Received: 10th August 2014 *Accepted: 14th November 2014* *Conflicts of Interest: None*

Source of Support: Nil

Original Research

Antigenic Heterogeneity in Granular Cell Ameloblastoma: An Immunohistochemical Study

Ramadoss Ramya[1], Peddanna Kondamari Sudheerkanth[2], Cherian Eapen[3], Varghese Kumaranthara Alex[4], Krishnan Rajkumar[5], Padmanaban Rajashree[6]

Contributors:

[1]Reader, Department of Oral Pathology, SRM Dental College, Chennai, Tamil Nadu, India; [2]Reader, Department of Oral Pathology, Mamata Dental College, Khammam, Telangana, India; [3]Reader, Department of Oral Pathology, St. Gregorius Dental College, Ernakulam, Kerala, India; [4]Professor, Department of Oral Pathology, Pushpagiri College of Dental Sciences, Thiruvalla, Kerala, India; [5]Professor & Head, Department of Oral Pathology, SRM Dental College, Chennai, Tamil Nadu, India; [6]Research Faculty, Department of Oral Pathology, SRM Dental College, Chennai, Tamil Nadu, India.

Correspondence:

Dr. Ramya R. 1170, 14th Street, Ashok Nagar, Chennai - 600 083, Tamil Nadu, India. Phone: +91-9840431293. Email: drramyascdc@gmail.com

How to cite the article:

Ramya R, Sudheerkanth PK, Eapen C, Alex VK, Rajkumar K, Rajashree P. Antigenic heterogeneity in granular cell ameloblastoma: An immunohistochemical study. J Int Oral Health 2015;7(3):49-52.

Abstract:

Background: Nature of granular cells in granular cell ameloblastoma (GCA) has always invoked considerable interest. The present study aims at antigenic characterization in five such cases with a panel of markers.

Materials and Methods: Tissue specimens of five patients with GCA were fixed in buffered formalin and later embedded in paraffin wax. Blocks were sliced into 3 µ thick sections for immunohistochemical analysis using a panel of markers CD68, Bcl2, S100, p53, cytokeratin (AE1/AE3), vimentin and desmin.

Results: All five cases were strongly positive for cytokeratin and CD68. S100 was negative in three cases and showed a mild positivity in two cases. Bcl2, p53, vimentin and desmin were negative in all the five cases.

Conclusions: This study presents a heterogenous nature of the granular cells; however, further validation is required with a larger sample size.

Key Words: Bcl2, CD68, cytokeratin (AE1/AE3), desmin, granular cell ameloblastoma, lysosomes, p53, S100, vimentin

Introduction

Ameloblastoma is the most common benign odontogenic tumor usually located in the jaw bone.[1] It is a tumor of the enamel organ that has not undergone differentiation to the point of formation of enamel.[2] Robinson has defined it as unicentric, non-functional, intermittent in growth, anatomically benign and clinically persistent. The tumor is locally invasive and leads to severe defacement.[3] The microscopic appearance of ameloblastoma is characterized by the presence of peripheral columnar cells with hyperchromatic, reversely polarized nuclei, arranged in a palisaded pattern.[4]

Conventional solid or multicystic ameloblastoma exhibits six microscopic subtypes namely follicular, plexiform, acanthomatous, granular cell, desmoplastic and basal cell ameloblastoma.[5] The follicular and plexiform patterns are the most frequent. Less common histopathologic subtypes include the acanthomatous, granular cell, desmoplastic, and basal cell.[1,6] Granular cell ameloblastoma (GCA) is one of the rare histological variants of ameloblastoma accounting for only 3.5% of ameloblastomas.[6]

GCA is characterized by presence of eosinophilic granules in the cytoplasm of stellate reticulum like cells.[7] Several studies have reported marked proclivity for recurrence.[6] However, aggressive behavior has been ruled out by recent studies implying that granular cells represent an evolution to a matured phase in the life cycle of ameloblastomas.[7,8] Despite numerous reports, granular cell change in ameloblastoma have always kindled considerable interest as to whether it is only a degenerative process or a portent of more aggressive course (Figure 1).[9,10]

Previous studies have carried out ultrastructural, histochemical and immunohistochemical methods to characterize the nature of the granular cells though the mechanism involved is poorly understood. The present study attempts to do an immunohistochemical analysis with a panel of markers to study the nature of granular cells in GCA. Due to its rarity accounting to 3.5%, literature search revealed that the majority of them were single case studies. This study is the first of its kind to report antigenic characterization in five such cases with a wide range of markers.

Materials and Methods

Case selection

Formalin-fixed paraffin-embedded tissue blocks of GCA were retrieved from the archives of Department of Oral and Maxillofacial Pathology, SRM Dental College, Chennai. The clinical data of the patients are listed in Table 1.

Immunohistochemical analysis

Immunohistochemical analysis was performed on 3 µ tissue sections on poly-L-lysine coated slides (Biogenex Life Sciences Limited, CA, US). Prediluted primary monoclonal mouse anti-CD68, anti-Bcl2, anti-S100, anti-p53, anti-cytokeratin antibody

Table 1: Clinical data of patients.

Case	Age	Sex	Location	Treatment	Recurrence
1	62	Male	Mandibular right premolar region	Resection	Once
2	59	Male	Mandibular left retromolar region	Resection	Nil
3	65	Female	Mandibular left retromolar region	Resection	Twice
4	45	Female	Mandibular right molar ramus region	Resection	Nil
5	38	Male	Mandibular right premolar region	Resection	Nil

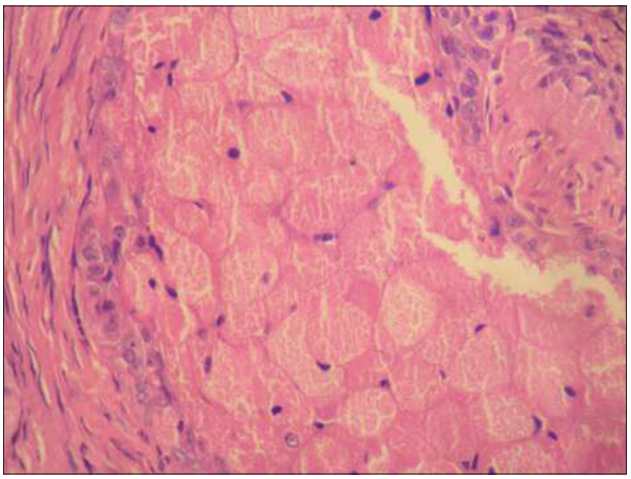

Figure 1: The granular cells exhibiting coarsely granular eosinophilic cytoplasm and small pyknotic nuclei replacing the central stellate reticulum cells (H and E, ×40).

(AE1/AE3), anti-vimentin and anti-desmin (Biogenex Life Sciences Limited, CA, US) were used, followed by the secondary super sensitive polymer HRP detection system (Biogenex Life Sciences Limited, CA, US). Diaminobenzidine was used as the chromogen and counterstained with Harris hematoxylin. Presence of brown colored end product at the site of target antigen was indicative of positive immunoreactivity. Evaluation of immunoreactivity was based on the staining intensity and was classified as weak, moderate, and strong. Localization of positively stained cells in peripheral ameloblast-like cells, central stellate reticulum like cells, and granular cells were also evaluated.

Results

Immunoreactivity of the markers used in the study is listed in Table 2. CD-68 expressed strong positivity in all the five cases. Positivity was observed only in the granular cells. Cytokeratin (AE1/AE3) expressed strong positivity in all the five cases by staining the peripheral cells, stellate reticulum like cells and granular cells. Bcl2, P53, vimentin and desmin exhibited negative staining in all the five cases.

Discussion

GCA accounts to 3.5% of all ameloblastomas.[11] The lesion presents with prominent change in cytoplasm of the stellate reticulum like cells to a very coarse, granular, eosinophilic

Table 2: Expression of CD68, cytokeratin, S-100, Bcl2, vimentin, desmin in GCA.

Markers used	Staining localization		
	Peripheral cells	Central cells	Granular cells
CD68	Negative	Negative	Strong
Cytokeratin	Strong	Strong	Strong
S-100	Negative	Negative	Mild
Bcl2	Negative	Negative	Negative
Vimentin	Negative	Negative	Negative
Desmin	Negative	Negative	Negative

GCA: Granular cell ameloblastoma

appearance.[5] Granular cells have also been found in other oral lesions like granular cell ameloblastic fibroma, congenital epulis and granular cell tumor.[12] Various concepts have been contemplated to ascribe the nature of oral granular cell lesions, foremost among them are odontogenic, fibroblastic, histiocytic, myoblastic, and neurogenic origins.[13]

The granular appearance has been attributed to lysosomes based on histochemical and ultrastructural findings.[14-16] Lysosomes are membrane-bound cell organelles found in most mammalian cells. They contain hydrolytic enzymes, which are capable of breaking down virtually all kinds of biomolecules, including proteins, nucleic acids, carbohydrates, lipids, and cellular debris. Presence of lysosomes represents increased cellular actions of the tumor ameloblasts to digest unwanted components.[17-19]

Considerable interest about the nature of granular cells in ameloblastoma ever since it was recognized has happened because of its reported aggressive behavior however recent literature reports speculate that the granular cell transformation in GCA may be associated with the aging phenomenon.[20,21]

The present study was carried out in five cases of GCA to ascertain the nature of the granules using a panel of markers CD68, Bcl2, S100, P53, Cytokeratin (AE1/AE3), vimentin and desmin. Strong positivity for cytokeratin and CD68 was noted in all the cases. S100 was negative in three cases and mildly positive in two cases. P53, Bcl2, vimentin and desmin were negative in all the five cases (Table 2).

The nature of granules in GCA in the previous studies has reported epithelial origin due to consistent positivity with cytokeratin and negativity with other mesenchymal markers (Figure 2). Presence of strong positivity with CD68 in granular cells indicates the presence of lysosomal aggregates (Figure 3).

Negative expression of antiapoptotic factors such as Bcl-2 and p53 proteins in granular cells indicate that there is increased apoptosis in the granular cells. This finding was similar to the report by Kumomoto *et al.* who reported apoptosis in the granular cells.[20] Contradictory to previous reports is the presence of a mild positivity with S100 unlike other

previously published reports (Figure 4). S100 is normally present in cells derived from the neural crest chondrocytes, adipocytes, myoepithelial cells, macrophages, langerhans cells, dendritic cells, and keratinocytes. Mild positivity of S100 could be suggestive of transdifferentiation of the cells. Such heterogenous presentation of granular ameloblastomas evokes more interest to further ratify its true nature.

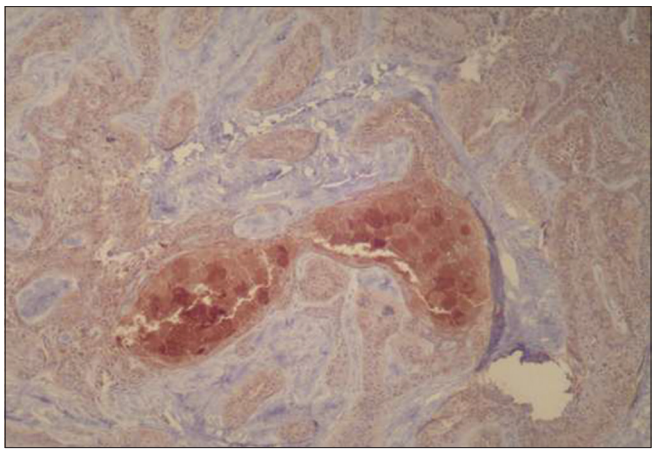

Figure 2: CD 68 exhibiting positivity in central granular cells (×20).

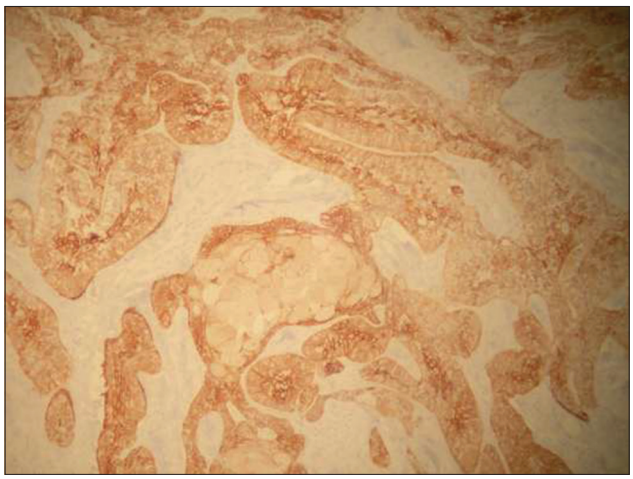

Figure 3: Cytokeratin positivity in peripheral, central and granular cells (×20).

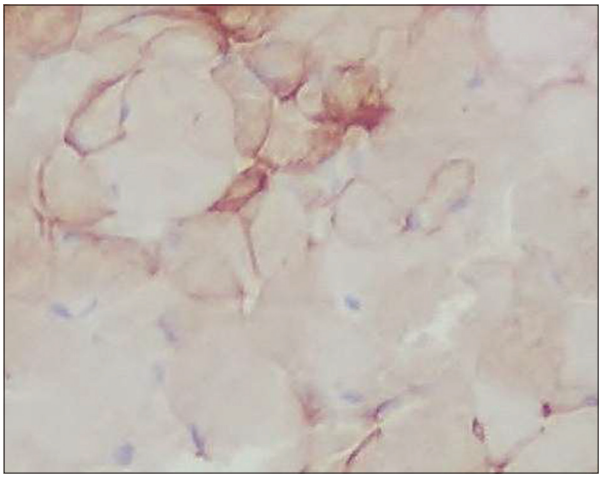

Figure 4: Mild positivity in granular cells with S100 (×40).

Conclusion

The current immunohistochemical panel could be evolved further for a better understanding of the nature of the granular cells in ameloblastomas. Further studies with more number of cases could help reason out the antigenic heterogeneity of GCA.

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