Comparative Study of Calretinin Expression in Dentigerous Cyst, Odontogenic Keratocyst, and Unicystic Ameloblastoma

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Abstract

Odontogenic cysts and tumors are aspects that often discussed and quite important in the field of either oromaxillofacial surgery or pathology. Radiographically, the dentigerous cyst, odontogenic keratocyst (OKC), and unicystic ameloblastoma have a similar appearance in the form of unilocular radiolucent lesions. As a results of histopathological examination with hematoxylin eosin staining, these three lesions can be distinguished, however, many pathologists are misdiagnosed because of their similarity. Calretinin is a calcium binding protein that has been widely used for markers of malignancy in human tissues, due to its role in cell apoptosis which causes cell proliferation.

This study aims to observe and compare Calretinin expression in dentigerous cysts, OKC, and unicystic ameloblastoma.

34 paraffin blocks of dentigerous cysts, OKC and unicystic ameloblastoma were obtained by consecutive sampling from medical record data in the Cipto Mangunkusumo Hospital, Oral Surgery Division which had confirmed histopathological results at the Department of Anatomical Pathology Cipto Mangunkusumo Hospital during the period 2015-2019. All samples were subjected to immunohistochemical staning using mouse monolclonal Calretinin antibodies.

13 samples of dentigerous cysts, 6 samples of OKC, and 15 samples monoklonal of unicystic ameloblastoma were obtained. The positive interpretation of Calretinin was 1 sample of dentigerous cyst and 11 samples of unicystic ameloblastoma. Using Chi-square test value of significancy was 0,001 with odds ratio 49,5 (95% CI = 4.88 to 501.7).

Calretinin was expressed in dentigerous cysts and unicystic ameloblastoma with different percentages, but not in OKC. Calretinin can be used as a marker for unicystic ameloblastoma

Experimental article (J Int Dent Med Res 2021; 14(3): 965-969)

Keywords: Calretinin, dentigerous cyst, odontogenic keratocyst, unicystic ameloblastoma, expression.

Received date: 03 March 2021 **Accept date:** 18 June 2021

Introduction

Odontogenic cysts and tumors are frequently discussed topics in the field of surgery and oromaxillofacial pathology.[1] In routine dental examination, it is common to find unilocular lesions in patients accidentally.[2] These unilocular lesions may represent dentigerous cysts, odontogeniccerocyst (OKC) or unicystic ameloblastoma.[3] Dentigerous cysts is the most common among these lesions, but with the inherent ability for the metaplastic change, it could turn into more aggressive lesions such as OKC or ameloblastoma. These lesions have an identical shape on plain radiograph that show a radiolucency with well-defined sclerotic borders, and commonly located in posterior mandible.[4,5] The additional CT imaging might be needed to gives the exact information about the size, origin, content, and the relationship of the lesion with the surrounding tissue.[6] On histopathological examination, using hematoxylin eosi (HE) staining these three lesions can be distinguished, however many pathologists are misdiagnosed because these three lesions have a similar appearance (mimicking).[7] Unicystic ameloblastoma has minimum criteria for diagnosing, that is the presence of ameloblastomatous epithelium lining a

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macrocystic space, but still it may mimicking the lining of dentigerous cyst or radicular cyst.[8]
It is important to differentiate these three lesions to determine a treatment plan. In general, surgical treatment of cystic lesions and odontogenic tumors is divided into two, conservative from enucleation to radical curatase and radicals from enblock resection to segmental resection. In cases with large tissue destruction and high recurrence, radical surgical treatment is preferable.[9]

The various biological behavior of tumors exist as the key to differentiate each other, so that the combination of clinical examination, routine histopathology, and the use of other diagnostic support such as immunohistochemical examination (IHC) might be needed.[10] Immunohistochemical examination is an examination that is often performed to detect antigem in cells of a tissue that has been cut using the principle of specific antibody-antigen binding when conventional HE tests are difficult to distinguish.[11] Calretinin is a calcium binding protein, which is distributed in neuronal and non-neuronal cells. Calretinin was not expressed in the cusp tops of rat molar teeth, but was expressed in the deep enamel epithelium and presecretory ameloblast. Calretinin has been established as a specific marker for tumors in mesotheliomas, which are malignancies in the lung organs.[12,13] In cysts and odontogenic tumors, calretinin is only expressed in ameloblastoma.[14,15] In Indonesia, there are not many studies regarding the use of Calretinin in odontogenic cysts and tumors.[16] In uniocular lesions such as dentigerous cysts, OKC and unicystic ameloblastoma, it is expected that calretinin can provide additional data in differential diagnosis so that the appropriate treatment can be decided for the three lesions.

Materials and methods

Ethical approval from the Faculty of Dentistry, Universitas Indonesia research ethics committee. The search for samples in the form of paraffin blocks was obtained from matching patient data with the diagnosis of dentigerous cyst surgery, OKC, and unilocular ameloblastoma in the Oral Maxillofacial Surgery division and the results of histopathological examination in the department of Pathology Anatomy Faculty of Medicine/ Cipto Manungkusumo Hospital for the period 2015-2019. All samples were stained using the mouse monoclonal antibody calretinin, the samples were cut with a thickness of 4 µm (Finesse ME +, Thermo Scientific) and then the samples were placed on a slide. Glass preparations were heated using slides warmer XH 2001Premiere at 60 suhuC for 30 minutes. The sample was then deparaffinized by inserting it into xylol I, xylol II and xylol III each for 5 minutes. Then, rehydration was carried out with graded alcohol, namely absolute alcohol, 96% alcohol and 70% alcohol for 4 minutes each. After that, it is washed under running water for 5 minutes. Samples were immersed in methanol with 0.5% hydrogen peroxidase for 30 minutes to inhibit endogenous peroxidase activity. After that the sample was immersed in Tris EDTA 0.01 M with a pH of 9.0 and heated with a microwave Electrolux 230 V - 50 Hz for 5 minutes at level 8 and 5 minutes at level 1. Then the sample was cooled to room temperature for ± 30-45 minute. After chilling, the samples were washed in PBS pH 7.4 for 5 minutes. The sample was then placed in a moist block and the treatment area was limited using a Biogear paraffin pen, blocking the background with a sniper for 15 minutes. Then the application of primary antibodies, namely mouse monoclonal antibodies against Calretinin antibody plus pronase (Novocastra, Leica).[16,17]

Samples that had undergone examination were observed under the Olympus BX41 microscope and the level of Calretinin expression from each group was assessed using a modified score system of the Rudraju score system (2019) using imageJ software, as a positive control preparations from the testis and unicistic ameloblastoma samples were used and negative control. is a dental follicle.[18] After taking the image under a light microscope with a magnification of 400x in two fields of view, the image is stored. The number of cells expressed by calretinin will be brown and the total cells in the two visual fields are added and used as a percentage.[16-18]

The number of cells was counted three times by two different obeservers. Twice by the same observer at different times of 3 days, and once by another observer. When taking pictures and counting the number of cells, the two observers were accompanied by an Anatomical Pathologist. Accumulate clinical or radiological

data including age, gender, location of tumor / cyst, surgical diagnosis, entering and tabulating data on a computer with Microsoft Office Excel 2016 software. Data processing by comparing the score measured from each sample and analyzed statistically with SPSS version 25.0

Results

Total of 34 samples consisted of 13 samples of dentigerous cysts, 6 samples of OKC, and 15 samples of unicistic ameloblastoma. In this study, the ROC Curve was used to obtain the area under the sample curve with a positive interpretation of calretinin. We found an under curve area of 0.846 (95% CI = 0.704 to 0.988) and then used the Youden Index to determine the cut-off point between sensitivity and specificity. Obtained a pint cut-off at coordinates 0.4 (sensitivity 73% and specificity 27%) so that it can be concluded that a sample with a percentage less than 0.4% is considered negative, or positive if greater is equal to 0.4%. From these results, it was obtained that 11 samples of unicystic ameloblasatoma and 1 sample in dentigerous cysts were interpreted as positive, but none of them were positive for calretinin. Of the 11 samples of unicistic ameloblastoma, 8 samples had a focal distribution pattern with the most staining on stellate reticulum cells as many as 9 samples.

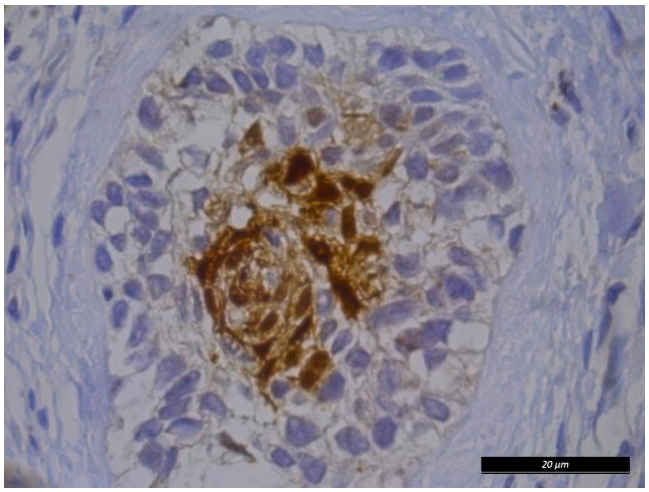

Figure 1. Immunopositive of Calretinin in unicystic ameloblastoma with focal distribution at nuclear-cytoplasm ameloblast cell (1000x).

In dentigerous cysts, 1 sample was positive for Calretinin, whereas in OKC none of them expressed Calretinin. Overall, the most stained cell samples were the stelate reticulum with a total of 10 samples from the total samples positive for Calretinin. The distribution pattern of calretinin was not found in the cytoplasm, but the same number was found in the nucleus and nucleus-cytoplasm in all calretinin positive samples (Figure 1, 2, 3), (Table 1, 2, 3, 4).

The difference in the proportion of calretinin expression between sample unicystic ameloblastoma and non unicystic ameloblastoma (dentigerous cyst) statistically using the Chi-square test was significant with p = 0.001 and odds ratio (OR) = 49.5, (95% CI = 4.88 to 501.7)

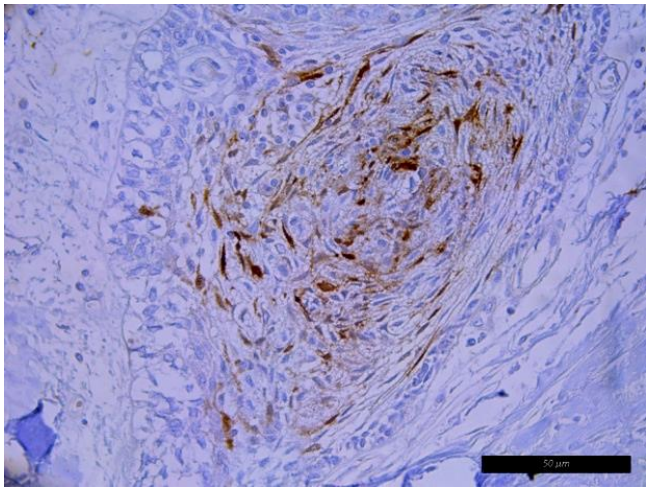

Figure 2. Immunopositive of Calretinin in unicystic ameloblastoma with focal distribution at nuclear-cytoplasm ameloblast cell (1000x).

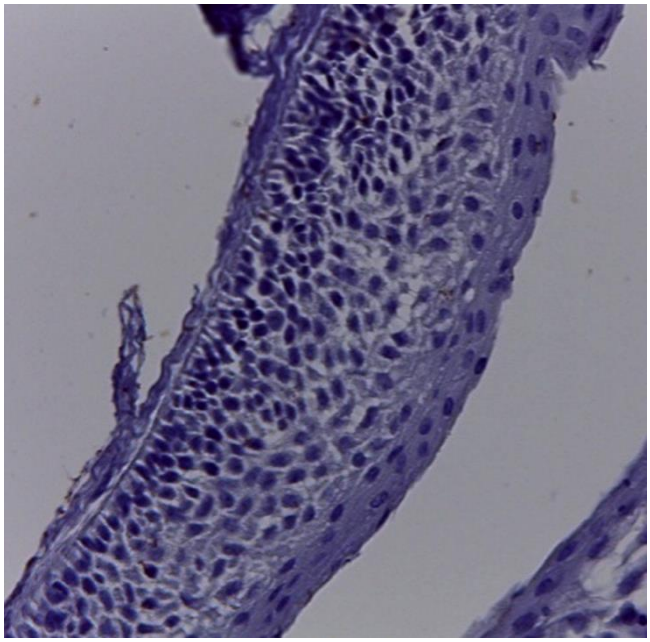

Figure 3. Imunonegative of Calretinin in OKC (400x).

Histopatology	33sample (n=34)	Percentage(%)
Dentigerous Cyst	13	58,8
OKC	6	17,6
Unicystic Ameloblastoma	15	44,2
Folicular	2	13,3
Achantomatous	1	6,7
Desmoplastic	1	6,7
Mix type	11	32,3

Table 1. Sample Distribution according to histopatology.

Histopatology	Location of Distribution		
	Nucleus	Cytoplasm	Nucleus and Cytoplasm
Kista Dentigerous (n=13)	6 (46,2%)	0 (0)	0 (0)
OKC (n=6)	1(16,7%)	0 (0)	0 (0)
Ameloblastoma Unicystic(n=15)	7 (46,7%)	0 (0)	6 (40,0%)
Folicular (n=2)	1 (50%)	0 (0)	1 (50%)
Achantomatous (n=1)	0 (0)	0 (0)	1 (100%)
Desmoplastic(n=1)	1 (100%)	0 (0)	0 (0)
Mix type (n=11)	7(63,6%)	0 (0)	4 (36,4%)

Table 2. Calretinin Distribution according to Localization.

Histopatology	Location		Stained Cell		Distribution pattern		
	Nucleus	Nucleus-cytoplasm	ameloblast	Stellate reticulum	both	focal	Diffuse
Dentigerous Cyst(n=13)	1(7,7%)	0	0	1(7,7%)	0(0)	1(7,7%)	0(0)
OKC(n=6)	0	0	0(0)	0	0(0)	0	0
Unicystic Ameloblastoma (n=15)	5(30%)	11(73,3%)	1(6,7%)	9(60%0	1(6, 7%)	8	3(20%)

Table 3. Immunopositive Calretinin Cell Distribution.

Histopatology	Calretinin Intepretation		P	OR
	Positifve	Negative		
Dentigerous Cyst (n=13)	1(7,7%)	12(92,3%)	0,001*	49,5
OKC(n=6)	0	6(100%)		
Unycystic Ameloblastoma (n=15)	11(73,3%)	4(26,7		

Table 4. Calretinin Intepretation.
* *Chi-square* , p< 0,05

Discussion

The interpretation of calretinin in unicistic ameloblastoma is in accordance with the research of Altini and Coleman (2000) which states that calretinin is only expressed in 81.5% of unicystic ameloblastoma samples.[17] On the other hand, Anandani (2014) stated that in cases of unicystic ameloblastoma the expression was as much as 50%. The staining in the ameloblastoma cells interpreted is stellate reticulum cells.[19] Chen J, Zhang Y and Denbesten P (2009) states that the stellate reticulum is a cell that provides nutrients for the ameloblast layer. The nutrients that are delivered also contain the calcium needed by the cells so that the Calretinin stain will give positive results.[20]

There was no calretinin expression in the cytoplasm in this study. Gotzos (1992) states that calcium ions play a role in the cell cycle. The quantity of calcium ions depends on the stage of the cell cycle. Calcium binding protein is the only protein that plays a role in the cell cycle.[21] The location of the CaBP (Calretinin) depends on the stage in the During the cell division phase that occurs in the cell nucleus, calcium is needed in large quantities either as a buffer or protein transport, so that caleretinin is expressed in the cell nucleus. Calretinin expression in the cytoplasm was also found at the mitotic stage, except at the anaphase and metaphase stages, which are still associated with the processes of the cell cycle.[22]

There were 4 samples (26.7%) of unicystic ameloblastoma did not express calretinin. Chen J, Zhang Y, Mendoza J, and Denbesten P (2009) states that calcium is needed for ameloblast cell differentiation, if the differentiation process of ameloblast cells is complete, the stellate reticulum cells will experience degeneration due to nutritional competition in tumor cells resulting in metabolic deficiency in stellate reticulum cells. This indicates that there is a difference in the concentration of calcium that causes calretinin not to be expressed.[20]

There was 1 sample of dentigerous cyst that expressed calretinin. According to Mistry (2001) states that dentigerous cysts and ameloblastomas are different because dentigerous cysts are formed after crown formation and enamel mineralization is complete, so that dentigerous cysts are not found in calcium along with calcium binding proteins.[13] Calretinn is not expressed on OKC, this is in accordance with Kalsoom's research (2015), but contrary to the research of Gaafar (2017) which

states that OKC can express calretinin.[23,25] This can result if the sample is small tissue or reactive epithelial neoplasm induced by inflammation, such as in unicystic ameloblastoma, and. it was also reported that OKC was derived from tooth-forming cells, which are the same as ameloblastoma, so it was difficult to distinguish between these two lesions.[24]

Conclusions

Calretinin was expressed in dentigerous cysts and unicystic ameloblastoma with different percentages, but not in OKC. Calretinin can be used as a marker for unicystic ameloblastoma.

Acknowledgements

The authors would gratefully like to thank the anonymous reviewers for their useful comments and for their vocational guidance.
This work was supported by Directorate of Research and Community Engagement Universitas Indonesia.

Declaration of Interest

The authors report no conflict of interest.

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