Original Article

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Histochemical Demonstration of Ghost Cells in Calcifying Odontogenic Cyst

Dipti A Tharani*, Ritesh B. Wadhwani**

Abstract

Aim/Background: Ghost cells are seen in calcifying odontogenic cyst (COC) and some other odontogenic and non-odontogenic lesions. There are varying opinions regarding their origin, nature, significance and relation in different lesions. It has been stated that ghost cells represent an abnormal type of keratinisation. This study was done with an aim to demonstrate the ghost cells in COC with one routine stain- hematoxylin-eosin; and three special stains- Ayoub Shklar, Mallory and van Gieson. *Materials and Methods:* Four tissue sections were made from paraffin embedded specimens of COC. Sections were stained with hematoxylin-eosin stain, Ayoub-Shklar stain, Mallory stain and van Gieson stain respectively. The stained sections were evaluated for efficacy of the four staining techniques to stain the ghost cells. *Results:* The ghost cells could be identified in the sections stained by all the four different stains used in this study. When compared among all the four stains, Ayoub-Shklar stain and Mallory stain appeared to be better than hematoxylin-eosin stain and van Gieson stain to discern the ghost cells from rest of the cystic epithelium. *Conclusion:* The ghost cells were stained positively by special stains used to demonstrate keratin. It indicates that they accumulate keratin in their cytoplasm during the pathological transformation process.

Keywords: Ghost Cells; Calcifying Odontogenic Cyst; Keratin.

Introduction

Ghost cells are the characteristic and distinctive histologic feature of calcifying odontogenic cyst (COC). The ghost cells, however, have been reported to occur in several other odontogenic lesions in addition to COC such as odontomas, ameloblastic fibromas, ameloblasstic fibro-odontomas, and solid/multicystic ameloblastomas. Furthermore, ghost cells with similar histomorphologic appearance to those in odontogenic lesions are found in craniopharyngiomas and the cutaneous calcifying epithelioma of Malherbe (pilomatricoma) [1].

The ghost cells are enlarged, ballooned, ovoid or elongated elliptoid epithelial cells. They are eosinophilic and although the cell outlines are usually well-defined, they may sometimes be blurred so that groups of them appear fused. A few ghost cells may contain nuclear remnants but these are in various stages of degeneration and in the majority all traces of chromatin have disappeared leaving only a faint outline of the original nucleus. The ghost cells represent an abnormal type of keratinisation and have an affinity for calcification. They have the same histological reactions as keratin [2].

The nature and content of these cells have been widely discussed over the years based on histomorphologic, conventional histochemical, and ultrastructural investigations. Consequently, various theories have been proposed. The ghost cells represent an abnormal type of keratinisation [3]. Some studies report that these cells may represent the product of coagulative necrosis of odontogenic epithelium [4]. Some investigators state that ghost cells represent simple cell degeneration or a form of enamel matrix. Takata et al have shown that ghost cells in COCs contain enamel-related proteins in their cytoplasm [5]. It has also been hypothesised that they are derived from the apoptotic process of

Author's Affiliation: *Assistant Professor **Professor, Department of Dentistry, Government Medical College and Hospital, Latur, India.

Reprints Requests: Dipti A. Tharani, A1/2 Doctors quarters, Government Medical College campus, Latur, Maharashtra, India. 413512. Email: diptitharani@gmail.com

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odontogenic cells [6].

Ayoub-Shklar and Mallory are special histochemical stains used to stain keratin specifically. Van Gieson stain, a trichrome stain for connective tissue, also stains keratinised epithelium.

As it is stated that the ghost cells represent an abnormal type of keratinisation, we carried out this study to demonstrate the ghost cells in COCs with one routine stain- hematoxylin-eosin; and three special stains- Ayoub-Shklar, Mallory and van Gieson.

Materials and Methods

Four sections of 4 micron thickness were made from paraffin embedded specimens of calcifying odontogenic cyst. Sections were stained with hematoxylin and eosin stain, Ayoub-Shklar stain, Mallory stain and van Gieson stain respectively.

Hematoxylin-Eosin Staining Technique

1. The section was deparaffinised in xylene and brought to water through decreasing grades of alcohol.
2. Then it was stained in Harri's hematoxylin for 5 minutes.
3. After water wash, the section was differentiated with 1% acid alcohol.
4. Bluing was done by keeping the slide in running tap water for 5 minutes.
5. It was then stained with eosin for 5 seconds.
6. Dehydrated with alcohol, cleared in xylene and mounted.

Ayoub-Shklar Staining Technique

Materials used

1. 5% acid fuschin solution
2. Aniline blue orange G solution

Aniline blue (water soluble)- 0.5 gm

Orange G- 2gm

Phosphotungstic acid- 1 gm

Distilled water- 100 ml

Staining Procedure

1. The section was deparaffinised in xylene and brought to water through decreasing grades of alcohol.
2. The slide was then placed in 5% acid fuschin solution for 3 minutes.
3. Then transferred to aniline blue orange G solution for 45 minutes.
4. Section transferred to 95% alcohol, 3 changes.
5. Section was dehydrated in alcohol, cleared and mounted.

Mallory Staining Technique

Materials used

1. 3% potassium dichromate solution
2. 0.1% acid fuschin solution
3. 1% phosphomolybdic acid
4. Mallory II solution

Staining Procedure

1. The section was deparaffinised in xylene and brought to water through decreasing grades of alcohol.
2. It was then placed in 3% potassium dichromate solution for 2 hours.
3. Then rinsed in distilled water several times until water remained clear.
4. The section was stained with 0.1% acid fuchsin for 15 seconds.
5. It was rinsed properly in distilled water.
6. Section was stained in 1% phoshomolybdic acid for 2.5 minutes.
7. Then rinsed properly in distilled water.
8. It was stained in Malory II solution for 25 seconds.
9. Then rinsed in distilled water, dehydrated, cleared and mounted.

Van Gieson Staining Technique

Materials used

1. Weigert's hematoxylin
2. van Gieson solution

Staining Procedure

1. The section was deparaffinised in xylene and brought to water through decreasing grades of alcohol.

2. It was stained in Weigert's hematoxylin for 1 min.
3. The section was then washed in tap water and differentiated with 1% acid alcohol.
4. Bluing was done by washing the section in running tap water.
5. Then it was stained with van Gieson solution for 3 minutes.
6. Dehydrated in alcohol, cleared and mounted.

The stained sections were evaluated for efficacy of the four staining techniques to stain the ghost cells.

Results

The ghost cells could be identified in the sections stained by all the four different stains used in this study. In hematoxylin and eosin stained section the ghost cells appeared as enlarged, elongated epithelial cells which were eosinophilic in appearance with well defined cell outlines and loss of nucleus in some cells (Figure 1,2).

When stained with Ayoub-Shklar stain, the ghost cells appeared as brilliant red with well defined cell outlines. The ghost cells could be differentiated from rest of the epithelial cells which were stained as blue (Figure 3,4).

In sections stained with Mallory stain, the ghost cells appeared as orange in colour and could be differentiated from rest of the non-keratinised epithelial lining which appeared blue in colour (Figure 5,6).

van Gieson stained sections showed ghost cells appearing yellow in colour with well defined cell outlines. They could be differentiated from rest of the non-keratinised epithelium and the underlying connective tissue component (Figure 7,8).

When compared among all the four stains, Ayoub Shklar stain and Mallory stain appeared better than hematoxylin-eosin stain and van Gieson stain to discern the ghost cells from rest of the cystic epithelium in COC.

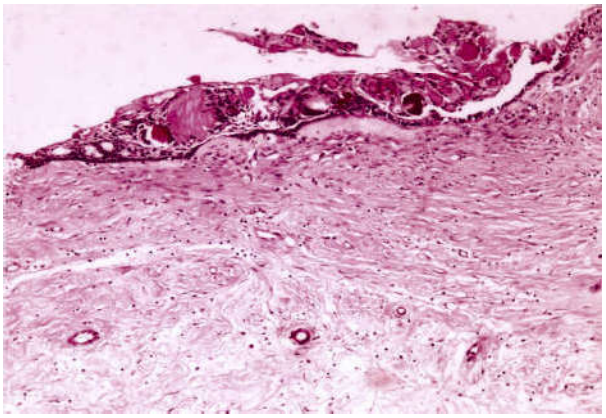

Fig. 1: H and E stain. Eosinophilic ghost cells appearing as enlarged, elongated epithelial cells (10x magnification)

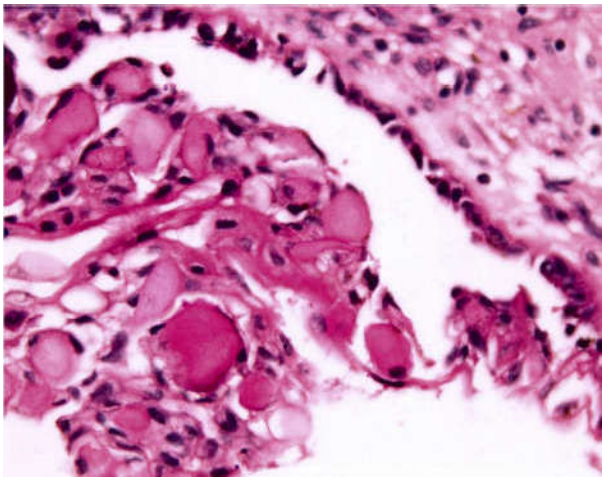

Fig. 2: H and E stain. High power view of ghost cells as seen in figure 1 (40x magnification)

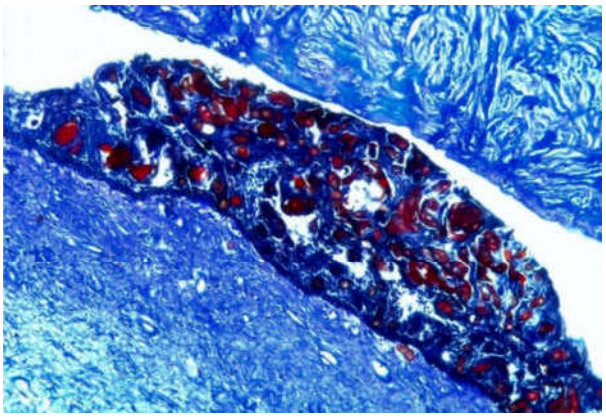

Fig. 3: Ayoub-Shklar stain. Ghost cells appearing as brilliant red in distinction to the rest of the blue staining epithelium (10x magnification)

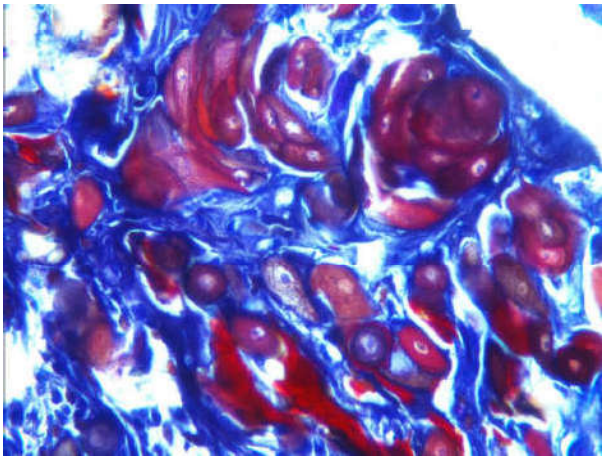

Fig. 4: Ayoub-Shklar stain. High power view of ghost cells as seen in figure 3 (40x magnification)

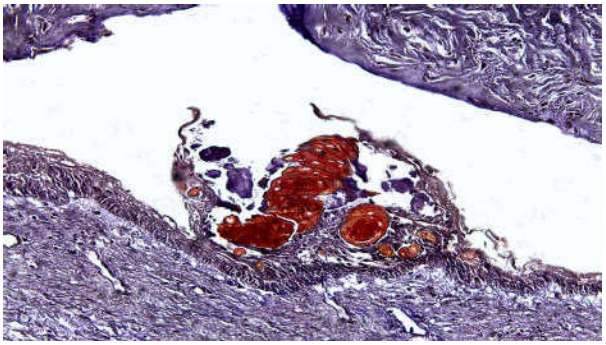

Fig. 5: Mallory stain. Ghost cells appearing as orange in distinction to the rest of the blue staining epithelium (10x magnification)

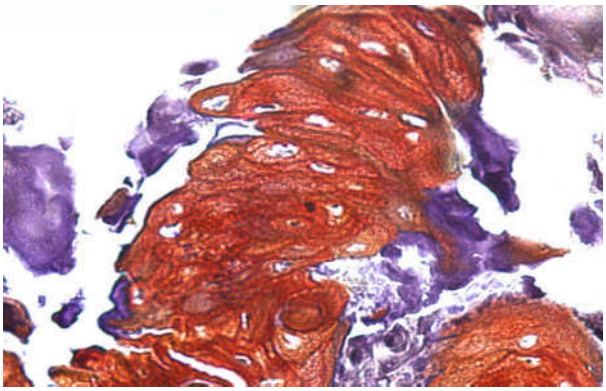

Fig. 6: Mallory stain. High power view of ghost cells as seen in figure 5 (40x magnification)

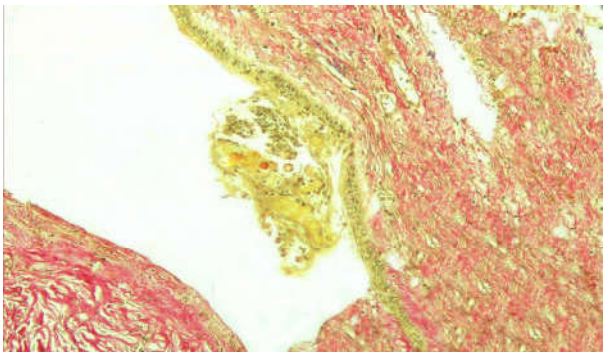

Fig. 7: van Gieson stain. Ghost cells appearing yellow in colour. Underlying connective tissue appearing red. (10x magnification)

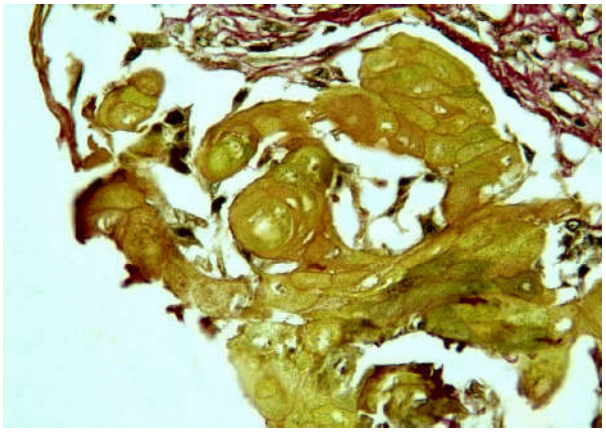

Fig. 8: van Gieson stain. High power view of ghost cells (40x magnification)

Discussion

Ghost cells are seen in COC and few other odontogenic and non-odontogenic lesions. They have been a topic of controversy since a long time. Controversy arises because of the fact that there are varying opinions regarding their origin, nature, significance and relation in different neoplasms.

Various theories have been put forward regarding the nature of ghost cells. There are different reports suggesting ghost cells as a form of true keratinisation [7], abnormal/aberrant keratinisation [3], cells which have lost their developmental and inductive effect [8], special form of degeneration [9], or formed as a result of coagulative necrosis of odontogenic epithelium [4]. Few investigators state that ghost cells represent enamel matrix which probably could not completely calcify because of the absence of odontoblasts and dentin [10].

Special stains are the stains that are used to visualize specific tissues and cellular structures. These are the dyes that bind to the cellular components either physically or by chemical bonds. Special stains for keratin are indicated to differentiate and emphasize small foci of abnormal keratinization. Examples include Ayoub-Shklar and Mallory. Trichrome stain such as van Gieson, used to stain connective tissue elements can differentiate epithelium from the underlying collagenous connective tissue.

In our study the ghost cells present in COCs were stained by hematoxylin-eosin stain and all the three special stains. The ghost cells were better seen in sections stained with Ayoub-Shklar and Mallory stains as compared to hamatoxylin-eosin and van Gieson stains.

As the former two stains are used specifically to stain keratin, the results of our study emphasise the fact that the ghost cells accumulate keratin in their cytoplasm during the pathological transformation process. This is in accordance with the previous literature on this particular kind of cell.

Thus the staining reaction of ghost cells appeared to be similar to keratin. But certain immunohisto-chemical investigations on cytokeratins in the ghost cells of COCs failed to demonstrate positive staining for different kinds of keratin [4, 11].

Hong et al expressed the opinion that the characteristics of ghost cells are compatible with the features of coagulative necrosis of odontogenic epithelium [4]. Based on immunohistochemical

investigations Takata et al concluded that ghost cells in COCs contain enamel-related proteins in the cytoplasm accumulated during the process of pathologic transformation [5]. Ultrastructural findings have revealed that these cells represent an aberrant or unusual form of keratin and not true keratin [12].

To gain insight into the true nature of ghost cells further studies at molecular level can be done.

References

1. Reichart PA, Philipsen HP. Odontogenic tumors and allied lesions. Quintessence Publishing Co. Ltd., London, UK, 2004.
2. Shear M, Speight P. Cysts of the oral and maxillofacial regions. 4th ed. Blackwell Munksgaard, Oxford UK, 2007.
3. Gorlin RJ, Pindborg JJ, Clausen FP, Odont, Vickers RA. The calcifying odontogenic cyst: A possible analogue of the cutaneous calcifying epithelioma of Malherbe. An analysis of fifteen cases. Oral Surg Oral Med Oral Pathol. 1962;15:1235–43.
4. Hong SP, Ellis GL, Hartman KS. Calcifying odontogenic cyst. A review of ninety-two cases with reevaluation of their nature as cysts or neoplasms, the nature of ghost cells, and subclassification. Oral Surg Oral Med Oral Pathol.1991;72:56–64.
5. Takata T, Zhao M, Nikai H, Uchida T, Wang T. Ghost cells in calcifying odontogenic cyst express enamel-related proteins. Histochem J. 2000;32:323-9.
6. Kim J, Lee EH, Yook JI, Han JY, Yoon JH, Ellis GL. Odontogenic ghost cell carcinoma: A case report with reference to the relation between apoptosis and ghost cells. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2000;90:630–5.
7. Smith JF, Blankenship J. The calcifying odontogenic cyst. Report of a case. Oral Surg Oral Med Oral Pathol. 1965;20:624–31.
8. Kerebel B, Kerebel LM. Ghost cells in complex odontoma: A light microscopic and SEM study. Oral Surg Oral Med Oral Pathol. 1985;59:371-8.
9. Chaves E, Pessôa J. The calcifying odontogenic cyst. Report of two cases. Oral Surg Oral Med Oral Pathol. 1968;25:849–55.
10. Freedmen PD, Lumerman H, Gee JK. Calcifying odontogenic cyst. A review and analysis of seventy cases. Oral Surg Oral Med Oral Pathol. 1975;40: 93–106.
11. Lukinmaa P-L, Leppaniemi A, Hietanen A, et al. Features of odontogenesis and expression of cytokeratins and tenascin-C in three cases of extraosseous and intraosseous calcifying odontogenic cyst. J Oral Pathol Med. 1997;26: 265-72.
12. Regezi JA, Courtney RM, Kerr DA. Keratinization in odontogenic tumors. Oral Surg Oral Med Oral Pathol. 1975;39:447-55.