Original Article

Comparison of calretinin expression in dentigerous cysts and ameloblastoma: An immunohistochemical study

ABSTRACT

Background: Calretinin is a calcium-binding protein of 29-kilodalton (kDa), which is widely expressed in normal human tissues and tumorigenic tissues. Its expression in the odontogenic epithelium during odontogenesis and in neoplastic odontogenic tissues has been demonstrated. Unicystic ameloblastoma poses a diagnostic challenge, as its histologic presentation can be sometimes mistaken for cystic odontogenic lesions. In the present study, an attempt is made to overcome the confusion encountered in the diagnosis of dentigerous cyst and unicystic ameloblastoma, using the expression of calretinin in both lesions and to compare this expression with conventional ameloblastoma to accurately diagnose and differentiate these lesions.

Materials and Methods: A total of eighty cases, in which twenty cases each of ameloblastoma, unicystic ameloblastoma, dentigerous cyst, and odontogenic keratocyst (OKC) were included in the study. Slides were made from the archival blocks of each case and were stained immunohistochemically with calretinin.

Results: Correlation between calretinin staining and histopathological diagnosis was done, and it was found that all twenty cases of ameloblastoma showed positivity for calretinin, whereas 17 of twenty cases of unicystic ameloblastoma showed positivity for calretinin staining. All the cases of OKC and dentigerous cyst were negative for calretinin.

Conclusion: Calretinin may serve as an important diagnostic adjunct in the differential diagnosis of ameloblastoma and cystic odontogenic lesions.

Keywords: Ameloblastoma, calretinin, odontogenic cyst

INTRODUCTION

Odontogenic cysts and tumors can originate from various cell layers of the tooth germ.[1] These lesions represent a wide spectrum of clinical characteristics ranging from benign to locally invasive or malignant.[2] On occasion, the morphological and histopathologic patterns of odontogenic cysts and tumors mimic each other, and it is very difficult for the histopathologist to make a definitive diagnosis.[3] There are many techniques that can be used to distinguish odontogenic cysts from ameloblastoma-like cell surface carbohydrates demonstration with blood group specificity, characterization of cytokeratin profiles, AgNORs, and quantifying cell proliferation markers as proliferating cell nuclear antigen (PCNA) and Ki67. These techniques have shown differences occurring between various cysts and unicystic ameloblastoma, but considerable overlap still exists, and none of the above techniques can be used to routinely distinguish these lesions.[4] Recently,

Anchal Varshney, Shivani Aggarwal, Sharanjeet Kaur Gill, Ashim Aggarwal[1], Yashmi Jaiswal[2], Jaishree Sharma

Department of Oral Pathology and Microbiology, Manav Rachna Dental College, [1]Department of Oral and Maxillofacial Surgery, Manav Rachna Dental College, Faridabad, Haryana, [2]Senior Consultant, Ahead Dental Clinic, New Delhi, India

Address for correspondence: Dr. Yashmi Jaiswal, H 23 Aaron Ville, Sector 48, Gurugram - 112 001, Haryana, India. E-mail: yashmijaiswal@gmail.com

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immunohistochemistry (IHC) with the use of proper markers has become very popular for the differentiation of these pathologic lesions. Several markers have been studied in odontogenic lesions as specific molecular markers are characteristic of particular cellular events.[5]

Calretinin is a calcium-binding protein (CaBp) of 29-kilodalton (kDa). CaBp acts as a mediator of signaling intracellular calcium ions, which have been considered as an important second messenger intervening in several cellular processes, which includes proliferation and differentiation.[6] Calretinin has been found as a specific immunohistochemical marker for neoplastic ameloblastic epithelium and has a role in the transition of the epithelial lining of the odontogenic cyst to ameloblastomatous epithelium. It may act as a diagnostic tool for differentiating cystic odontogenic lesions from ameloblastic tumors.[7]

Unicystic ameloblastoma is those cystic lesions that have clinical, radiographic, or gross features of jaw cyst, but on histological examination, they show a typical ameloblastomatous epithelium lining the cyst cavity, with or without luminal or mural tumor proliferation. As this tumor shows considerable similarities with dentigerous cyst and at times, both lesions become histologically indistinguishable.[8]

In the present study, an attempt is made to overcome the confusion encountered in the diagnosis of dentigerous cyst and unicystic ameloblastoma, using the expression of calretinin in both lesions and to compare this expression with conventional ameloblastoma to accurately diagnose and differentiate these lesions.

MATERIALS AND METHODS

Case selection

The present study was conducted on the archival formalin-fixed paraffin-embedded tissue blocks of ameloblastoma, unicystic ameloblastoma, dentigerous cyst, and odontogenic keratocyst (OKC) from the Department of Oral Pathology and Microbiology, Manav Rachna Dental College, Faridabad. The study was conducted according to ethical guidelines approved by the Institutional Ethical Committee, Manav Rachna Dental College.

Study design

A total of eighty cases, in which twenty cases each of ameloblastoma, unicystic ameloblastoma, dentigerous cyst, and OKC were included in the study. Slides were made from the archival blocks of each case and were stained immunohistochemically with calretinin.

Immunohistochemistry

Four micrometres thick sections were deparaffinized in xylene, and they were rehydrated by immersion in a graded series of ethanol. Micro sections were then dipped in freshly prepared 3% H2O2 for 20 min to block endogenous peroxidase activity and rinsed with phosphate-buffered saline for 5 min. Then, antigen retrieval was done using citrate buffer (pH-6.0) in a pressure cooker, for two to four whistles, and then the solution is allowed to bench cool to room temperature, and then the sections were washed in tris buffered saline (TBS) (pH 7.2–7.6) for two changes for 2 min each. The sections were then incubated with a prediluted primary calretinin antibody (Biogenex Ind. Pvt. Ltd) at room temperature overnight in a humid chamber. Sections were then washed in TBS thoroughly for 2–4 min. The super enhancer of the secondary antibody was added for 30 min and then washed in Tris wash buffer thoroughly for 2–4 min. Then DAB (1 drop) was added for 10 min. For visualization, sections were incubated with HRP for15 min. The sections were then lightly counterstained using Mayer's hematoxylin for 10 s, after which sections were gently washed in running tap water for 60 s. Mounting was done in DPX mounting.

Immunohistochemical analysis

The sections stained with calretinin antibody were evaluated for the presence, localization, distribution, and intensity of immunoreactive cells. The presence was evaluated to estimate whether the staining is positive or negative and if positive, which epithelial layer is stained. Localization was evaluated whether the staining is in the nucleus (N), cytoplasm ©, or both. Distribution was evaluated as being either focal (involving <50% of positive cells) or diffused (involving >50% of positive cells). The intensity was graded depending on the number of positive cells seen, 0– no staining, 1– weak staining, 2– moderate staining, and 3– intense staining.

Statistical analysis

The data were analyzed using the Epi-info version 6.0 and Statistical Package for the Social Sciences version 16 (SPSS Inc. version 16, Chicago, Illinois). The statistical test used was the Chi-square test for comparison of categorical variables. The *P* value was taken statistically significant when <0.05 (confidence interval: 95%).

RESULTS

Correlation between calretinin staining and histopathological diagnosis were performed, and it was found that all twenty cases (100%) of ameloblastoma showed positivity for calretinin [Figure 1] whereas 17 (85%) out of twenty cases of unicystic ameloblastoma showed positivity for calretinin

staining [Figure 2]. All the cases of OKC and dentigerous cyst were negative for calretinin OKC [Figure 3], Dentigerous cyst [Figure 4]. Thus, there was a significant difference in the distribution of staining for calretinin in the different odontogenic cysts/tumors (Chi-square value = 69.742, $P < 0.001$) [Table 1].

The intensity of calretinin staining was compared between different subtypes of ameloblastoma and unicystic ameloblastoma using the Chi-square test. Of 20 cases of ameloblastoma comprising of acanthomatous, follicular, and plexiform subtypes, 6 cases had score 1, and 14 cases had score 2. Of 20 cases of unicystic ameloblastoma, 3 cases had score 0, 10 cases had score 1, and 7 cases had score 2. The intensity of calretinin staining was compared using the Chi-square test. There was a statistically significant difference in the distribution of calretinin staining in different subtypes of ameloblastoma and unicystic ameloblastoma.(Chi-square value = 2.265, $P = 0.043$) [Table 2].

The distribution of calretinin staining patterns was compared between different subtypes of ameloblastoma and unicystic ameloblastoma. Of twenty cases of ameloblastoma, 6 cases showed focal distribution [Figure 5] of calretinin staining, and 14 cases showed diffuse pattern [Figure 6]. Of 20 cases of unicystic ameloblastoma, 17 cases showed a focal distribution pattern and 3 cases were negative for calretinin. Thus, there was a statistically significant difference in the distribution pattern of calretinin staining in different subtypes of ameloblastoma and unicystic

Table 1: Correlation of calretinin staining in histopathologically diagnosed cases

Histopathological diagnosis	Total number of cases	IHC staining	
		Positive	Negative
Ameloblastoma	20	20	
Unicysticameloblastoma	20	17	3
OKC	20		20
Dentigerous cyst	20		20

IHC: Immunohistochemistry, OKC: Odontogenic keratocyst

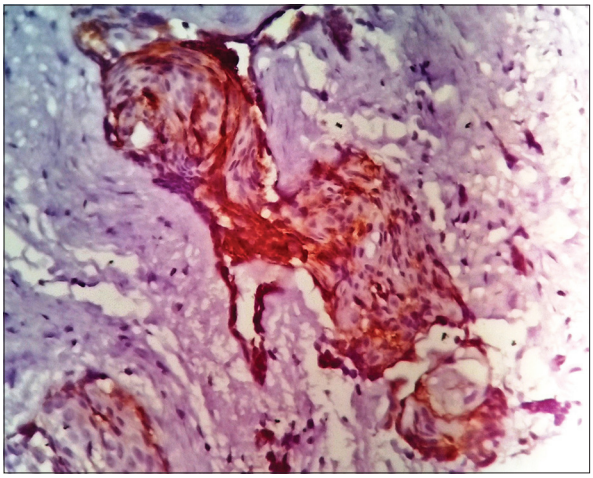

Figure 1: Positive calretinin staining in the ameloblastic follicle (×40)

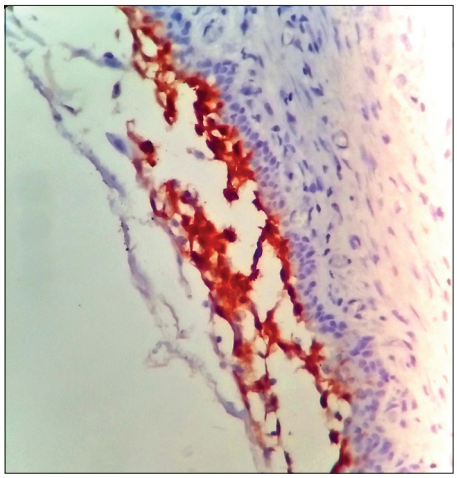

Figure 2: Positive calretinin staining in the lining of unicystic ameloblastoma (×40)

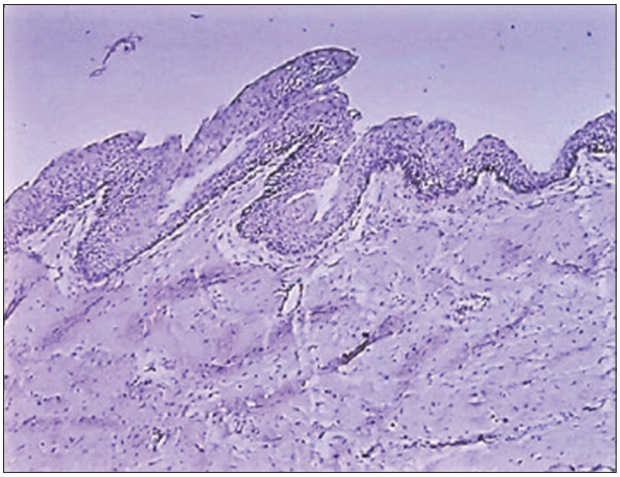

Figure 3: Negative calretinin staining in odontogenic keratocyst (×10)

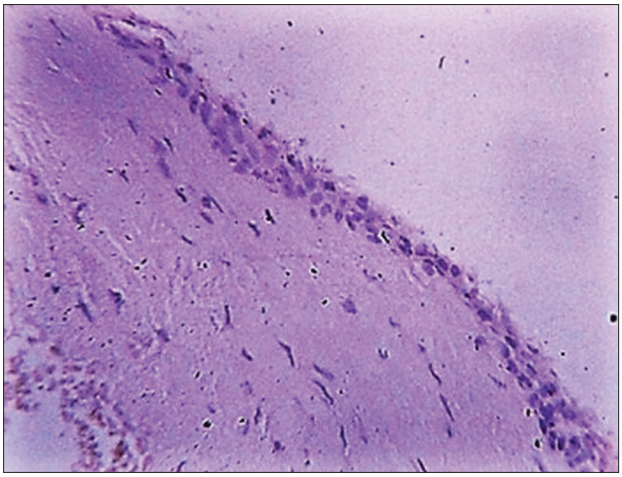

Figure 4: Negative calretinin staining in the dentigerous cyst (×40)

Table 2: Intensity of calretinin staining in different subtypes of ameloblastoma and unicystic ameloblastoma

Intensity	Acanthomatous ameloblastoma (%)	Follicular ameloblastoma (%)	Plexiform ameloblastoma (%)	Unicystic ameloblastoma (%)
0	0 (0.0)	0 (0.0)	0 (0.0)	3 (15.0)
1	3 (23.1)	2 (66.7)	1 (25.0)	10 (50.0)
2	10 (76.9)	1 (33.3)	3 (75.0)	7 (35.0)
3	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Total	13 (100.0)	3 (100.0)	4 (100.0)	20 (100.0)
χ^2, *P*		2.265, 0.043*		

Table 3: Distribution of calretinin staining in subtypes of ameloblastoma and unicystic ameloblastoma

Pattern	Acanthomatous ameloblastoma (%)	Follicular ameloblastoma (%)	Plexiform ameloblastoma (%)	Unicystic ameloblastoma (%)
Focal	3 (23.1)	2 (66.7)	1 (25.0)	17 (85.0)
Diffuse	10 (76.9)	1 (33.3)	3 (75.0)	0 (0.0)
No	0 (0)	0 (0)	0 (0)	3 (15)
Total	13 (100.0)	3 (100.0)	4 (100.0)	20 (100.0)
χ^2, *P*		2.269, 0.043*		

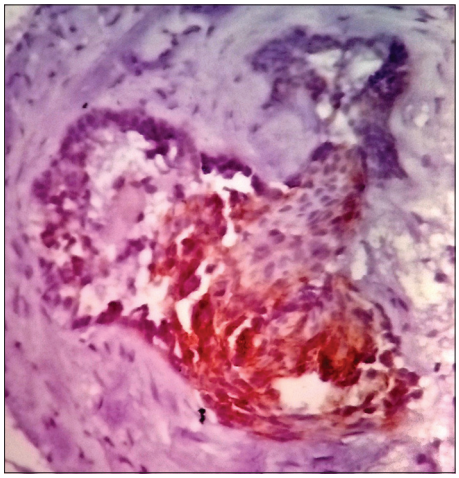

Figure 5: Focal pattern of distribution of calretinin staining in ameloblastoma (×40)

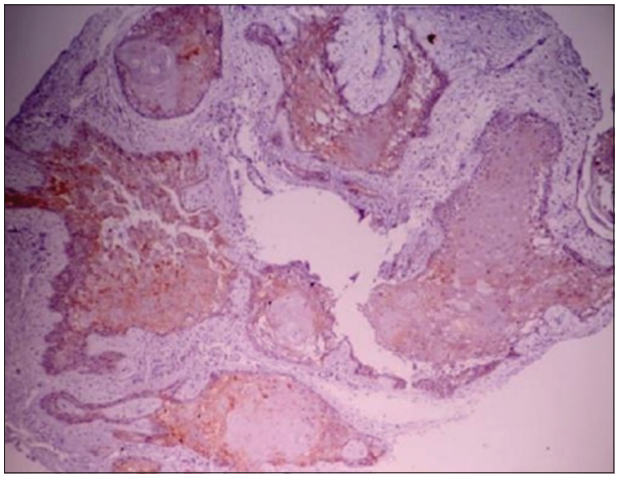

Figure 6: Diffuse pattern of distribution of calretinin staining in ameloblastoma (×10)

ameloblastoma (Chi-square value = 2.269, *P* = 0.043) [Table 3].

DISCUSSION

Odontogenic cysts and tumors are a diverse group of lesions that arise from the tooth forming apparatus and its remnants.[9] They have variable clinical and biological behavior, which depend on the degree of inductive tissue interaction. Ameloblastoma is a benign, locally aggressive epithelial odontogenic tumor that has the capability of becoming malignant and can produce metastasis to various distant sites such as lungs and kidneys. OKC is an aggressive cyst which has a neoplastic behavior.[10,11] It is characterized, histologically, by a palisaded basal cell layer of columnar cells and a surface of corrugated parakeratin, sometimes showing spongiosis, which resembles closely to the stellate reticulum. It is usually about 5–8 cell layers in thickness without any rete ridges formation. In small incisional biopsies, if the cystic epithelium displays reactive changes induced by inflammation, it can closely resemble unicystic ameloblastoma histologically. Thus, at times, both lesions become histologically indistinguishable.[12]

Unicystic ameloblastoma is a single cystic lesion. It shares common clinical and radiographical features with other odontogenic lesions making its diagnosis very difficult. Dentigerous cyst, OKC, residual cyst, adenomatoid odontogenic tumor, giant cell lesions, and sometimes ameloblastoma can become the possible differential diagnosis of uniystic ameloblastoma.[13] Differentiating unicystic ameloblastoma from dentigerous cyst poses a great challenge. In dentigerous cyst, expansion of the buccal cortex occurs as the cyst expands towards the most dependent part, i.e., buccally. Ameloblastoma usually grows buccally and lingually.[14] Histologically, unicystic ameloblastoma is

lined in some areas, but rarely entirely, by the odontogenic epithelium of ameloblastic appearance and stratified squamous epithelium in the remaining areas.[15] In fact, such squamous metaplasia is a relatively common phenomenon in unicystic ameloblastoma and most of these lesions are lined by such nondescript epithelium; it can create diagnostic confusion with odontogenic cysts.[16]

There are many techniques that can be used to distinguish odontogenic cysts from Ameloblastoma-like cell surface carbohydrates demonstration with blood group specificity, characterization of cytokeratin profiles, AgNORs, quantifying cell proliferation markers as PCNA and Ki67. These techniques have shown differences occurring between various cysts and unicystic ameloblastoma, but considerable overlap still exists, and none of the above techniques can be used to routinely distinguish these lesions.[6]

Calretinin is widely distributed in many normal and neoplastic human tissues. Its expression in the nervous system has been extensively used by neuroanatomists, and it represents by far the most specific and sensitive marker for both benign and malignant mesothelial cells. Calretinin is now established as a marker of neuronal differentiation in the central nervous system tumors.[17] Expression of calretinin in many normal human tissues and other human neoplasms has been investigated and its role as a specific immunohistochemical marker has to be elucidated.[18]

The present study assessed the expression of calretinin in twenty cases each of ameloblastoma, unicystic ameloblastoma, dentigerous cyst, and OKC comprising 62 males and 18 females with the mean age of males being 40.2 years and females 39.7 years. The results demonstrated frequent expression of calretinin in the epithelium of both unicystic ameloblastoma and ameloblastomas, whereas, no case of OKC and dentigerous cyst lining showed positive staining for calretinin. These findings were in accordance with the studies done by Altini *et al*.,[17] Coleman *et al*.,[16] DeVilliers *et al*.[12] and Sundaragiri *et al*.[6] who found positive staining in both unicystic ameloblastoma and ameloblastoma, whereas none of the odontogenic cysts linings showed positive staining. Alaeddini *et al*.[19] also found positive calretinin immunoreactivity for ameloblastoma when compared to the calcifying epithelial odontogenic tumor, adenomatoid odontogenic tumor, ameloblastic fibroma, and odontogenic myxoma, stating that this protein may have a role in the transition of the dental lamina remnants to ameloblastoma. They hypothesized that calretinin may be one of the factors responsible for the differences between this aggressive neoplasm and other odontogenic tumors studied. In contrast to the above findings, D'Silva *et al*.[18] and Piattelli *et al*.[20] observed positive staining of the cystic lining epithelium and keratin flakes in the cystic lumen of OKC. These authors attributed this finding to the aggressive biologic behavior of OKC.

In the present study, immunopositivity was seen exclusively in the stellate reticulum like epithelium in both the unicystic ameloblastoma and ameloblastomas which was similar to the findings of Altini *et al*.,[17] DeVilliers *et al*.,[12] Sundaragiri *et al*.,[6] Alaeddini *et al*.[19] and Mistry *et al*.[21] In a study by Mistry *et al*.[21] on developing rat molars, calretinin immunoreactivity was present in the inner enamel epithelium and pre secretory ameloblasts from the late cap stage onwards. In the cap and the late cap stages, many of the specimens were immunopositive for calretinin in the stellate reticulum. However, in the peripheral layers of the ameloblastic islands, calretinin immunoreactivity was not observed as was the case in normal tooth germs. As the enamel organ has been proposed to be one of the possible origins of ameloblastoma, this peculiar distribution of calretinin in the ameloblastic epithelium is noteworthy.[22]

The distribution pattern of calretinin immunopositive cells was recorded in the present study. It was found that unicystic ameloblastoma showed a focal distribution pattern for calretinin, whereas in ameloblastoma both focal and diffuse patterns were observed. These findings were similar to the study of Anandani *et al*.[4] and Mistry *et al*.[21] According to Shivapathasundaram B *et al*.[23] Unicystic ameloblastoma has an epithelial lining which is present only focally, and the rest of it is lined by stratified squamous epithelium. This focal presence of epithelium in unicystic ameloblastoma may be the reason for the focal distribution pattern for calretinin staining. This was in contrast to the earlier studies done by DeVilliers *et al*.,[12] and Alaeddini *et al*.,[19] where most of the cases showed diffuse staining in unicystic ameloblastoma.

The intensity of staining was weak to moderate in most of the cases of unicystic ameloblastoma in our study. This was in accordance with the study of Altini *et al*.[17] who stated that the better the differentiation of the epithelium was, the lesser the expression of calretinin. They found little or no immunostaining in those cases of unicystic ameloblastomas that were lined by typical ameloblastic epithelium, while the epithelium, which completely lacked ameloblastic features frequently expressed calretinin. Hence, they indicated that calretinin expression in some cells varied according to their metabolic activity. However, Coleman *et al*.[16] observed intense positive staining in both the areas of the nondescript epithelial lining and the areas with typical ameloblastic

features in unicystic ameloblastoma, which indicated that although the cyst linings may have lost their typical ameloblastic features, the cells still have retained their immunophenotypic characteristics resulting in the continued expression of calretinin.

In the present study, few nonepithelial cells that stained positive for calretinin were also observed in the connective tissue stroma in the cases of ameloblastoma and unicystic ameloblastoma. Altini *et al*.[17] and Coleman *et al*.[16] also noticed such darkly stained cells in the tumor epithelium and the fibrous connective tissue walls. These later were interpreted as being mast cells or Langerhans cells, both of which have been found to be occurring in ameloblastomas.

The present study suggests that calretinin may be used as a specific immunohistochemical marker for neoplastic ameloblastic epithelium as calretinin positivity was observed exclusively in ameloblastomas. Hence, it may serve as an important diagnostic adjunct in the differential diagnosis of ameloblastoma and cystic odontogenic lesions. Although the calretinin expression in neoplastic ameloblastic epithelium could be statistically established through this study, owing to limited sampling, yet, we would like to propose further exploration into this innovative system.

CONCLUSION

The diagnosis of various odontogenic cysts and tumors exclusively count on histopathological examination. Various crucial factors such as detection of neoplastic or dysplastic changes and assessment of proliferation potential, cannot be judged with this conventional method. With the advent of IHC, a novel technique, these limitations can be overcome, and it may assist to determine the transition from normal to neoplastic tissue.

Many studies have been conducted on the expression of different proteins in ameloblastomas using many techniques such as immunocytochemistry, *in situ* hybridization, and cytogenetic analysis. There is still no completely reliable technique for identifying the ameloblastic epithelium.

This study suggests that calretinin may be used as a specific immunohistochemical marker for ameloblastic epithelium and serve as an important diagnostic aid in differentiating cystic odontogenic lesions and ameloblastic tumors. It is of utmost importance in the treatment planning of the patients as both entities carry different treatment protocols with potentially serious functional and esthetic consequences. However, the use of calretinin as an early marker to predict the neoplastic changes in the odontogenic cyst could be answered only through further research.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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