assessed quantitatively in reduced enamel epithelium, dentigerous cyst and unicystic ameloblastoma.

Aim: The aim of present study is to assess expression of CK 19, a stem cell marker in reduced enamel epithelium, dentigerous cyst and unicystic ameloblastoma, quantitatively.

Materials and Methods: The present study is carried out with 15 samples in each group. Reduced enamel epithelium is derived from the patients undergoing treatment for impacted teeth. Histopathologically diagnosed cases of dentigerous cyst and unicystic ameloblastoma were considered for the study. With the help of Olympus BX 43 microscope, with ProgRes microscope camera, the 45 slides obtained were examined. The region of interest was selected in each slide and number of cells positively stained was counted. Data were analyzed using SPSS software version 23. Descriptive for scale data, One way anova with post hoc Tukey’s test for intergroup comparison.

Results: The results showed significant P value < 0.05. Expression of CK 19 was highest in reduced enamel epithelium, followed by dentigerous cyst and unicystic ameloblastoma.

Conclusion: CK 19 can be used as diagnostic marker to differentiate between odontogenic cyst and tumor.

Keywords: Cytokeratin 19, cytokeratins, immunohistochemistry, odontogenic tumors, reduced enamel epithelium

Access this article online

Quick Response Code:  
Website: www.jomfp.in  
DOI: 10.4103/jomfp.JOMFP_316_19

The first signs of tooth development appear during the 6th week of gestation. At this stage, the oral epithelium thickens along the future dental arches. The primitive...
oral cavity or stomodeum is lined by an oral ectoderm or primitive oral epithelium. Each band of epithelium is called primary epithelial band; it divides into dental lamina and vestibular lamina. Remnants of the dental lamina are known to be the progenitors of certain cysts and tumors.

The triggering factors and cells precisely involved in the pathogenesis of cysts and tumors are unknown. Various studies showed that the incidence of odontogenic cysts and tumors is common in jaws, in which odontogenic cysts are even more common in occurrence.

Odontogenic cysts are classified into developmental and inflammatory cysts based on their origin. The origin of the developmental cysts is varied and is mainly attributed to the remnants of tooth germ. Dentigerous cyst is the most common developmental cyst that forms in up to 25% of all jaw cysts. Ameloblastoma is the common odontogenic tumor occurring in the jaws, with incidence ranging up to 60%. Ameloblastoma is divided into three types based on clinical, radiological and histological features as solid ameloblastoma, unicystic ameloblastoma and desmoplastic ameloblastoma.

Various studies have been performed to analyze the histopathogenesis of odontogenic cysts and tumors. One of such studies is related to detect antigen–antibody reaction of target cell, that is, immunohistochemistry (IHC). The biomarkers used in IHC stain specific cellular components of interest or rarely the entire cell.

There is a vast array of biomarkers used to stain different sites, thereby helpful in diagnosing and evaluating the prognosis of the disease. Cytokeratins are the intermediate filaments found at the epithelial–epithelial cell junctions. The expression of these different cytokeratins and their combinations was found to be unique to certain neoplasms. IHC studies are known to be widely used in final diagnosis, prognosis and research.

The present study is an IHC study aimed at detecting the cytokeratin 19 (CK19) in reduced enamel epithelium (REE), dentigerous cyst and unicystic ameloblastoma.

The aim of the present study is to evaluate the expression of CK19 in REE, dentigerous cyst and unicystic ameloblastoma. The present study is also aimed to find a correlation between REE and dentigerous cyst and REE and unicystic ameloblastoma.

**MATERIALS AND METHODOLOGY**

The present study was conducted at G. Pulla Reddy Dental College and Hospital, Kurnool. The study involved comparison of CK19 expression in REE, dentigerous cyst and unicystic ameloblastoma. Histopathologically diagnosed cases of dentigerous cyst and unicystic ameloblastoma were taken from the archives of oral pathology and microbiology department. REE was retrieved from the patients undergoing surgery for impaction. Cases with impacted teeth completely embedded in the socket, were selected.[Figure 1]. Infected dentigerous cysts and partially erupted teeth visible in the oral cavity were excluded from the study.

For REE, the tissue was obtained during the surgical procedure for impaction. The tissue surrounding the impacted tooth which attached to the cementoenamel junction of the tooth was isolated. The tissue was then put immediately into a container containing 10% neutralized buffered formalin. The tissue was left overnight, that is, 24 h for optimal fixation. After tissue processing and embedding, routine H and E staining procedure was carried out for all sections. The slides were examined under a microscope and histologically diagnosed slides with REE were included in the study. IHC was carried out on all the selected samples of REE, dentigerous cyst and unicystic ameloblastoma.

All the obtained 45 slides were examined under BX 43 microscope by Olympus manufacturers, with ProgRes microscope camera ProgRes® CapturePro microscope camera software from Jenoptic solutions software under surgery for impaction ×40 [Figure 2]. A total of 15

| Group | n  | Minimum | Maximum | Mean | SD  |
|-------|----|---------|---------|------|-----|
| REE   | 30 | 39.0    | 100.0   | 81.89| 16.10|
| DC    | 30 | 2.0     | 99.0    | 64.20| 23.58|
| UA    | 30 | 1.0     | 98.0    | 56.77| 33.13|

SD: Standard deviation, REE: Reduced enamel epithelium, DC: Dentigerous cyst, UA: Unicystic ameloblastoma

| Group (I) | Group (J) | Mean difference (I-J) | SE  | Significant |
|-----------|-----------|-----------------------|-----|-------------|
| REE       | DC        | 17.6889*              | 6.7683| 0.028*      |
| REE       | UA        | 25.1222*              | 6.7683| 0.001*      |
| DC        | UA        | 7.4333                | 6.5878| 0.499 (NS)  |

*The mean difference is significant at the 0.05 level. NS: Not significant (P>0.05), SE: Standard error, REE: Reduced enamel epithelium, DC: Dentigerous cyst, UA: Unicystic ameloblastoma
images were obtained. The total cell count per 100 cells was intended to measure in each image. Cell counting was carried out using software named QuPath Version 0.1.2 [Figure 3]. (developed by University of Edinburgh). The image properties were automatically detected by the software, as a H-DAB-stained slide. All the images were analyzed. The ROI was selected in the image, and the number of cells selected was taken as standard, that is, 100 cells. The number of cells stained positive and the number of cells stained negative were calculated.

RESULTS

Data were analyzed using SPSS software version 23. The software is well known and doesn’t require company name. If needed please add, SPSS software is developed by IBM. Descriptive for scale data, one way anova is performed and post hoc Tukey’s test is done for intergroup comparison.

Table 1 shows the mean positive percentage of cells showing the expression of CK19.

There was a statistically significant difference in the expression of CK19 in various groups ($P = 0.001$) (Figure 4). ANOVA comparison explained the overall significance. To know the individual pair-wise comparisons, post hoc test should be done [Figure 5 and 6] [Table 2].

Table 3 shows the individual pair-wise comparisons. There was a statistically significant difference between the expression of CK19 in between REE and dentigerous cyst (DC) ($P = 0.028$) and REE and UA ($P = 0.001$). There was no statistically significant difference in the expression between DC and UA ($P = 0.499$).

Table 4 shows the order of expression of CK19, which is REE > DC ≥ UA.

There was a statistically significant negative correlation between group and CK19 expression [Table 5].
Akhila, et al.: Expression of CK 19 in reduced enamel epithelium, dentigerous cyst and unicystic ameloblastoma

The epithelium associated with odontogenic cysts and tumors is derived from one of the following sources:

1. The REE of the tooth crown
2. Epithelial rests of Malassez, which are remnants of the Hertwig root sheath
3. Epithelial rests of Serres, which are remnants of the dental lamina
4. The tooth germ itself, which includes the enamel organ, dental papilla and dental sac.

The exact etiology and pathogenesis of odontogenic tumors is unfamiliar although recent studies have shown several molecular alterations in the formation of odontogenic tumors. Advanced research has shown that alteration in key genes is responsible for the growth of cells. Sonic Hedgehog (SHH) signaling molecules such as smoothened homolog (SMO), phenylthiocarbamide (PTC) and zinc finger protein (GLI1) have been observed in a number of odontogenic tumors.[2‑4]

IHC is an important application to determine monoclonal as well as polyclonal antibodies distribution in the tissue of interest in health and disease. IHC is widely used for the diagnosis of cancers and tumors.

Moll et al. classified a total of 19 human epithelial keratins with variable molecular weights within 40–70 kDa range, and subsequently an additional keratin was identified, CK20.[5] CK19 is the smallest one and it is exceptional because, unlike other cytokeratins, it lacks the typical domain.

Owing to the histopathogenesis of both dentigerous cyst and unicystic ameloblastoma related to REE, the expression of CK19 in dentigerous cyst and unicystic ameloblastoma was consistent with $P = 0.499$.

Significant $P$ values were observed on comparing REE with dentigerous cyst and REE and unicystic ameloblastoma ($P = 0.001$). Various similar studies were carried out using CK19 as a marker for prognosis and final diagnosis of lesions involving epithelial tumors, such as hepatocellular carcinoma, cholangiocarcinoma, pancreatic tumors and breast carcinomas. Expression of CK19 in odontogenic tumors and cysts was also assessed and the usefulness of the marker was proven. The results showed that the expression of CK19 decreased from normal tissue, dentigerous cyst and tumor, with lowest expression of CK19 in tumor. Hence, CK19 can be used as a prognostic and a diagnostic marker for odontogenic lesions.

The use of CK19 as a stem cell marker has been recently known and is more useful in understanding the histogenesis of the lesions. The present study emphasizes the significance of CK19 as a stem cell marker in odontogenic lesions also. The expression of CK19 was correlated with...
the histogenic origin of tissues arising at the site. As REE is predominantly involved with the occurrence of dentigerous cyst and its involvement with unicystic ameloblastoma is rare, the findings of the present study coincided with that of the literature.

CK19 is an epithelial marker of simple epithelia, predominantly similar to that of odontogenic cystic lining in dentigerous cyst. It is discernible in simple epithelia and basal cells of nonkeratinized stratified squamous epithelia. In a study by Bhaskar et al., CK19 expression was more significantly present in dentigerous cyst when compared to that of odontogenic keratocyst and radicular cyst. In our study, CK19 was expressed at a significant percentage of 64% in dentigerous cyst next to REE. Tsuji et al. and Stoll et al. in their study found positive expression for CK19 in dentigerous cyst. They concluded that CK19 is a marker of odontogenic epithelium.

Comparison of the staining intensities of different panel markers between the odontogenic cyst (DC) and odontogenic tumor (ameloblastoma) confirms and supports that considerable difference exists between these two lesions in terms of their clinical characters and biologic behavior. Hence, IHC analysis used in this study may be useful in differentiating DC and ameloblastoma.

Tsuji et al. and Stoll et al. in their study found positive expression for CK19 in dentigerous cyst. They concluded that CK19 is a marker of odontogenic epithelium. Yajima-Himuro et al. assessed the expression of CK19 in tooth germs, odontogenic epithelial tumors and nonodontogenic epithelial tumors. CK19 was expressed in the cytoplasm of the odontogenic epithelial cells. Immunoreactivity for CK19 was detected in all epithelial cells of enamel organ and dental lamina. In their study, whole epithelial cells of enamel organ and dental lamina were diffusely positive for CK19. Expression of CK19 was diffusely present in all neoplastic cells of ameloblastomas. In the present study also, the expression of CK19 was observed in the components of enamel organ, that is REE. According to their study, CK19 was one of the characteristics of epithelial odontogenic tumors, which correlated with the present study.

Odontogenic epithelial stem cells in humans are present in the active dental lamina in postnatal life, in remnants of dental lamina (the gubernaculum cord), in the epithelial cell rests of Malassez and in REE. The junctional epithelium and REE were proven to be odontogenic in origin and to have epithelial stem cell potential as reported by Yajima-Himuro et al. in 2014. Cytokeratin, the intermediate filament, can serve as a marker system for epithelial cells at different stages of cellular differentiation.

Cytokeratin markers can provide valuable supporting evidence in combination with standard marker system to subfractionate populations of stem, progenitor and differentiated cells in epithelial neoplasms. Overexpression of CK19 in REE reflects the immaturity of the tumor cell lineage, and may disrupt terminal differentiation and indicate the ability of cells to multiply.

CONCLUSION

Hence, CK19 may be used as a stem cell marker for the identification of cell of origin and also for understanding the pathogenesis of odontogenic cysts and tumors in a detailed aspect, thereby increasing the therapeutic approaches. CK19 can also be used as one of the diagnostic markers to differentiate between odontogenic cyst and tumor. Similar studies have to be undertaken to effectively rule out its importance in a broader perspective with a large sample size and appropriate molecular assays.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

REFERENCES

1. Nalabolu GRK, Mohiddin A, Hiremath SKS, Manyam R, Bharath TS, Raju PR. Epidemiological study of odontogenic tumours: An institutional experience. J Infect Public Health 2017;10:324-30.
2. Thesleff I. Epithelial-mesenchymal signalling regulating tooth morphogenesis. J Cell Sci 2003;116:1647-8.
3. Akagi T, Iida Y, Nakanishi H, Terada N, Morooka S, Yamada H, et al. Microvascular Density in Glaucomatous Eyes With Hemifield Visual Field Defects: An Optical Coherence Tomography Angiography Study. Am J Ophthalmol 2016;168:237-49.
4. Marianna Be. Molecular genetics of tooth development. Curr Opin Genet Dev 2005;19:504-10.
5. Moll R, Divo M, Langbein L. The human keratins: biology and pathology. Histochem Cell Biol 2008;129:705-33.
6. Bhakhar VP, Shah VS, Ghanehi MJ, Gosavi SS, Srivastava HM, Pachore NJ. A Comparative Analysis of Cytokeratin 18 and 19 Expressions in Odontogenic Keratocyst, Denticigerous Cyst and Radicular Cyst with a Review of Literature. J Clin Diagn Res 2016;10:ZC85-9.
7. Tsuji K, Wato M, Hayashi T, Yasuda N, Matsushita T, Ito T, et al. The expression of cytokeratin in keratoctytic odontogenic tumour, orthokeratinized odontogenic cyst, denticigerous cyst, radicular cyst and dermoid cyst. Med Mol Morphol 2014;47:156-61.
8. Stoll C, Stollenwerk C, Riediger D, Mittermayer C, Alfer J. Cytokeratin expression patterns for distinction of odontogenic keratoctysts from dentigerous and radicular cysts. J Oral Pathol Med 2005;34:558-64.
9. Yajima-Himuro S, Oshima M, Yamamoto G, Ogawa M, Furuya M, Tanaka J, et al. The junctional epithelium originates from the odontogenic epithelium of an erupted tooth. Sci Rep 2014;4:8467.