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EXPRESSION OF CALRETININ IN AMELOBLASTOMA AND ODONTOGENIC CYST IN A.W. SJAHRANIE GENERAL HOSPITAL SAMARINDA

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ABSTRACT

Background : *Ameloblastoma and odontogenic cysts have similar clinical and radiographic features but different treatments. Diagnosis is established by histologic features, but sometimes this step have difficulty making appropriate assessment because epithelial cells can show various variations. Preoperative misdiagnosis may result in inefficient treatment. In such situations, immunohistochemistry (IHC) with the use of proper markers might be needed for the differentiation of these lesions.* ***Purpose :*** *This study aimed to assess calretinin expression in ameloblastoma and various odontogenic cysts so that calretinin can be used as a specific diagnostic marker for ameloblastoma.* ***Method :*** *This was a retrospective analytical study of immunohistochemical examination of calretinin expression in ameloblastoma and odontogenic cysts. A total of eighty cases, in which thirty four cases of ameloblastoma and forty six cases of odontogenic cysts were included in the study. Slides were made from the paraffin blocks of each case and were stained immunohistochemically with calretinin.* ***Results :*** *In ameloblastoma, almost all subjects expressed calretinin, i.e. 33 (97.1%), followed by radicular cyst 7 (46.7%), odontogenic cyst 2 (40.0%), then dentigerous cyst 6 (26.1%) and OKC 0 (0.0%). The results of the analysis of the Chi-Square test showed a significant difference with a p-value < 0.001.* ***Conclusion :*** *The study concluded that calretinin was mainly expressed in ameloblastoma, whereas majority of odontogenic cyst groups showed negative for calretinin in various percentages. Calretinin may be a specific immunohistochemical marker for ameloblastoma.*

Keywords : *Ameloblastoma, Calretinin, Immunohistochemical, Odontogenic cyst, Odontogenic tumor*

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INTRODUCTION

Oral and maxillofacial lesions must be accurately identified and diagnosed to perform appropriate management. When abnormal tissue growth is found, several essential and systematic steps must be taken to identify and characterise the tissue. Therefore, the initial diagnosis of oral pathological lesions must be made correctly. Pathological lesions of the oral cavity can be broadly classified into the following main categories: (1) Cysts and cyst-like lesions of the jaws; (2) Benign tumours of

the jaws; (3) Malignant tumours; (4) Benign lesions of the oral soft tissues.[1,2] The prevalence of jaw cysts may be related to the large amount of epithelium that proliferates in the bone during tooth formation and along the line where the surface of the embryological jaw processus fuses. Jaw cysts can be divided into two types: (1) those arising from the odontogenic epithelium (i.e., odontogenic cysts) and (2) those arising from oral epithelium trapped between the fused processus during embryogenesis. An odontogenic tumour is a lesion that originates from epithelial, ectomesenchyme and mesenchymal elements that are still present or have tooth-forming parts. Both classification groups state that odontogenic tumours and odontogenic cysts are a group of lesions arising from the tooth-forming apparatus or its remnants, which are of odontogenic epithelial or ectomesenchymal origin with varying degrees of inductive tissue interaction.[3,4] Although many tumours of the jaws have many similarities in clinical, radiological, or histopathological features, their management may also be similar. Many benign, non-aggressive tumours can be treated conservatively with enucleation, curettage or both. Tumours and cysts almost have the same radiographic appearance but the management of each is different, it is necessary to find a specific marker to distinguish between the two types of cases. One of the most helpful supporting examinations is an immunohistochemical examination with appropriate markers for differentiating these pathological lesions.[5] This aims to avoid overdiagnosis or underdiagnosis so that clinicians can perform efficient treatment.

Calretinin is a 29 kDa calcium-binding protein of the EF-hand family. It is expressed in a wide array of standard and tumorigenic tissues. The dual functions of calretinin include as a Ca2+ buffer protein, which regulates the concentration and controls the distribution of Ca2+ in cells, and also as a Ca2+ sensor protein, which means a transmitter or mediator of the intracellular Ca2+ signal delivery process in cell proliferation and differentiation. During tooth seed formation, calretinin is focally expressed in the dental lamina, outer enamel epithelium, stellate reticulum and stratum intermedium. Its expression in the odontogenic epithelium during odontogenesis and in neoplastic odontogenic tissues has been demonstrated.[6] Calretinin expression was seen in epithelial-derived tissues during odontogenesis of rat molar tooth seeds, in dental pulp nerve elements, periodontal ligament and oral tissue viscerosensory nerve fibres, suggesting that this protein may play a role in enamel formation.[7]

Several studies of calretinin protein expression in the nervous system have been widely used as a specific and sensitive marker in benign and malignant mesothelial cells.[8,9] Calretinin has been suggested to be a specific immunohistochemical marker for ameloblastic lesion. In a study by Sharma et al. (2020) in 80 cases of odontogenic tumours, including ameloblastoma, unicystic ameloblastoma, dentigerous cyst and odontogenic keratocyst, calretinin was only expressed in ameloblastoma.[5] It is biological role remains unknown, but this protein act as a second messenger in the control of abnormal cell cycle proliferation. Based on this background, the present study aimed to assess calretinin expression in ameloblastoma and various odontogenic cysts so that calretinin can be used as a specific diagnostic marker in order to differentiating ameloblastoma from other odontogenic cyst lesions.

MATERIAL AND METHODS

The research design was an analytical retrospective study of immunohistochemical examination of Calretinin expression in ameloblastoma and odontogenic cysts. Research has received permission from the Health Research Ethics Commission of A.W. Sjahranie General Hospital No. 358/KEPK-AWS/IX/2021. The study subjects were paraffin block specimens of ameloblastoma and odontogenic cyst stored in the Anatomical Pathology section of A.W. Sjahranie General Hospital Samarinda, Indonesia during 2015-2021. These specimens were each subjected to histopathological examination for diagnosis. Identifying the patients used in these samples is kept confidential to fulfil the ethical aspects of patient confidentiality.

Sample selection was based on the completeness of data and tissue preparations of odontogenic tumours and cysts. Representative paraffin block samples were prepared with two new preparations, one each for Hematoxylin Eosin and Calretinin antibody staining. Immunohistochemical staining with Calretinin antibody was performed at the Anatomical Pathology Department of dr. Sardjito Hospital Yogyakarta, Indonesia. Paraffin block ameloblastoma samples and odontogenic cysts in the Anatomical Pathology Section of A.W. Sjahranie General Hospital and primary antibody polyclonal rabbit Calretinin from Ab clonal. Paraffin blocks were cut to 4 millimicrons thick using a rotary microtome as

preparation material for immunohistochemical (IHC) staining. Observation with a Nikon Eclipse E600 light microscope (USA) using the presence assessment criteria of immunoreactive cells was performed at the Anatomical Pathology department of A.W. Sjahranie General Hospital. Presence was assessed to see whether the staining was positive or negative and, if positive, which part of the epithelium was stained. The data obtained were then analysed using the Chi-square test with a significance of $P < 0.05$.

RESULTS

The research was conducted with samples from the Anatomical Pathology Laboratory of A.W. Sjahranie General Hospital from 2015-2021. In this study, the immunohistochemical staining results met the criteria: 34 slides for ameloblastoma types and 46 slides for odontogenic cyst types.

Table 1. Calretinin expression in ameloblastoma and several type of odontogenic cysts

Diagnosis	Calretinin expression	
	Positive	Negative
Ameloblastoma	33 (97.1%)	1 (2.9%)
Radicular Cyst	7 (46.7%)	8 (53.3%)
Odontogenic Cyst	2 (40.0%)	3 (60.0%)
Dentigerous Cyst	6 (26.1%)	17 (73.9%)
OKC	0 (0.0%)	3 (100.0%)

P < 0,05

The presence or absence of positive cells assessed calretinin expression in this study. If there was an expression, the subject was included in the positive group; if there was no expression, the subject was included in the opposing group. In ameloblastoma, almost all subjects expressed calretinin, i.e. 33 (97.1%), followed by radicular cyst 7 (46.7%), odontogenic cyst 2 (40.0%), then dentigerous cyst 6 (26.1%) and odontogenic keratocyst (OKC) 0 (0.0%). To determine the difference in calretinin expression in the various diagnosis groups, statistical analysis was performed using the Chi-Square test. The results of this Chi-Square test showed a significant difference with a p-value < 0.001 ($p < 0.05$) (table 1).

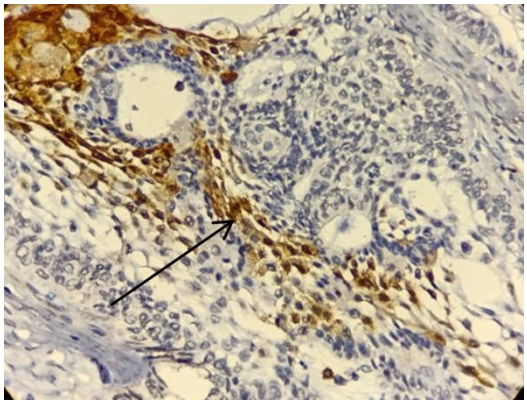

Figure 1. Calretinin expression in ameloblastoma. Black arrows indicate stellate reticulum-like cells (immunohistochemical staining)

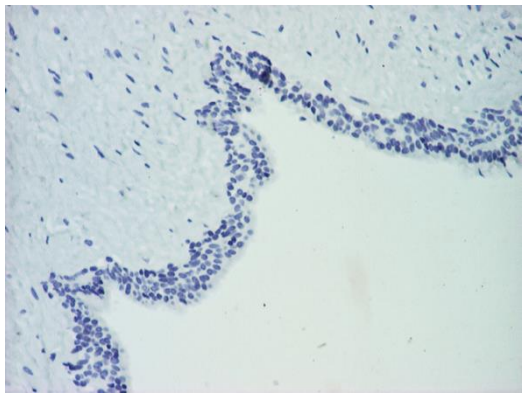

Figure 2. Negative calretinin expression in odontogenic cyst (immunohistochemical staining; 100x magnification)

DISCUSSION

In this study, 33 ameloblastoma cases (97.1%) were positive for calretinin immunohistochemical staining. Research conducted by Prassana et al (2022) showed that 100% of ameloblastoma cases were positive for this painting.[10] A study conducted by Anandani et al. (2014) was only able to show 50% of cases of unicystic ameloblastoma. It achieved maximum results of 100% of cases of multicystic ameloblastoma positive for calretinin painting.[7] A study by Koneru et al. (2014) also supported the previous study that 90% of ameloblastoma cases were positive for this antibody.[11] Varshney et al (2020) mentioned that in ameloblastoma, staining occurs mainly in the stellate reticulum, and there is no staining in ameloblast-like cells. In contrast to regular rat molar teeth, calretinin expression mainly occurs in the inner enamel epithelium, excretory and secretory ameloblasts, and the stellate reticulum.[12] In this study, it was

found that positive staining in the form of ribbons was found in the cytoplasm of stellate reticulum-like cells. The stellate reticulum is a cell that provides nutrients for the ameloblast layer.[13] The nutrients delivered also contain the calcium cells needed, so the staining with calretinin will yield positive results.

Ameloblastoma is bordered by epithelium that varies from having typical ameloblastic characteristics to metaplasia and an overall nondescript appearance consisting of multiple layers of non keratinized squamous cells. Typical ameloblastic characteristics include basal palisade columnar cells and stellate reticulum. Squamous metaplasia is a relatively frequent phenomenon in unicystic ameloblastoma and is mainly bordered by nondescript epithelium. This study showed that almost all cases of ameloblastoma had nondescript epithelium, and the area showed positive staining for calretinin.[14] Areas with nondescript epithelium boundary walls will be stained more strongly and diffusely compared to the epithelium that has ameloblastic characteristics or it can be said that even though the metaplastic cyst wall has lost the typical ameloblastic picture, the cells still leave immunophenotype characteristics that are still able to express calretinin.

In this study, 4 groups of odontogenic cyst produced positive and negative staining for calretinin immunohistochemical staining. The presence of positive staining for calretinin immunohistochemical staining requires a review of each diagnosis in the cyst group. This aims to reduce bias regarding the certainty of diagnosis or transformation of cysts into ameloblastoma. Dentigerous cysts were formed after crown formation and enamel mineralisation is complete. In contrast to ameloblastoma, dentigerous cysts arises from reduced enamel epithelium and do not contain calcium so that calretinin has no role in calcium transport.[10] A total of 6 cases of dentigerous cyst (26,1%) showed positive expression on calretinin immunohistochemical staining. Each of these cases was reviewed for histopathological examination with Hematoxylin Eosin and a diagnosis of a dentigerous cyst with mixed type ameloblastoma changes was obtained. This also supports the existence of areas of ameloblastoma found in dentigerous cyst that can also express calretinin. Calretinin is mainly expressed during the differentiation stage of enamel formation.[13] Only a weak calretinin band is seen at the secretion stage, so no other bands are detected during the maturation stage. The opinion expressed supports this study's results; calretinin plays a role in enamel formation, so in true dentigerous cysts, no positive expression of calretinin is found. This study concluded that calretinin was mainly expressed in ameloblastoma, whereas the majority of odontogenic cyst groups showed negative for calretinin in various percentages. Calretinin may be a specific immunohistochemical marker for ameloblastoma.

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