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Original Research Paper

Oral Pathology

EVALUATION OF CALRETININ EXPRESSION IN ODONTOGENIC CYSTS AND TUMORS - AN IMMUNOHISTOCHEMICAL STUDY

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ABSTRACT

Background: Calretinin (calbindin) acts as calcium binding protein, it functions as a regulator of various physiological and pathological processes like phosphorylation of target proteins, extracellular matrix remodeling, cell proliferation, apoptosis and gene regulation. Hence, its assessment in odontogenic tumors like Ameloblastoma was carried out. **Aims and Objectives:** To evaluate, assess, correlate and compare the Calretinin immune positivity in tumors and cysts of odontogenic origin. **Materials and Methods:** The present study comprised of 65 samples, fifteen cases each of Dentigerous cysts, Odontogenic Keratocyst, Unicystic Ameloblastoma and 20 cases of Ameloblastoma. All the slides were stained immunohistochemically using Calretinin. **Results and Observation:** The localization, staining intensity and percentage of positivity for Calretinin showed a statistical significant increase from DC to OKC to UA and Ameloblastoma which was 16.9, 31.9, 44.6, and 48.7 respectively. Stellate reticulum cells within Ameloblastoma showed intense immunostaining when compare with other tumors and cysts of odontogenic origin. **Conclusion:** calbindin can be used as diagnostic marker in Conventional Ameloblastomas. It also helps to know the biological behavior of these tumors,

KEYWORDS : Ameloblastoma, Calretinin, Dentigerous cyst, Immunohistochemistry, Odontogenic keratocyst, Unicystic Ameloblastoma.

INTRODUCTION

The multiformity of tumors of odontogenic origin reflects the complex development of the normal pattern of odontogenesis. The cysts of odontogenic origin also shows an aberration at some stages of odontogenesis.[1] Mechanisms that activate the proliferation of epithelial remnants are not known but a variety of developmentally and sub cellular factors are required for their differentiation and leads to tumor formation.[2]

Ameloblastoma is a destructive tumor of odontogenic origin that formed from epithelium of odontogenic origin within the mature fibrous stroma instead of odontogenic ectomesenchyme.[3] It has a aggressive growth pattern locally, more than 70% cases undergo malignant alteration and 2% metastasize to different sites.[4] It accounts for about 14% cases of all jaw tumors and cysts and it is the most prevalent odontogenic tumor in developing countries.[5] It is the second most common benign epithelial-odontogenic tumor which constitutes for about 1% of oral tumors and about 18% of odontogenic tumors.[6]It may arise from cell remnants in dental lamina, from a developing enamel organ, from the epithelial lining of an odontogenic cyst, or from the basal cells of the mucosa of oral cavity.[3]

Amongst the cysts, Odontogenic Keratocyst (OKC) attains with special considerations because of its aggressive nature, in the lower degree when it is compared with ameloblastoma.[10] there are radiographic and clinical similarities between Odontogenic Keratocyst and Ameloblastoma it may also reflect at histological stage if the sample of tissue is small and neoplastic epithelium shows reactive changes caused by inflammation.[7]

Squamous metaplasia is a relatively frequent phenomenon of Unicystic-Ameloblastoma and many of these lesions are lined by such nondescript epithelium, which can create diagnostic confusion with odontogenic cysts.[6] In such situations, more specific techniques might be needed for the aid of diagnosis, and to avoid underdiagnosis or overdiagnosis, leading towards inefficient treatment.[8]

Calretinin - 29 kDa protein encoded by CALB2 gene - an immunopositive marker for neoplastic ameloblastic epithelium, there is change in appearance among lesions because of its diverse histopathological characteristics and their developmental origin.[2]It may present only in the cells that present directly within the path of calcium transport on the way of enamel matrix acts as calcium ferry.[2]It is expressed as neoplastic epithelial cells and developing tooth buds in odontogenic tumors. It can also expressed in cell rest of malassez, remnants of serres and lining of cysts of odontogenic origin.[2]

Very few studies exist regarding calbindin expression in tumors and cysts of odontogenic origin. Thus, our study is aimed to asses the Calretinin positivity in selective tumors and cysts to understand its possible role in their pathogenesis and biologic behavior.

MATERIALS AND METHODS:

This present study was undertaken using 65 (N=65) tissue blocks embedded in paraffin wax of histologically diagnosed cases of Dentigerous cyst (DC) (Group-I, N= 15), Odontogenic Keratocyst (OKC) (Group-II, *N* = 15), Unicystic Ameloblastoma (UA) (Group III, *N* = 15), Ameloblastoma (Group IV, N=20). These tissue samples are obtained from archives of Department of Oral Pathology, Microbiology and Forensic Odontology, Government Dental College and Hospital

(GDCH), Hyderabad after obtaining institutional ethical clearance (**Reg No.ECR/300/Inst/AP/2013/RR-16(GDCH - IEC/PG/1924- dated 13-11-2019)** and consent from all the subjects.

These samples are processed as per the standard protocols. Two 3 micron thick sections were obtained from formalin fixative and tissue blocks embedded within paraffin wax. One section was stained with the hematoxylin and eosin and another was immunostained with primary antibody Calretinin **(Biogenex, Monoclonal Rabbit Anti-Calretinin)**

Methodology for Immunohistochemistry:

Immunohistochemical staining procedure for paraffin embedded tissue sections was performed as per instructions given by Biogenex IHC protocol.

3μm thick sections were taken from selected tissue blocks followed by deparaffinization and the sections are placed in EZ- retriever system of the antigen retrievals containing retrieval buffer and treated at 95°C for five cycles..Further slides were treated with Poly Excel hydrogen peroxide for ten minutes to stop endogenous peroxidase activity. Then tissue sections were incubate with a prediluted primary antibody towards Calretinin (Biogenex Rabbit Monoclonal) for a period of 30 minutes in room temperature and the tissue sections were than incubated with secondary antibody i.e., Poly Excel Poly HRP in room temperature - 10 minutes and tissues were completely covered with freshly prepared substrate chromogen solution (Poly Excel Stunn DAB) for five minutes. The sections are immersed in Harris hematoxylin for two minutes for blueing. The sections are kept immersed in xylene bath and later were mounted using DPX.

RESULTS:

The sum of 65 paraffin-embedded tissue blocks were chosen for this study which includes 15 tissue blocks each of DC (Group I), OKC (Group-II), UA (Group III) and 20 cases of the Ameloblastoma (Group IV).According to our study, the mean age of patients included in DC (Group I),OKC (Group II), UA (Group III), Ameloblastoma (Group-IV) was 30.60, 27.33, 34.13, 31.60 years respectively (Table 1).

Table 1: Age distribution across groups I, II, III, and IV

	Mean	Standard deviation	P value
Group I	30.6000	14.57885	0.606
Group II	27.3333	12.29789	
Group III	34.1333	15.58785	
Group IV	31.6000	13.14854	

Kruskal wallis test shows stastistically significant value

According to gender distribution in DC, OKC and UA 12(80%), 6(40%) and 11(73.3%) were males while 3(20%), 9(60%) and 4(26.6%) were females respectively. Out of Twenty cases in Ameloblastoma 11 (55%) were males and (9 %) were females (Table 2).

Table 2: Gender distribution across groups I, II, III, and IV

	Females	males	P value
Group I	3	12	0.096
Group II	9	6	
Group III	4	11	
Group IV	9	11	

Fisher Exact test, p value <0.05 was considered statistically significant

The mean percentage of Calretinin positive cells was calculated using one way ANOVA test in DC (Group I), OKC (Group-II) and UA (Group III) and Ameloblastoma is 16.9%, 31.9%, 44.67% and 48.70% respectively. The 'p' value obtained was 0.046* which shows statistical difference in percentage of immunopositive cells from DC to OKC to UA to Ameloblastoma (Table 3).

Table 3: Percentage of Calretinin positive cells among Group I, II, III and IV

	Mean	Standard deviation	P value
Group I	16.9867	35.18199	0.046*
Group II	31.9067	46.75921	
Group III	44.6733	34.93569	
Group IV	48.7000	31.39214	

Kruskal wallis test, p value <0.05 was considered statistically significant

In this study when we observed for the localization of the staining, immunopositive expression are seen within the suprabasal Stellate-reticulum like cells of about 72% cases of UA and in stellate-reticulum like cells in follicles of about 77% cases of Ameloblastomas. Location in Calretinin expression within the cell was compared using Chi square test among all groups. In DC 20% cases showed Cytoplasm expression , in OKC 26.6% cases showed Cytoplamic and 6.6% showed both in the cytoplasm and nucleus expression, in UA 33.3% cases showed cytoplamic expression, 26.6% cases showed both cytoplasm and nucleus expression. Out of twenty cases of Ameloblastoma, 50% cases showed cytoplasm expression and 30% cases showed both cytoplasm and nucleus expression. There is statistically difference with a 'p' value (0.011*) in location of Calretinin immunoexpression from cytoplasm to combined cytoplasm and nucleus to predominantly cytoplasm from DC to Ameloblastoma (Table 4)

Table 4: Location of Calretinin within cell in Groups I, II, III and IV

	Cytoplasm	Nucleus + cytoplasm	No	P value
Group I	3	0	12	0.011*
Group II	4	1	10	
Group III	5	4	6	
Group IV	10	6	4	

Fisher Exact test, p value <0.05 was considered statistically significant

In this study, the intensity of staining in Calretinin is calculated using Chi square test. Among the 15 cases with each DC, OKC and UA 3(20%), 4(26.6%) and 1(6.6%) cases showed mild positivity respectively 1(6.6%), 7(46.6%) cases of OKC and UA showed moderate intensity (**Figure 2 and 2a**), 1(6.6%) case of UA show intense staining (**Figure 3 and 3a**) and out-of 20 cases in Ameloblastoma , mild intensity of positivity was seen in 5(25%) cases, moderate in 5(25%) cases, intense in 6(30%) cases (**Figure 4 and 4a**). There was statistically significant difference with a 'p' value (0.000*) in intensity in staining from DC to Ameloblastoma (Table 5)

Table 5: Intensity of calretinin staining among Groups I, II, III, IV

	Mild	Moderate	Intense	No	P value
Group I	3	0	0	12	0.000*
Group II	4	1	0	10	
Group III	1	7	1	6	
Group IV	5	5	6	4	

Fisher Exact test, p value <0.05 was considered statistically significant

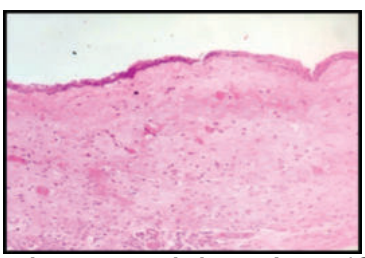

Figure 2: The photomicrograph showing lining of OKC. (H and E, 20X)

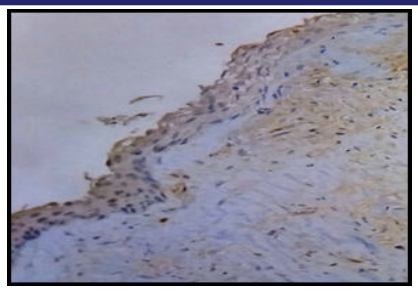

Figure 2a: The photomicrograph showing moderate expression of Calretinin in OKC. histochemistry, 20X) magnification

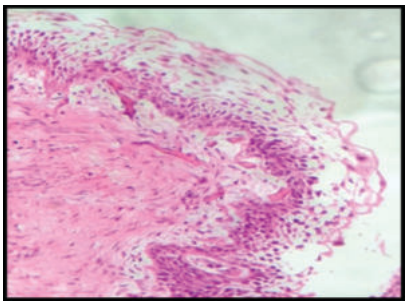

Figure 3: The photomicrograph of UA. (H and E, 20X)

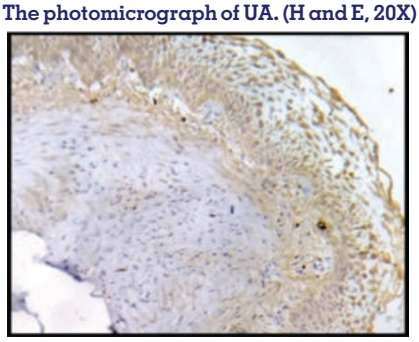

Figure 3a: The photomicrograph showing moderate expression of Calretinin in UA. (Immunohistochemistry, 20X)

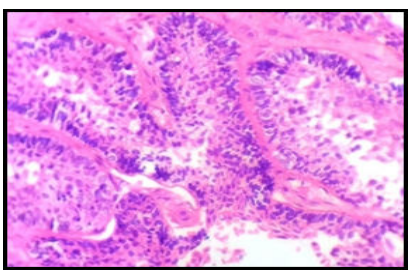

Figure 4: The photomicrograph of Ameloblastoma. (H and E, 20X)

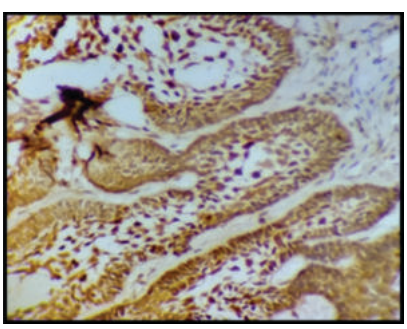

Figure 4a: The photomicrograph showing intense expression of Calretinin in Ameloblastoma. (Immuno histochemistry,20X) magnification

According to our study Pattern of staining was compared using **Fisher Exact test** among all groups. Out of 15 cases each in DC, OKC, UA 3(20%), 5(33.3), 8(53.3%) immunopositive cases showed diffuse staining and 1(6.6%) case of UA showed focal pattern of staining. Out-of 20 patients of Ameloblastoma 6 cases showed focal pattern whereas 10 cases showed diffuse pattern of staining of calbindin (Table 6,Figure 4)

Table 6: Comparison of pattern of staining among Group I (Dentigerous cyst), Group II (Odontogenic keratocyst), Group III (Unicystic ameloblastoma) and Group IV (Conventional Ameloblastoma)

	Focal	Diffuse	No	P value
Group I	0	3	12	0.002*
Group II	0	5	10	
Group III	1	8	6	
Group IV	6	10	4	

Fisher Exact test, p value <0.05 was considered statistically significant

DISCUSSION:

The jaws are host to a large variety of cysts and neoplasm's. The tumors and cysts of odontogenic origin are the most common lesions formed with the tooth-producing apparatus or with its remnants.[11] They might origin with odontogenic epithelium and from the ectomesenchyme with different degrees of the inductive tissue interactions.[9]Many benign jaw tumors and several cysts of odontogenic origin can exhibit a biologically aggressive course, often mimicking malignancies and sometimes making diagnosis difficult.[10] Therefore new molecular markers that assist in predicting prognosis and aggressiveness of tumors must be studied extensively in an individualized manner.

Calretinin, has a widespread distribution within the normal and neoplastic-tissues, same as in odontogenic epithelium during odontogenesis.[11]It is seen in tooth buds and also in neoplastic epithelial cells in some tumors of odontogenic origin. A few studies, hence, were conducted to evaluate its expression in tumors and cysts of odontogenic origin. It expressed as a diagnostic marker for Ameloblastoma. It has a role as a calcium sensor, buffer and also been established as an apoptotic factor.[12]Calcium signals play a main role in several cellular functions like Gene expression, Synaptic transmission, Cell cycle progression and apoptosis. There levels must be accurately controlled in spatial location and signal kinetics which is done by many calcium-binding proteins located throughout the cytoplasm.[13]

Mutation in calbindin gene be occurred and by the positivity of certain levels of calbindin gene in mRNA stage in ameloblastic cells of ameloblastoma but short of its protein. This might be accountable for short of functional maturation. It can also result in an difference in calcium levels which can effect further genetic imbalance in this cells, thereby having a role in tumorigenesis. This study was undertaken to assess and compare the efficacy of calbindin protein in tumors and cysts of odontogenic origin and to use this as a diagnosti - marker by evaluating its immunoexpression.[14]

In this study the number of immunopositive cells for Calretinin was calculated among which the cysts showed less percentage of positive cells whereas the tumors showed more percentage of positive cells. These are in accordance with study done by **D Silva et al (2013) and Imran et al (2016)** which showed more number of immunopositive cells in Ameloblastoma, UAs compared to other odontogenic cysts like DCs and OKCs suggesting that calbindin is expressed in the odontogenic epithelium of tooth germs at various stages of tooth development.[1,2]

In this study the number of immunopositive cells gradually increased from Group I to Group IV. There were no studies

done till date for evaluating the number of calretinin immunopositive cells in tumors and cysts of odontogenic origin.

In this study, staining intensity of calbindin showed stastistical significant difference among tumors and cysts of odontogenic origin. Which was in support to the study conducted by **Rudraraju et al (2019), Thakre et al (2017) and Sundaragiri et al(2010)** in which many of the cases in Ameloblastomas showed intense positivity when compared with other tumors and cysts suggesting the intense Calretinin positivity in enamel organ at early-late bell stages of physiological development of tooth which is similar to that seen in the stellate reticulum cells of ameloblastomas.[7,8,9] This findings were aganist the study done by **Koneru et al (2014)** in which the OKC shows strong staining positivity suggesting the function of calbindin with the regulation of its cell cycle, in apoptosis showing its aggressive behaviour.[15]

According to localization of Calretinin, positive expression it is seen in stellate reticulum cells in follicles of Ameloblastomas and UA. This may be due to retaining of immunophenotypic features of stellate reticulum cells in Ameloblastomas. In accordance to the study conducted by **Kalsoom et al (2015), Altini et al (2003) and Colemon et al (2001)** where most cases of UA and Ameloblastoma showed positive expression in stellate reticulum cells suggesting the role of calbindin as an antiapoptotic-proliferating protein in its outer layer and proapoptotic differentiating factor in stellate reticulum areas.[16,17,18] A study done by **D'Silva et al (2013)** in which Calretinin expression was also seen in peripheral ameloblast like cells along with star shaped cells suggesting the role of its expression in odontogenic-epithelium of tooth germs at various stages of development .[1]

In this present study, location of calbindin expression within the cell was evaluated which showed stastistical difference in which many cases showed cytoplasmic expression. These findings are in accordance of the study conducted by **Anandani et al (2014) and Varshney et al (2020)** which suggests the role of calbindin in extracellular matrix remodelling, cytoskeleton dynamics, apoptotic proteins and cell-cycle regulating factors in cytoplasm.[19,20] This findings were aganist the study done by **Piatelli et al (2003)** in which many of the cases of tumors and cysts of odontogenic origin showed both nuclear and cytoplasmic expression, which suggests that role of calbindin as a calcium-binding protein helps in cell-proliferation, phosphorylation of target proteins and genetic regulation.[21]

According to pattern of staining 50% cases expressed diffuse pattern of staining in the ameloblastoma and UA when compare to other cysts which was similar to the study conducted by **Alaeddini et al (2008)** in which many cases of UA and Ameloblastoma showed diffuse pattern of staining. This suggests the absence of mutations in Calretinin gene in stellate reticulum cells of UA and Ameloblastoma.[13]

Our study is different with the other studies conducted by **Pawar et al (2015)** in which focal positivity was observed in UA compared to solid / multicystic ameloblastomas. The cases of UA which are lined with ameloblastic epithelium showed no/little calbindin positivity because the better differentiation of epithelium the lesser expression of calbindin. [22]

CONCLUSION:

Calretinin has a role in Ameloblastoma where its level of expression and activity in Ameloblastoma shows Calretinin as a diagnostic tool. It also helps to know the biological nature of this tumor. Hence, it is considered as a most specific marker for Ameloblastomas. Negative expression in ameloblast-like cells might be due to its mutation, leading to lack of cytodifferentiation and may also have a role in the destructive nature of this tumor. The intense positive staining of this marker in ameloblastoma when compared to DC and OKC shows its aggressive nature. Calbindin expression in proliferating cells can be related to the destruction of this tumor and their response to various individual treatment strategies.

REFERENCES:

1. D Silva S, Sumathi MK, Balaji N, Shetty NK, Cheeramelil J (2013). Evaluation of Calretinin expression in Ameloblastoma and Non-Neoplastic Odontogenic Cysts-An immunohistochemical study. J Int Oral Health,5(6),42-8.
2. Imran A, Tanveer S, Ranganathan K, Rao UK, Joshua E, Thavarajah R (2016). Expression of calretinin and cytokeratin 19 in radicular cyst, dentigerous cyst, odontogenic keratocyst and ameloblastoma. J NTR Univ Health Sci.,5(2),11822.
3. Sciubba JJ, Eversole LR, Slootweg PJ. Odontogenic tumours. In: Barnes L, Eveson JW, Reichart P, Sidransky Deds (2005). World Health Organization classification head and neck tumours. IARC Press: Lyon, pp, 283–328.
4. Odukoya O, Effiom OA (2008). Clinicopathological study of 100 Nigerian cases of ameloblastoma. Niger Postgrad Med J;15: 1–5.
5. Lasisi TJ, Adisa AO, Olusanya AA (2013). Appraisal of jaw swellings in a Nigerian tertiary healthcare facility. J Clin Exp Dent,5,42– 7.
6. Nagi R, Sahu S, Rakesh N (2016). Molecular and genetic aspects in the etiopathogenesis of ameloblastoma: An update. J Oral Maxillofac Pathol,20,497-504.
7. Sundaragiri KS, Chawda J, Gill S, Odedra S, Parmar G (2010). Calretinin expression in unicystic ameloblastoma: An aid in differential diagnosis. J Oral Biosci,52(2),164-9.
8. Rudraraju A, Venigalla A, Babburi S, Soujanya P, Subramanyam RV, Lakshmi KR (2019). Calretinin expression in odontogenic cysts and odontogenic tumors and the possible role of Calretinin in pathogenesis of Ameloblastoma. J Oral Maxillofac Pathol,23,349-55.
9. Thakre GR, Desai RS, Mamatha GS, Chaudhari SM (2017). Role of Calretinin in the Pathogenesis of Odontogenic Cysts and Tumors. Sch. J. Dent. Sci,4(8),362-368
10. Piattelli A, Fioroni M, Iezzi G, Rubini C (2003). Calretinin expression in odontogenic cysts. J Endod, 29,394-6.
11. Fernandes AM, E. C. B. Duarte, F. J. G. S (2005). Pimenta et al.,"Odontogenic tumors: a study of 340 cases in a Brazilian population," Journal of Oral Pathology and Medicine , 34(10),583–587.
12. Fukumashi K, Enokiya Y, Inoue T (2002). Cytokeratin expression of constituting cells in ameloblastoma. Bull Tokyo Dent Coll,43,13-21
13. Alaeddini M, Etemad-Moghadam S, Baghaii F(2008). Comparative expression of calretinin in selected odontogenic tumors: A possible relationship to histogenesis. Histopathology;52,299-304
14. Lugli A, Forster Y, Haas P, Nocito A, Bucher C, Bissig H, *et al(2003).* Calretinin expression in human normal and neoplastic tissues: A tissue microarray analysis on 5233 tissue samples. Hum Pathol,34,994-1000
15. Koneru A, Hallikeri A, Sreekanth G, Krishnapillai R, Prabhu S (2014), Immunohistochemical expression of calretinin in ameloblastoma, adenomatoid odontogenic tumor, and keratocystic odontogenic tumor: a comparative study. appl immunohistochem mol morphol,22(10),762-67
16. Colemon H, Altini M,Ali H, Doglioni C (2001).Use of calretinin in the diagnosis of unicystic ameloblastoma. Blackwell science limited, histopathology,312-7.
17. Kalsoom F et al (2015). calretinin expression in the differential diagnosis of ameloblastoma and keratocystic odontogenic tumour. Pak armed forces med j,65(1),8-12.
18. Altini M, Coleman H, Doglioni C, Favia G, Maiorano E (2000). Calretinin expression in ameloblastomas. Histopathology,37,27-32
19. Anandani C, Metgud R, Singh K (2014). Calretinin as a diagnostic adjunct for ameloblastoma. Pathology research international,1-7
20. Varshney A, Aggarwal S, Gill SK, Aggarwal A, Jaiswal Y, Sharma J (2020). Comparison of calretinin expression in dentigerous cysts and ameloblastoma: An immunohistochemical study. Natl J Maxillofac Surg,11,224-30.
21. Piattelli A, Fioron M, Lezzi G, Rubini C (2003). Calretinin Expression in Odontogenic Cysts. J of endodontics;29(6):394-96.
22. Pawar V M, Patel S, Pathak J, Swain N, Hosalkar R, Iyer J (2015). Immunohistochemical Evaluation of Calretinin and Cytokeratin-19 in Odontogenic Keratocyst and Ameloblastoma: A Retrospective Study. J of Contemp Dentistry;5(2):98-103