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CASE REPORT

Granular cells in ghost cell odontogenic lesions: an unusual and unexpected finding

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Abstract:

This study aimed to present two cases of ghost cell odontogenic lesions (GCOL) with granular cells, an unusual finding, in order to contribute to their immunohistochemical characterization and compare results with previous reports. One case corresponded to a calcifying odontogenic cyst (COC) in a 44-year-old man and the other to a dentinogenic ghost cell tumor (DGCT) presenting in an 84-year-old woman. Both lesions were located in the mandible. Microscopically, COC showed a cystic wall lined by ameloblastic/ameloblastomatous epithelium with ghost cells, and DGCT showed ameloblastomatous proliferation with ghost cells and dentinoid/osteodentin matrix. In addition, the presence of cells with abundant granular cytoplasm was observed within the epithelial lining in the cystic lesion and trapped in the dentinoid matrix in the neoplastic lesion. In both cases, the granular cells were positive for AE1-AE3, S100, and CD68; additionally, granular cells in the DGCT were positive for CK19, amelogenin, β-catenin, E-cadherin, vimentin, and lysozyme. The immunohistochemical profile suggests an epithelial origin of granular cells with an increase in lysosomes possibly associated with a degenerative process. Further studies are necessary to clarify the origin of these cells.

Keywords: Ghost cell; Odontogenic lesions; Calcifying odontogenic cyst; Dentinogenic ghost cell tumor; Granular cells; Immunohistochemistry.

INTRODUCTION

Ghost cell odontogenic lesions (GCOL) comprise a group of lesions that present a wide variety of clinical-radiographic characteristics but differ in their biological behaviour, prognosis and clinical course. On microscopic examination, they typically exhibit ghost cells that tend to calcify. Gorlin et al. reported the presence of ghost cells in cystic lesions, which they termed "calcifying odontogenic cyst", and proposed the latter as a new pathological entity in 1964[1,2]. In 1981, Praetorius et al. recognized the existence of two entities, a cyst and a tumor, and suggested the term "dentinogenic ghost cell tumor" for the neoplastic lesion[3]. According to the 5th and latest classification of the World Health Organization (WHO), ghost cell odontogenic lesions comprise the following entities: calcifying odontogenic cyst (COC), dentinogenic ghost cell tumor (DGCT), and ghost cell odontogenic carcinoma (GCOC)[4]. Microscopic diagnosis of these lesions is established by the presence of ameloblastic-ameloblastomatous proliferation, clusters of ghost cells that can calcify, and dentinoid-osteodentin matrix in contact with the epithelium[4]. In addition, the unusual presence of cells of uncertain nature with abundant cytoplasm containing granulations has been reported[5-8].

Statement of Clinical Significance
Ghost cell odontogenic lesions are rare, especially those of a neoplastic nature. The presence of granular cells in these lesions is an unusual and unexpected finding. Case reports contribute to the general understanding of these lesions.

The aim of this study was to present two new cases of GCOL with granular cells, a cystic and a neoplastic lesion, to contribute to the immunohistochemical characterization of these cells, and compare the results with those reported in the literature.

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CASE REPORTS

Case 1

A 44-year-old man presented with a 3 cm wide swelling in an edentulous region distal to the right lower lateral incisor and covered by clinically normal-appearing mucosa. Duration of the lesion was unknown. The patient had no relevant familial or medical history. The panoramic radiograph showed a unilocular radiolucent osteolytic lesion with well-defined contours and focal radiopacity associated with the impacted lower right canine. The basal cortical bone showed thinning, expansion, and perforation. The presumptive clinical-radiographic diagnosis was dentigerous cyst; complete surgical excision of the lesion was performed. Microscopic examination showed a cyst wall lined with ameloblastic-ameloblastomatous epithelium of varying thickness with ghost cells and cells with abundant pale cytoplasm containing eosinophilic granulations and oval nuclei, which were mostly eccentric. An ameloblastomatous island with granular cells similar to those of the epithelial lining was observed within the wall. A giant cell reaction associated with ghost cells, some of which were calcified, was observed in the subepithelial chorion. Granular cells were positive for PAS-diastase and negative for alcian blue, Congo red, and thioflavin T (Figure 1). The immunohistochemical results are shown in Table 1 and illustrated in Figure 2. Diagnosis was calcifying odontogenic cyst with granular cells.

Case 2

An 84-year-old female patient presented for consultation with a swelling of one year duration in the anterior region of the mandible. Intraoral inspection revealed a 3 x 5 cm tumor covered by normal mucosa and involving the left and right incisor and canine region. The patient had no relevant familial or medical history. Multislice computed tomography showed a unilocular radiolucent osteolytic lesion with ill-defined contours and radiodense foci. Cortical expansion, fenestration, and destruction, along with soft tissue involvement, were observed. The presumptive clinical-radiographic diagnosis was malignant tumor. Microscopic examination

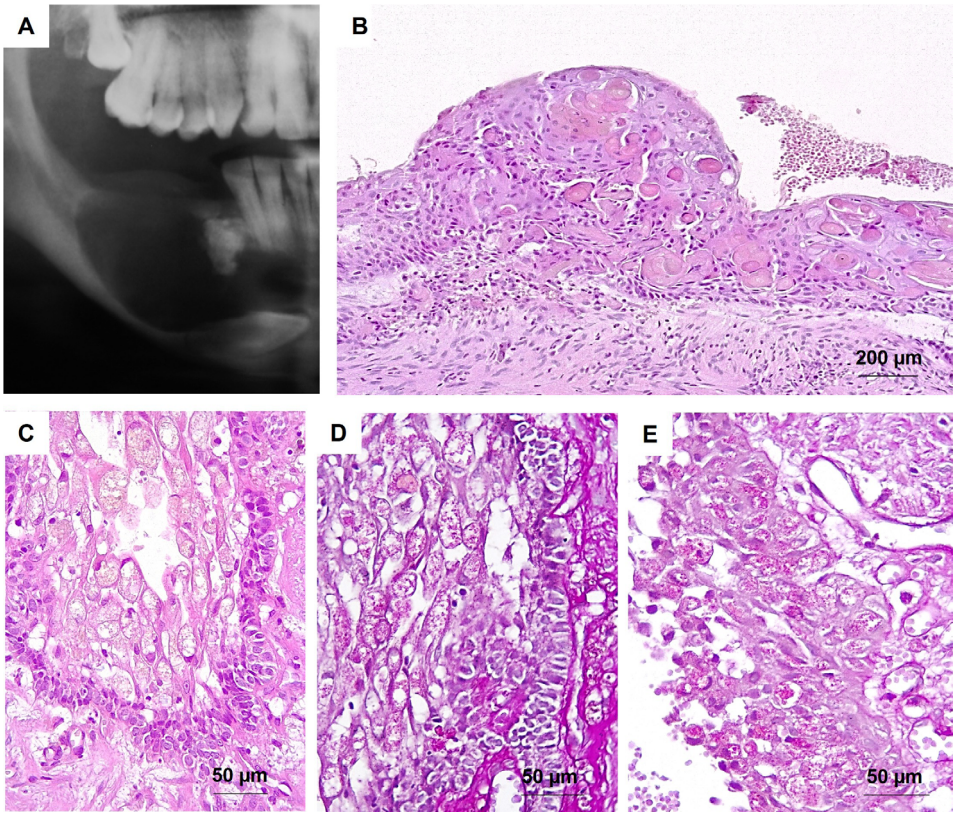

Figure 1. Calcifying odontogenic cyst with granular cells. **(A)** Panoramic radiograph. A unilocular osteolytic lesion with radiopaque focus can be observed. **(B)** Microscopy image showing the cystic wall with ghost cells in the epithelial lining, H-E, Orig. Mag. X100. **(C)** Ameloblastomatous epithelium exhibiting clusters of granular cells, H-E, Orig. Mag. X400. **(D and E)** Note positivity of granular cells for PAS staining, Orig. Mag. X400.

Table 1. Calcifying odontogenic cyst with granular cells. Immunohistochemistry.

		AE1-AE3	CK19	β-catenin	S-100	vimentin	CD68	CD1a	p63	SOX2	Ki-67
COC	EL	(+) Diffuse	(-)	(+) Focal M-C	(+) Focal	(-)	(-)	(-)	(-)	(-)	<1%
	GC	(+) Focal	(-)	(+) Focal M	(-)	(-)	(+) Focal	(-)	(-)	(-)	
	GrC	(+) Focal	(-)	(-)	(+) Focal	(-)	(+) Focal	(-)	(-)	(-)	

COC: Calcifying odontogenic cyst; EL: epithelial lining. GC: ghost cells. GrC: granular cells. (+): positive. (-): negative. M: membrane expression. C: cytoplasmic expression.

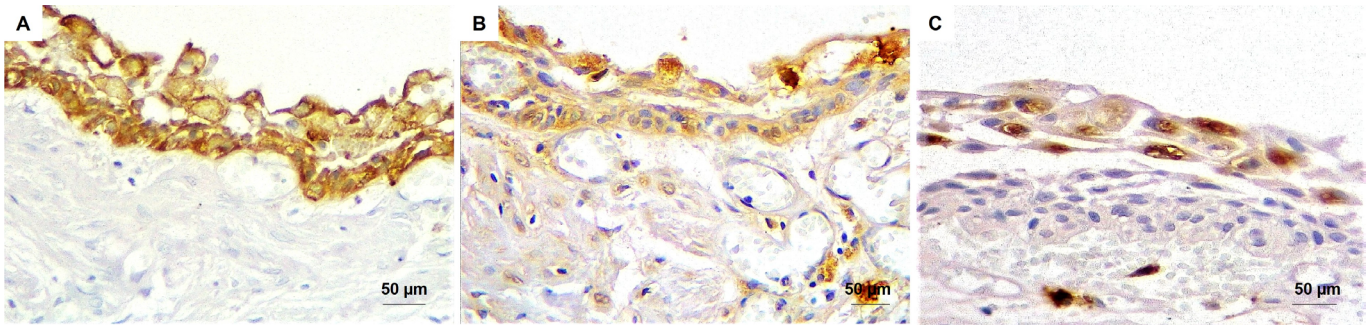

Figure 2. Calcifying odontogenic cyst with granular cells. Immunohistochemistry in granular cells. Orig. Mag. X400. **(A)** AE1-AE3. **(B)** S100. **(C)** CD68.

revealed an ameloblastomatous neoplastic proliferation consisting of nests and cords of columnar and hyperchromatic peripheral cells with inverted polarity, some of which showed subnuclear vacuolization. The central cells exhibited a stellate appearance resembling the stellate reticulum of the enamel organ. Some sectors showed a cribriform pattern, pseudo-ductal structures and whorls/morules. Large accumulations of ghost cells that were calcified focally and dentinoid/osteodentin matrix formation were identified. Ghost cells and granular cells trapped in the dentinoid matrix were observed in some sectors. The granular cells showed abundant cytoplasm with eosinophilic granulations and large oval central or eccentric nuclei (Figure 3). These cells were PAS diastase positive and alcian blue negative. Both granular and ghost cells showed focal fluorescence with thioflavin T, resembling that observed in keratins. Polarization of dentinoid/osteodentin with Congo red was similar to polarization seen in woven bone; ghost cells showed pinkish-white birefringence similar to keratin, and granular cells were negative. Results of the immunohistochemical studies are shown in Table 2 and Figure 4. Diagnosis was dentinogenic ghost cell tumor with granular cells. The treatment of choice was mandibular resection.

DISCUSSION

The first report of calcifying odontogenic cyst with granular cells included three cases documented by David and Buchner in 1976[5]. The cases corresponded to two men aged 52 and 62 years with a lesion in the mandible and a 12-year-old girl presenting a swelling in the maxilla associated with an impacted tooth. In addition to the typical histomorphological features of COC, all three cases showed balloon cells with wide pale eosinophilic cytoplasm with granulations and round, sometimes eccentric, nuclei. Congo red staining of granular cells showed intense birefringence, which the authors interpreted as fibrillar protein material similar to the amyloid found in calcifying epithelial odontogenic tumor[5]. Table 3 summarizes the reported cases of GCOL with granular cells.

In the two cases reported here, the granular cells were Congo red-negative. Both the COC and DGCT were negative for thioflavin T, and granular cells of DGCT showed focal fluorescence similar to that of keratin, indicating that the intracytoplasmic granulations were not amyloid-like material.

The fourth case was reported in 1985 by Ng and Siar in a publication entitled "Clear cell change in a calcifying odontogenic cyst" and involved a 29-year-old female patient with a lesion in the mandible[6]. The authors found polyhedral cells with slightly granular or clear cytoplasm and small ovoid nuclei, which were often displaced towards the periphery. They posited that these clear-granular cells originated from odontogenic epithelium undergoing an aberrant degenerative process.

There are only two reports of granular cells in dentinogenic ghost cell tumor, and in both cases the

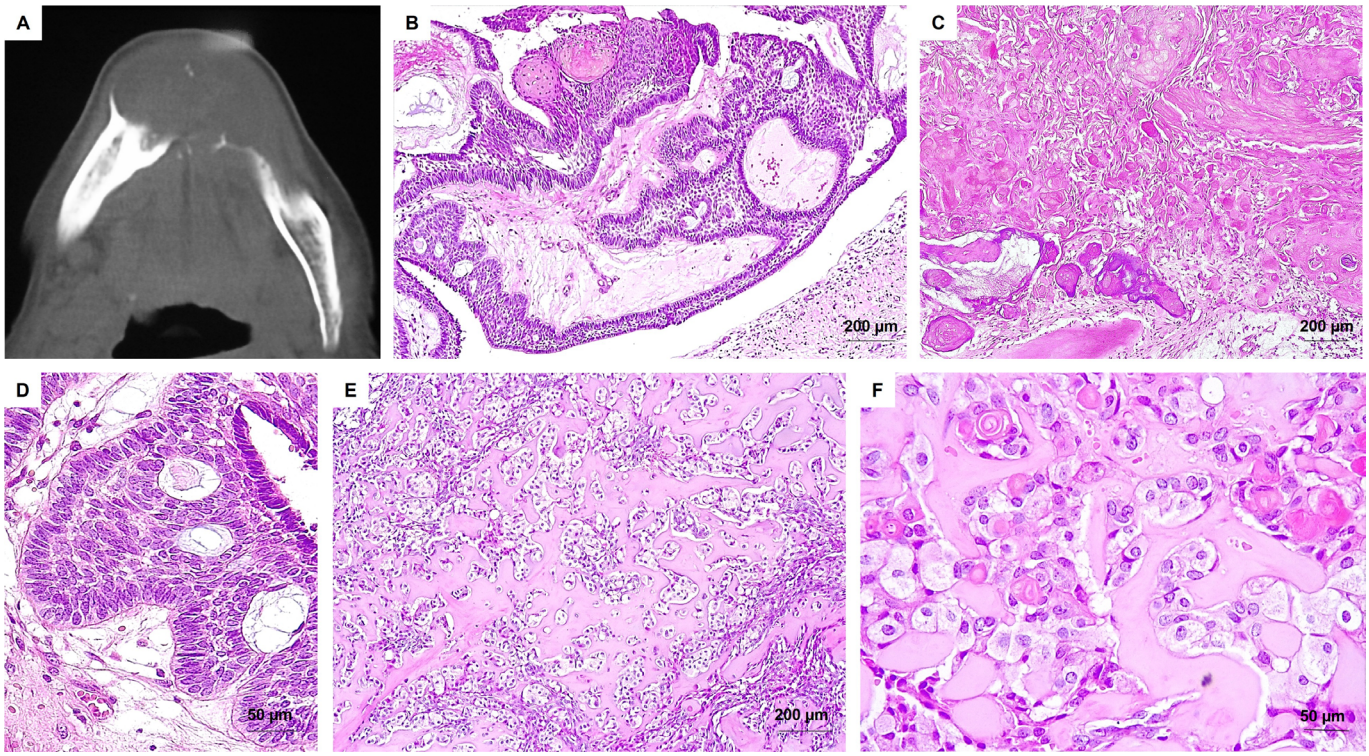

Figure 3. Dentinogenic ghost cell tumor with granular cells. **(A)** CT, axial section, note the destructive osteolytic lesion in the anterior mandibular region. **(B)** Microscopic image: ameloblastomatous proliferation and ghost cell focus can be observed, H-E, Orig. Mag. X100. **(C)** Note large clusters of focally calcified ghost cells, H-E, Orig. Mag. X100. **(D)** Ameloblastomatous proliferation with a ductal appearance, H-E, Orig. Mag. X400. **(E and F)** Note ghost cells and granular cells trapped in osteodentin-like material, **(E)** H-E, Orig. Mag. X100; **(F)** H-E, Orig. Mag. X400.

Table 2. Dentinogenic ghost cell tumor with granular cells. Immunohistochemistry.

		AE1-AE3	CK19	amelogenin	β-catenin	E-cadherin	S-100	vimentin	CD68	CD1a	lysozyme	TOM-20	BCL-2	p63	BRAFp V600E	SOX-2	Ki-67
	EP	(+) Diffuse	(+) Focal	(+) Focal	(+) Focal M-C-N	(+) Diffuse	(+) Focal	-	(-)	(-)	(-)	(+) Focal	(+) Focal	(+) Focal	(+) Focal	(-)	≤5%
DGCT	GC	(+) Diffuse	(+) Focal	(+) Focal	(+) Focal M	(+) Focal	(+) Focal	-	(+) Focal	(-)	(+) Focal	(-)	(-)	(-)	(-)	(-)	
	GrC	(+) Focal	(+) Focal	(+) Focal	(+) Focal M-C-N	(+) Focal	(+) Focal	(+) Focal	(+) Focal	(-)	(+) Focal	(-)	(-)	(-)	(-)	(-)	

DGCT: Dentinogenic ghost cell tumor; EP: epithelial proliferation. GC: ghost cells. GrC: granular cells. (+): positive. (-): negative. M: membrane expression. C: cytoplasmic expression. N: nuclear expression.

authors referred to them as clear cells[7,8]. The first, published in 2004, involved a 63-year-old man with a swelling of 10 years duration in the anterior edentulous region of the mandible. Microscopic examination revealed the presence of ameloblastomatous proliferation with a large number of ghost cells, dentinoid material, and islands of clear or finely granulated cells separated by fine sheets of fibrous tissue stroma[7]. The second case was published in 2016 and corresponded to an 18-year-old female with a lesion in the left posterior region of the maxilla. Histologic examination showed a solid tumor with ameloblastic follicles with diverse morphology, ghost cells, and clear cells, some of which had a granular appearance[8]. Consistent with our findings, the granular cells in both the aforementioned cases stained positive for AE1-AE3 and CK19[7,8].

Although the cells of interest are described as clear or granular-like in all four reports (two COC and two DGCT), we agree with the authors in that they correspond to the same cell type[5-8]. We understand that the presence of granular intracytoplasmic material is their most significant morphological feature,

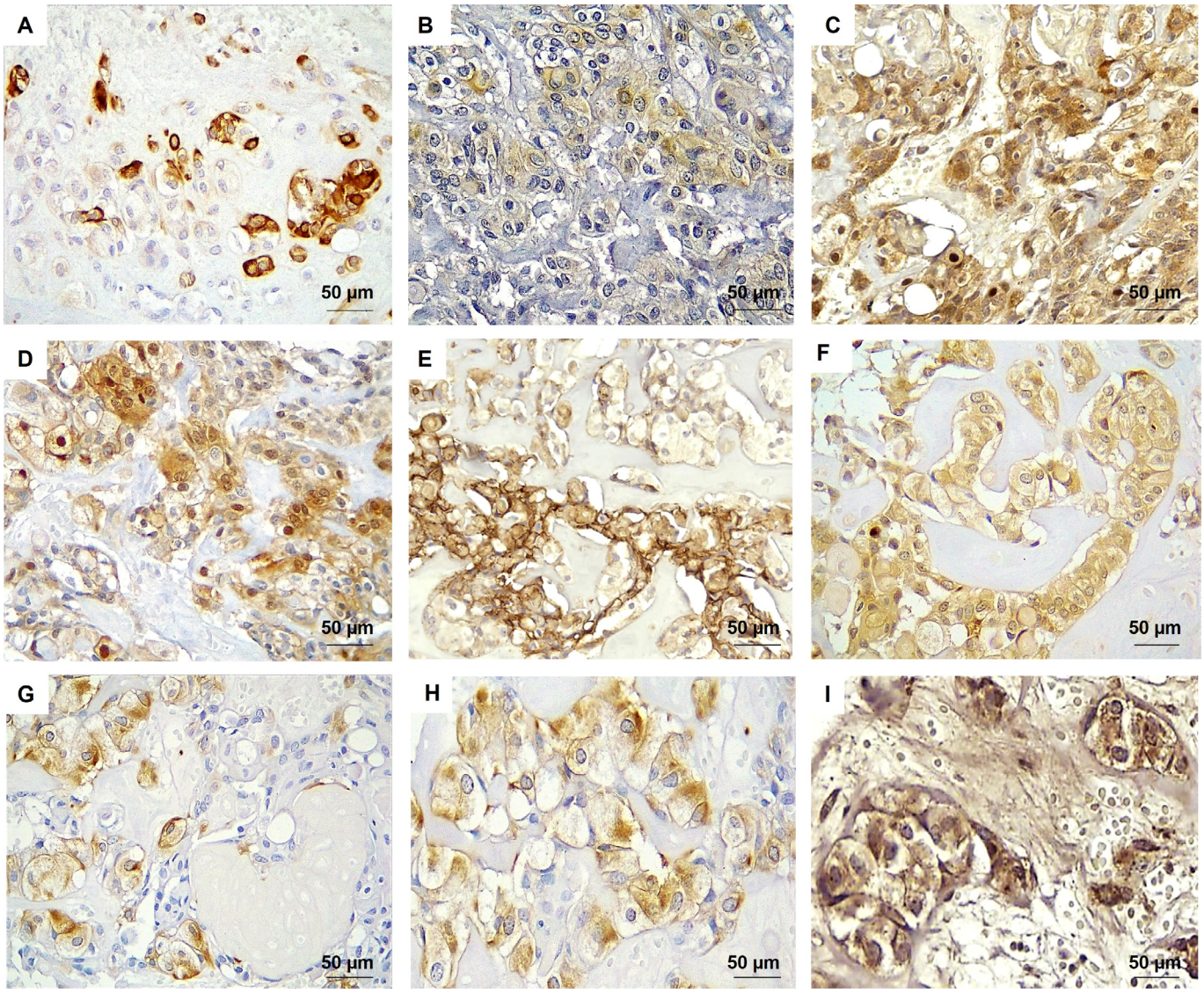

Figure 4. Dentinogenic ghost cell tumor with granular cells. Immunohistochemistry in granular cells. Orig. Mag. X400. **(A)** AE1-AE3. **(B)** CK19. **(C)** Amelogenin. **(D)** Focal positivity of β-catenin in the membrane, cytoplasm, and nucleus. **(E)** E-cadherin. **(F)** S100. **(G)** Vimentin. **(H)** CD68. **(I)** Lysozyme.

and they should therefore be termed granular cells to avoid confusion.

Furthermore, ghost cells remain a subject of debate regarding their origin and biological characterization[4,9,10]. Microscopically, these cells present an eosinophilic cytoplasm with a characteristic clear zone corresponding to the absence of their nucleus. They may appear individually, in small groups, or as large cell masses often displaying calcification. Different hypotheses have been suggested to explain their nature, including hypoxia and degeneration, coagulative necrosis, metaplasia, abnormal terminal differentiation or apoptosis, abortive formation of the enamel matrix, and aberrant keratinization. The results of histochemical and immunohistochemical staining support this last hypothesis[9,10].

Different histochemical, immunohistochemical, and ultrastructural studies of head and neck lesions showing granular cells have been conducted in an attempt to contribute to the understanding of their etiopathogenesis. However, findings are controversial and under debate[11-22]. The presence of granular cells was reported in several lesions of odontogenic origin, such as ameloblastoma, ameloblastic fibroma, odontogenic fibroma, and odontoma[4,11-14], as well as in more controversial entities in which granular cells are an essential morphologic component, as is the case of granular cell odontogenic cyst (GCOC)[15-17] and granular cell odontogenic tumor

Table 3. Reported cases of ghost cell odontogenic lesions with granular cells.

COC	Sex	Age	Clinical	Location	X-ray	Microscopy	HC/IHC	Treatment	Follow-up
David and Buchner[5] (3 cases)	M	52	NR	Mandible (right second premolar to left first molar)	Radiolucent lesion	Cystic epithelial lining with ghost cells and granular cells	Granular cells: PAS + Congo red + STB + (weak)	NR	NR
	F	12	NR	Maxilla (left second molar)	Radiolucent lesion associated with an impacted tooth	Cystic epithelial lining with ghost cells and granular cells	Granular cells: PAS + Congo red + STB + (weak)	NR	NR
	M	62	NR	Mandible (left canine to right canine)	Radiolucent lesion	Cystic epithelial lining with ghost cells and granular cells	Granular cells: PAS + Congo red + STB + (weak)	NR	NR
Ng and Siar[6]	F	29	Swelling	Mandible (right first premolar to right second molar)	NR	Cystic epithelial lining with ghost cells; nests, cords, and islands of granular cells	Granular cells: Alcian Blue – Mucicarmine – PAS-diastase –	Enucleation and curettage	NR
Dentinogenic ghost cell tumor									
Yoon et al.[7]	M	63	Painless swelling, 10 years duration	Mandible (anterior edentulous región)	CT: multilocular soft tissue density mass. Perforation of the bucco-lingual cortical bone. Expansion into the floor of the mouth	Ameloblastomatous proliferation, ghost cells, dentinoid material, sheets and islands of finely granular epithelial cells	Granular cells: PAS + (weak) Mucicarmine – AE1/3 + CK19 + S100 –	Marginal mandibulectomy	No recurrence after 3 years and 2 months
Urs et al.[8]	F	18	Painful swelling, 4 months duration	Maxilla (left posterior región)	CT: well-defined and heterogeneously enhanced expansile lesion in relation to the unerupted third molar	Ameloblastomatous proliferation, ghost cells, sheets and islands of granular cells	Granular cells: PAS + Mucicarmine – CK19 +	Segmental resection	No recurrence after 1 year and 4 months

COC: calcifying odontogenic cyst; HC: histochemistry. IHC: immunohistochemistry. STB: standardized toluidine blue. NR: not reported.

(GCOT)[18,19]. It must be pointed out that the latter have not been recognized by the WHO as pathological entities[4].

GCOC was first described by Gold and Christ in 1970[15]. They reported the case of a 38-year-old female with a mandibular lesion. Histologically, the luminal surface was lined with large epithelial cells with granular cytoplasm. The authors considered that the presence of granular cells in the basal layer was a metabolic phenomenon rather than a degenerative process and suggested that the entity could correspond to a unicystic variant of granular cell ameloblastoma[15]. Years later, Abaza et al.[17] documented the recurrence of the original case reported by Gold et al.[15] They found no histologic evidence of granular cells in the recurrent lesion. Analyzing the case retrospectively, the cystic

lesion may have been a granular cell ameloblastoma[17]. In 1973, Buchner reported another case of GCOC in a 21-year-old man. Microscopically, the cyst surface showed epithelial cells transitioning to granular cells and, occasionally, ghost cells. Despite morphological similarities with COC, the authors ruled out diagnosis of COC based on the predominance of granular cells and few ghost cells and considered diagnosis of unusual granular cell ameloblastoma[16].

GCOT is a rare odontogenic tumor. Some authors consider it as a separate pathological entity, whereas others understand it corresponds to a subtype of granular cells of other odontogenic tumors[18,19]. Immunohistochemical studies in granular cells of GCOT showed they were positive for vimentin and negative for cytokeratins, suggesting a mesenchymal origin. Additionally, they were positive for CD68, possibly due to the presence of phagocytic vacuoles. Positive expression of other markers like HLA-DR, CD1a, lysozyme, alfa-1-antitrypsin, and alfa-1-antichymotrypsin suggests macrophagic differentiation[18,19].

The presence of granular cells in ameloblastoma was first described in 1918 by Krompecher. The granular cells in this tumor are positive for cytokeratins, CD68, lysozyme, and alpha-1antichemotrypsin, but negative for acid phosphatase, Beta-glucuronidase, vimentin, desmin, S100, neuron-specific enolase, and CD15, indicating an epithelial origin with a lysosomal component. These findings suggest that the cytoplasmic granulations could be due to an increase in apoptosis and phagocytosis associated with adjacent neoplastic cells[20]. Radiographically, the two cases presented here showed radiolucent lesions with radiopaque areas. Morphologically, both exhibited ghost cells, and the tumor showed dentinoid material deposition, ruling out the diagnosis of granular cell ameloblastoma.

Based on ultrastructural studies, it was posited that the granulations in these cells could correspond to mitochondria or lysosomes[13,21]. Navarrete and Smith were the first to describe the ultrastructure of granular cells in an ameloblastoma. They identified pleomorphic and osmiophilic granules that were limited by a single membrane and resembled lysosomes[13]. Hamperl suggested that the cytoplasmic granules in granular cell ameloblastoma corresponded to mitochondria[21]. A large number of ultrastructural and immunohistochemical studies in granular cells of ameloblastomas and GCOT strongly support the presence of lysosomes[18,20].

Positive Beta-catenin expression was observed in the epithelium in both the cases reported here (COC and DGCT), as well as in the granular cells of the DGCT, suggesting an etiopathogenic mechanism involving the Wnt/β-catenin pathway. This molecular pathway is involved in various embryonic and homeostatic processes, including key events during odontogenesis[22]. The β-catenin protein, encoded by the CTNNB1 gene, is the main component of this molecular pathway, and mutations in this gene have been associated with various tumorigenic processes, including those of odontogenic origin. In 90% of COC cases, mutations in the CTNNB1 gene were identified, mainly in codons 32, 33, 34 and 37 of exon 3[23]. Similar mutations were also found in some GCOC cases, and one case of DGCT showed a mutation in codon 3[24]. These molecular findings are often associated with immunohistochemical expression of cytoplasmic and nuclear β-catenin observed in the cystic epithelium and neoplastic epithelium. Furthermore, previous studies have reported a loss of β-catenin expression in ghost cells[9,23,24]. The etiopathogenic mechanisms leading to GCOL development, and particularly ghost cell formation, still need to be elucidated. In both cases, sections were demineralized with 7% nitric acid, making the implementation of molecular techniques unfeasible.

It should be noted that granular cells can occur in non-odontogenic oral lesions, such as congenital epulis and granular cell tumor, and can be found in multiple salivary gland lesions[20,25].

Granular cells are similar in their morphological appearance but can have a different cell lineage or follow different differentiation pathways. It has been posited that the granular cells in ameloblastomas could be of epithelial origin, mesenchymal-macrophagic origin in GCOT, and neural origin in granular cell tumors, whereas their origin in other pathologies remains uncertain[18,20]. In both the cases shown here, granular cells were positive for AE1-AE3; this finding added to the documented expression of CK19, amelogenin, β-catenin, and E-cadherin in DGCT lend support to the epithelial origin of these cells. Positive CD68 expression in both cases and positive lysozyme expression in the neoplastic case confirm an increase in lysosomes. In turn, negative TOM-20 expression in granular cells of DGCT rules out the presence of mitochondria. Given that odontogenesis is regulated by signaling pathways that involve the oral epithelium and neural crest of ectomesenchymal tissues[26], S100 expression in the odontogenic epithelium and granular cells in both cases is not an unexpected finding.

CONCLUSION

To our knowledge, these two cases of ghost cell odontogenic lesions represent the fifth case of calcifying

odontogenic cyst and the third case of dentinogenic ghost cell tumor showing granular cells reported in the literature.

The immunohistochemical profile of the granular cells found in these two cases of ghost cell odontogenic lesions suggests an epithelial origin with an increase in lysosomal vacuoles/vesicles, possibly associated with a degenerative process. Further studies are necessary to clarify the genesis of these cells.

AUTHORS' CONTRIBUTIONS

LFP: conceptualization, investigation, writing – original draft, writing – review & editing. **ES:** resources, writing – review & editing. **FMS:** resources, writing – review & editing. **LFS:** resources, writing – review & editing. **VPP:** resources, writing – review & editing. **RBM:** resources, writing – review & editing. **MLP:** conceptualization, investigation, methodology, resources, supervision, writing – original draft, writing – review & editing.

CONFLICT OF INTEREST STATEMENT

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Competing interests: The authors have no relevant financial or non-financial interests to disclose.

Ethics approval: This work has obtained ethics approval from the Ethics Committee at Facultad Odontología, Universidad Buenos Aires (Nr006/2019).

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