Original Article
Pak Armed Forces Med J 2020; 70 (Suppl-1): S10-14

# ANALYSIS OF CD10 EXPRESSION IN THE EPITHELIAL LINING OF ODONTOGENIC CYSTS

**Anam Ali, Muhammad Asif, Iram Ali*, Bismah Ahmad, Muhammad Tahir Khadim, Farhan Akhtar, Muhammad Sajjad Abbasi****

Armed Forces Institute of Pathology/National University of Medical Sciences (NUMS) Rawalpindi Pakistan, *The Hamdard College of Medicine & Dentistry (HCMD) Karachi Pakistan, **CMH Multan Institute of Medical Sciences/National University of Medical Sciences (NUMS) Multan Pakistan

## ABSTRACT

***Objective***: To investigate the expression of CD10 in the epithelium of Odontogenic keratocyst (OKC), Dentigerous and radicular cyst.

***Study Design***: Cross sectional analytical study.

***Place and Duration of Study***: Armed Forces Institute of Pathology, Rawalpindi, from Jan 2017 to Dec 2017.

***Methodology***: In this study, total of sixty cases were included with 20 of each Radicular cyst, Dentigerous cyst and Odontogenic keratocyst. Sections were stained with Haematoxylin and eosin (H&E) followed by Immunohistochemistry (IHC) staining for CD10 antibody. SPSS version 20 was used to analyse the results. Expression of CD10 was evaluated.

***Results***: Out of total 60 cases, 41 (68.3%) were male and 19 (31.7%) were female patients with age ranges from 11 to 75 years. Mandible was reported in 38 (63.3%) of the cases followed by maxilla in 22 (36.7%) of the cases. In this study 7 (38%) cases of Odontogenic keratocysts showed negative epithelial CD10 expression as compared to Dentigerous cysts and Radicular cysts (45% & 25%). In Odontogenic keratocysts 9 (45%) of the cases showed basal layer of positivity of epithelium. While in dentigerous cyst 11 (55%) of the cases showed CD10 expression in the superficial layer of epithelium and the entire epithelium was positive in almost all 15 (75%) cases of radicular cyst. Intensity of staining was moderate in maximum number of the cases of Odontogenic keratocyst and radicular cyst (40% & 35%) as compared to dentigerous cyst (20%). There was a statistically significant association between odontogenic cysts and epithelial CD10 expression ($p$=0.001).

***Conclusion***: Majority of odontogenic cysts showed positivity for CD10 marker. Odontogenic keratocysts showed basal, DCs showed surface and RCs showed full thickness positivity for this marker.

**Keywords**: CD10, Dentigerous cysts, Odontogenic keratocysts, Radicular cyst.



## INTRODUCTION

Odontogenic cysts originated from rests of odontogenic epithelium that might remain in the oral cavity following development of tooth[1]. These are one of the major source of bone destruction of jaw[2]. The overall occurrence of all oral and maxillofacial cysts is 90% and approximately 20% of all pathological jaw lesions. Radicular cyst has highest incidence in Pakistani population followed by dentigerous and OKC. Odontogenic cysts are more common in males than females. They can be found in all age group with a wide age range from 5 to 65 yearsbut most commonly seen in 2nd to 3rd decade of life[4]. The most common location for dentigerous cyst and OKC are mandible, and radicular cyst common in maxilla. Dentigerous cyst is a developmental odontogenic cyst, which is created by collection of fluid between crown of unerupted tooth and enamel epithelium. It is most commonly associated with unerupted tooth and frequently involve maxillary canine and third molar[3,4].

Odontogenic keratocyst OKC is another type of developmental odontogenic cyst that arise from the remnants of dental lamina in the jaw before the completion of odontogenesis. Previously WHO has classified OKC as developmental odontogenic cysts. In 2005 OKC was reclassified

**Correspondence: Dr Anam Ali,** House No. 165-C, Housing Colony Jannah Chowk, Gujranwal Road, Hafizabad Pakistan
*Email: dr.anamali@yahoo.com*
*Received: 06 Feb 2019; revised received: 15 Aug 2019; accepted: 16 Aug 2019*

under the heading of tumour but recently it was again classified as cystic lesion. Among odontogenic cysts, OKC has high recurrence rate, locally aggressive nature and specific histological features. Majority of OKCs show chromosomal abnormalities. This finding favours the neoplastic nature of OKC. Furthermore, loss of allele at some loci noted in OKC was same as that noted in squamous cell carcinoma[5]. PTCH gene mutation also has been noted in isolated OKC. This further supports the explanation of malignant transformation in OKC. Neoplastic and hamartomatous changes can occur at any stage of odontogenesis. In literature, 30 cases of odontogenic cysts were reported with malignant transformation of the epithelial lining of these cysts especially dentigerous cyst and OKC[11]. Different studies showed development of SCC, unicystic ameloblastoma and intraosseous mucoepidermoid carcinoma arising from previously diagnosed cases of dentigerous cyst and they also emphasized that each sample should be submitted for pathology examination even clinically present as typical odontogenic cyst[6,7].

The CD10 is a neutral endopeptidase also known as CALLA (common acute lymphoblastic leukemia antigen). Which is the prototype of metallopeptidases group which comprise the erythrocyte surface antigen KELL, endothelin converting enzyme (ECE-1 and ECE-2) and PEX gene. It is obtained from the brush border of kidney of rabbit. Physiological function of CD10 is to cleave a wide variety of biological peptides. CD10 has prognostic, diagnostic and therapeutic value in leukemic disorders[7,8]. The expression of CD10 is also noted in other malignancies like melanoma, nephroblastoma and children's neuroblastoma and in other epithelial tumors of adults. The presence of CD10 on epithelial cells in tumour area may be associated with cancer progression. The expression of CD10 is most commonly seen in different malignancies and showed that there is an association between histological grade, tumour size and expression of CD10 and their expression might be helpful to assess the disease status and prognosis of lesion. Thus, CD10 play a significant role in many biochemical and physiological processes of human body and can provide a promising treatment approaches for various dieases[9,10]. So, the objective of this was to investigate the epithelial expression of CD10 in OKC, dentigerous and radicular cyst.

## METHODOLOGY

This cross sectional analytical study was carried out at Histopathology department, Armed Forces Institute of Pathology, Rawalpindi from January 2017 to December 2017. Institutional Review board (IRB) approval has been taken. In this study, a total of sixty cases were included with 20 of each Radicular, Dentigerous cyst and OKC. The sample size was calculated by using Openepi calculator according to following parameters, anticipated population proportion 3.51%[11,12], Margin of error 5% and confidence level 95%. Sampling technique was non-probability consecutive. All cases of Dentigerous cyst, Radicular cyst and odontogenic keratocyst (OKC) diagnosed on hematoxylin and eosin staining irrespective of gender and age of patient were included. Scanty tissue and poorly fixed specimen were not included in this study.

Formalin fixed paraffin embedded tissue sections of OKC, Dentigerous and Radicular cyst were selected. Paraffin embedded blocks were cut into thin section of 3 to 5 micron and histopathological diagnosis of each case was assessed from freshly prepared H & E sections. After establishing diagnosis, immune-marker monoclo-nal antibody CD10 (Rabbit, catalogue no: PA0270; Leica Biosystem, UK) ready to use kit was applied. According to manufacturer guidelines, on each case, immunohistochemical staining of D10 was performed and tissue sections were fixed in incubator for 3 hours at 600. Then slides were deparaffinized and rehydrated in xylene and alcohol. Pressure cooker method (Heat induced epitope retrieval) was used for antigen retrieval at 1000 C temperature for 15 to 20 min in stock solution of 28 gm of tris and EDTA which were mixed in 1 litter distilled water. Hydrophobic boundaries were created by circle the tissue

with pap pen. Peroxidase blocker and PBS buffer solution were applied on slides. Primary antibody (Rabbit) was applied followed by PBS buffer solution and secondary antibody, Horse Reddish peroxidase, DBA chromogen and mounted with DPX. Immunostaining of CD10 was assessed and results were then analyzed with SPSS version 20.0. Frequency and percentages were calculated for qualitative variables. Chi square and Fisher exact test was applied for association. $p<0.05$ was taken as significant.

### Evaluation of CD10 Staining

Epithelial CD10 expression was analysed as described by Deepa *et al*, 2014[13] at 10 high power field (x400). Pattern of staining was brown membranous and cytoplasmic. Expression of CD10 was evaluated as following criteria: Negative, Positive in basal layer, Positive in superficial epithelium, Positive in entire epithelium. Intensity of staining was also noted as Negative, Weak, Moderate and strong.

## RESULTS

Total number of 60 cases were included in this study with 20 of each odontogenic keratocyst, dentigerous cysts and radicular cyst. Forty-one (68.3%) were male and 19 (31.7%) were female patients with age ranges from 11 to 75 years. Most commonly noted site was mandible in 38 (63.3%) of cases followed by maxilla, which was noted in 22 (36.7%) of cases. The number of cases that showed negative epithelial CD10 expression were high in dentigerous cysts 9 (45%) as compared to OKC and Radicular 7 (35%), 5 (25%) respectively. In OKC CD10 expression was mainly seen in basal layer of epithelium. While in dentigerous cyst majority of cases showing superficial epithelial CD10 expression; in the radicular cyst entire epithelium was positive in almost all of cases. Intensity of staining was also studied (table-I).

**Table-I: Immunoreactivity of CD10 (Number and Intensity) positive cells in Epithelial lining of OCs.**

| OCs | Epithelial Expression | | | | Intensity of CD10 staining | | | |
|---|---|---|---|---|---|---|---|---|
| | Negative | Positive in Basal Layer | Positive in Superficial epithelium | Positive in Entire epithelium | Negative | Weak | Moderate | Strong |
| **OKCs** | 7 (35%) | 9 (45%) | 1 (5%) | 3 (15%) | 7 (35%) | - | 8 (40%) | 5 (25%) |
| **DCs** | 9 (45%) | - | 11 (55%) | - | 4 (20%) | 9 (45%) | 4 (20%) | 3 (15%) |
| **RCs** | 5 (25%) | - | - | 15 (75%) | 5 (25%) | 3 (15%) | 7 (35%) | 5 (25%) |
| ***p*-value** | <0.01 | | | | >0.01 | | | |

OCs*= Odontogenic cysts, OKCs*= Odontogenic keratocyst, DCs*= Dentigerous cysts, RCs*= Radicular cysts.

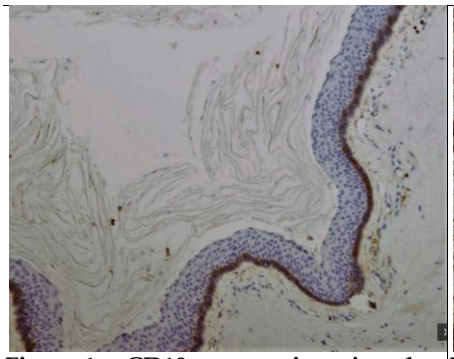

**Figure-1: CD10 expression in the epithelial lining of OKC: The image showing cytoplasmic and membranous staining in basal layer of epithelium with strong intensity (IHC CD-10 x100).**

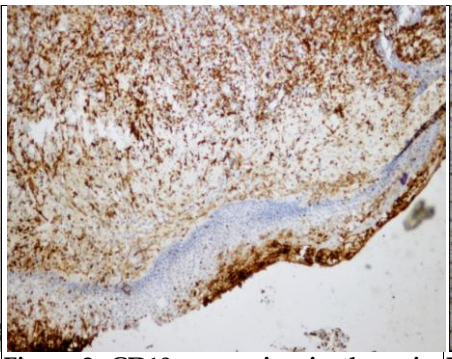

**Figure-2: CD10 expression in the epithelial lining of Dentigerous cyst: The image showing cytoplasmic and membranous staining in superficial cells of epithelium with moderate intensity (IHC CD-10 x100).**

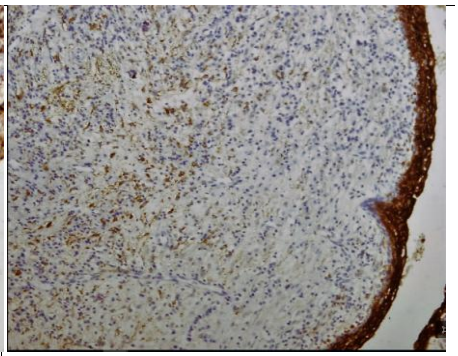

**Figure-3: CD10 expression in the epithelial lining of Radicular cyst: The image showing cytoplasmic and membranous staining in Full thickness epithelium with strong intensity (IHC CD-10 x100).**

## DISCUSSION

Approximately 90% of malignancies arise as carcinomas in the epithelial lining of tissue[14,15]. The epithelial lining may be the internal surface of organ such as breast, prostate and may be external surface of the skin. While other 10% of cancers originating as sarcoma from connective tissue and as leukemia from blood[16,17]. Although odontogenic cysts are benign lesions, carcinomatous degeneration has been described in the literature with an incidence that ranges from 0.13% to 3%[18-20]. The epithelial linings of OKC and dentigerous cyst are most commonly associated with malignant transformation such as mucoepidermoid carcinoma, squamous cell carcinoma, and odontogenic tumors like adenomatoid odontogenic tumour and ameloblastoma[12]. CD10 has been recognized as a membrane metalloendopeptidase, which distributes signalling peptide[21]. Initially CD10 was used only to differentiate between haematological malignancies however, currently it is widely used in different neoplasms. CD10 has significant role in the development of cancer and their progression[15].

In present study, brown membranous and cytoplasmic staining of CD10 expression was mainly seen in the superficial cells of epithelial lining of dentigerous cyst. While in OKC, majority of cases showed positivity in basal layer of epithelium and in radicular cyst entire epithelium showed positivity in most of cases. These results were in accordance with the study of Masloub *et al*1[23], who suggested that CD10 expression in dentigerous cyst might indicate the potential neoplastic activity of the epithelial lining of that cyst. Deepa *et al*[13], conducted a similar study and reported that majority of cases in their study showed CD10 positivity in the entire epithelium of radicular cyst. While superficial cells of epithelial lining were positive in 15% of cases of dentigerous cyst and in OKC's basal layer of the epithelium was positive in 20% of cases. They reported that the variation in the CD10 expression in the epithelial linings of these cysts may be due to proliferative potentiality of cells of epithelial lining of inflammatory cyst or cyst with secondary inflammation. They also suggested that in radicular cyst, CD10 expression in most of the epithelium might indicate some relation between CD10 and inflammation. Tadbir *et al*[24] also reported epithelial expression of CD10 in OKC, dentigerous cyst and ameloblastoma and the results showed that epithelial CD10 expression increased from dentigerous cyst (2.05 ± 1.5) to OKC (8.7 ± 2.2) to ameloblastoma (9.9 ± 2.1). While CD10 expression in dentigerous cyst was mainly seen in the superficial layer of epithelial lining. Similarly, in 2015, a local study conducted in University of health sciences, Lahore by Anjum *et al*[25], on epithelial CD10 expression in dentigerous cyst and ameloblastoma and reported that in all cases of dentigerous cyst, CD10 expression was seen in superficial cells of epithelial lining and concluded that high immunoreactivity in the superficial layer of the epithelial lining might be due to its neoplastic potential. In present study, majority of cases of OKC's and radicular cysts showed moderate intensity of staining as compared to dentigerous cyst.

We suggest further studies should be carried out to rule out the role of inflammation and CD10 in odontogenic cysts.

## ACKNOWLEDGEMENT

We would like to acknowledge all the studied patients for their cooperation. We wish to pay special gratitude to our families and friends for their continuous support and motivation.

## CONCLUSION

Majority of odontogenic cysts showed positivity for CD10 marker. OKC showed basal, DCs showed surface and RCs showed full thickness positivity for this marker.

## CONFLICT OF INTEREST

This study has no conflict of interest to be declared by any author.

## REFERENCES

1. Stucker FJ, De Souza C, Kenyon GS, Lian TS, Draf W, Schick B. Rhinology and facial plastic surgery. Springer Science & Business Media 2009 Mar 28. https://books.google.com.pk/books/about/Rhinology_and_Facial_Plastic_Surgery.html?id=EccLuc0mLWoC&redir _esc=y [Access 16 Apr 19].

2. Kamrujjaman M, Hasan S, Khan AD, Noor HT, Hasnat A. Clinicopathological evaluation of odontogenic jaw cysts. Updat Dent Coll J 2015; 5(2): 30-39.
3. Corso DG, Righi A, Bombardi M, Rossi B, Dallera V, Pelliccioni GA, et al. Jaw cysts diagnosed in an Italian population over a 20-year period. Int J Surg Pathol 2014; 22(8): 699-06.
4. Awan MU, Babar A, Ibrahim MW. Pattern and presentation of odontogenic jaw cysts: A clinical experience. Pak Armed Forces Med J 2017; 67(1): 102-06.
5. Paul R, Paul G, Prasad RK, Singh S, Agarwal N, Sinha A. Appearance can be deceptive: Dentigerous cyst crossing the midline. Nati j Maxillofac surg 2013; 4(1): 100-03.
6. Nayak MT, Singh A, Singhvi A, Sharma R. Odontogenic keratocyst: what is in the name?. J Natur Sci Biol Med 2013; 4(2): 282-85-91.
7. Passi D, Singhal D, Singh M, Mishra V, Panwar Y, Sahni A. Odontogenic Keratocyst (OKC) or Keratocystic Odontogenic Tumor (KCOT)-Journey of OKC from cyst to tumor to cyst again: a Comprehensive review with recent updates on who classification (2017). Int J Curr Res 2017; 9(1): 54080-86.
8. Henley J, Summerlin DJ, Tomich C, Zhang S, Cheng L. Molecular evidence supporting the neoplastic nature of odontogenic keratocyst: a laser capture microdissection study of 15 cases. Histopathol 2005; 47(6): 582-86.
9. Song YL, Zhang WF, Peng B, Wang CN, Wang Q, Bian Z. Germline mutations of the PTCH gene in families with odontogenic keratocysts and nevoid basal cell carcinoma syndrome. Tumor Biology 2006; 27(4): 175-80.
10. Kalburge JV, Latti B, Kalburge V, Kulkarni M. Neoplasms associated with dentigerous cyst: An insight into pathogenesis and clinicopathologic features. Arc Med Heal Sci 2015; 3(2): 309-13.
11. Gay-Escoda C, Camps-Font O, López-Ramírez M, Vidal-Bel A. Primary intraosseous squamous cell carcinoma arising in dentigerous cyst: Report of 2 cases and review of the literature. J Cli Exper Dent 2015; 7(5): e665-70.
12. Sreenivas SD, Lalita CS, Harsha G, Rao CV. Multiple pathology in a single lesion: AOT associated with dentigerous cyst. J maxillofac oral surg 2015; 14(1): 215-21.
13. Deepa K, Munisekhar MS, Suri C, Rajalbandi SK, Pradeep MR, Gothe P. Comparision of immunohistochemical expression of CD10 in odontogenic cysts. J clin diagn res 2014; 8(11): ZC35-ZC38.
14. Zhang LL, Yang R, Zhang L, Li W, Mac Donald-Jankowski D, Poh CF. Dentigerous cyst: a retrospective clinicopathological analysis of 2082 dentigerous cysts in British Columbia, Canada. Int J Oral Maxillofac Surg 2010; 39(9): 878-82.
15. Aiad HA, Hanout HM. Immunohistochemical expression of CD10 in cutaneous basal and squamous cell carcinomas. J Egypt Natl Canc Inst 2007; 19(3): 195-01.
16. Mishra D, Singh S, Narayan G. Role of B cell development marker CD10 in cancer progression and prognosis. Mol biol inter 2016; 2016(1): 01-09.
17. Oefner C, D'Arcy A, Hennig M, Winkler FK, Dale GE. Structure of human neutral endopeptidase (neprilysin) complexed with phosphoramidon1. J Mol Biol 2000; 296(2): 341-49.
18. Maguer Satta V, Besançon R, Bachelard Cascales E. Concise review: neutral endopeptidase (CD10): a multifaceted environment actor in stem cells, physiological mechanisms, and cancer. Stem Cells 2011; 29(3): 389-96.
19. Wtodek J. Clinical Significance of CD10 Expression in Cancer. Int Clin Pathol J 2017; 5 (1): 192-94.
20. Frank SA. Dynamics of cancer: incidence, inheritance, and evolution. Princeton University Press; 2007 Jul 22. https://www.amazon.com/Dynamics-Cancer-Incidence-Inheritance-Evolutionary/dp/ 0691133662
21. Borras-Ferreres J, Sánchez-Torres A, Gay-Escoda C. Malignant changes developing from odontogenic cysts: A systematic review. J Clin Experim Dentistr 2016; 8(5): e622.
22. Hormozi E, Fard VN, Naseri MA, Jahromi NH, Keshani F. Comparison of immunohistochemical expression of CD10 in keratocystic odontogenic tumor and ameloblastoma. Den Res J 2016; 13(2): 110-16.
23. Masloub SM, Abdel-Azim AM, Elhamid ES. CD10 and osteopontin expression in dentigerous cyst and ameloblastoma. Diagnostic Pathol 2011; 6(1): 44-51.
24. Tadbir AA, Geramizadeh B, Ranjbaran H. CD10 expression in dentigerous cyst, odontogenic keratocyst and ameloblastoma. Asian J Biol Sci 2013; 6(4): 221-27.
25. Anjum R, Naseem N, Saif R, Nagi A. Expression of CD10 in dentigerous cysts and ameloblastomas A reliable Prognosticator. Pak Oral Dental J 2015; 35(3): 1-4.