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## RESEARCH ARTICLE

### IDENTIFICATION OF NATURE OF SECRETORY MATERIAL USING HISTOCHEMICAL STAIN

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#### ABSTRACT

**Aims:** Odontogenic lesions comprise a complex group of lesions of diverse histopathologic types and clinical behaviour. These tumours are characterized by the presence of secretory material. Some of these secretory materials may show calcification. The secretory materials have been attributed to a wide range of material including enamel, dentin and cementum or bone, however the exact nature is still unknown. Methods like IHC though available are expensive, technique sensitive and questionable in certain circumstances. To identify the nature of secretory material in selected odontogenic tumours by using a histochemical stain called modified Gallego's stain using hard tissues of the tooth as the control.

**Methods:** The study will constitute a total of 12 histopathological proven cases (3 cases each) of various odontogenic tumours like adenomatoid odontogenic tumour, calcifying epithelial odontogenic tumour, calcifying odontogenic cyst and cemento-ossifying fibroma containing secretory material as identified in haematoxylin and eosin stained sections. The tissue sections from the above cases will be subjected to a histochemical stain called as modified Gallego's stain using hard tissues of the tooth as the control.

**Results:** Adenomatoid odontogenic tumour, calcifying epithelial odontogenic tumour, calcifying odontogenic cyst we found dentinoid like material and cemento-ossifying fibroma we found bone and cementum like material.

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## INTRODUCTION

Odontogenic tumours (OT) are lesions that are derived from either ectomesenchymal or epithelial elements that may demonstrate varying inductive interactions.<sup>1</sup> Lesions of odontogenic origin range from hamartomatous proliferations to malignant neoplasms (Neville *et al.*, 2002; Cawson and Evenson, 2008). Few of these are also characterized by formation of secretory material which mainly results because of inductive phenomena between ectomesenchyme and odontogenic epithelium. There are several hypothesis regarding the origin of these secretory component, however the nature of these are still controversial. In routine haematoxylin and eosin stains, secretory elements, in odontogenic tumours with or without calcification pose problems in determining its origin. New methods like IHC are technique sensitive, expensive and questionable in certain circumstances. Thus the aim of our present study is to determine the nature of these secretory material present in selected odontogenic tumours and demonstrating secretory material using modified Gallego's histochemical stain.

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## MATERIALS AND METHODS

This study constituted a total of 12 histopathological proven cases, 3 cases each of adenomatoid odontogenic tumour (AOT), calcifying epithelial odontogenic tumour (CEOT), calcifying odontogenic cyst and Cemento-ossifying fibroma retrieved from the archives of Department of Oral Pathology and Microbiology, SDM College of Dental Sciences and Hospital, Dharwad. Ground sections and fetal tooth germ were selected as control tissue. After examining the haematoxylin and eosin stained sections of the selected odontogenic tumours, the control tissues and sections of the selected odontogenic tumours were stained with modified Gallego's stain. Comparison between the staining characteristics of control and study groups was done and the secretory material categorized accordingly in every odontogenic tumour. The inclusion criteria comprised of paraffin embedded tissue sections of odontogenic tumours containing secretory material, fetal tooth germ and ground section that were available in the Department archives. While odontogenic tumours which do not contain secretory material & sections which exhibited folds, not allowing appropriate examination of the sections were excluded from the study.

## RESULTS

On staining the sections of the control and study groups with modified Gallegos' stain, interpretation was done based on the comparison of the staining characteristics of the control sections with that of the sections of the study groups.

### The following observations were made

- The ground sections & fetal tooth germ stained with Modified Gallego's stain demonstrated different colours for the different hard tissues of the tooth and bone (Table 1). Enamel exhibited pink colour; dentin and bone in various shades of green and cementum red colour (Figure 1 and 2).
- In Adenomatoid odontogenic tumours, the secretory material observed in shades of green was interpreted as dentin like material (Figure 3).

- In calcifying epithelial odontogenic tumour, the green coloured secretory material was also interpreted as dentin like material (Figure 4).
- In Calcifying odontogenic cyst, the green coloured secretory material was interpreted as dentin like material (Figure 5).
- In Cemento-ossifying fibroma, both green and red colour of the secretory material was attributed to the secretory material resembling bone like material and cementum like material respectively (Figure 6; Table 2).

## DISCUSSION

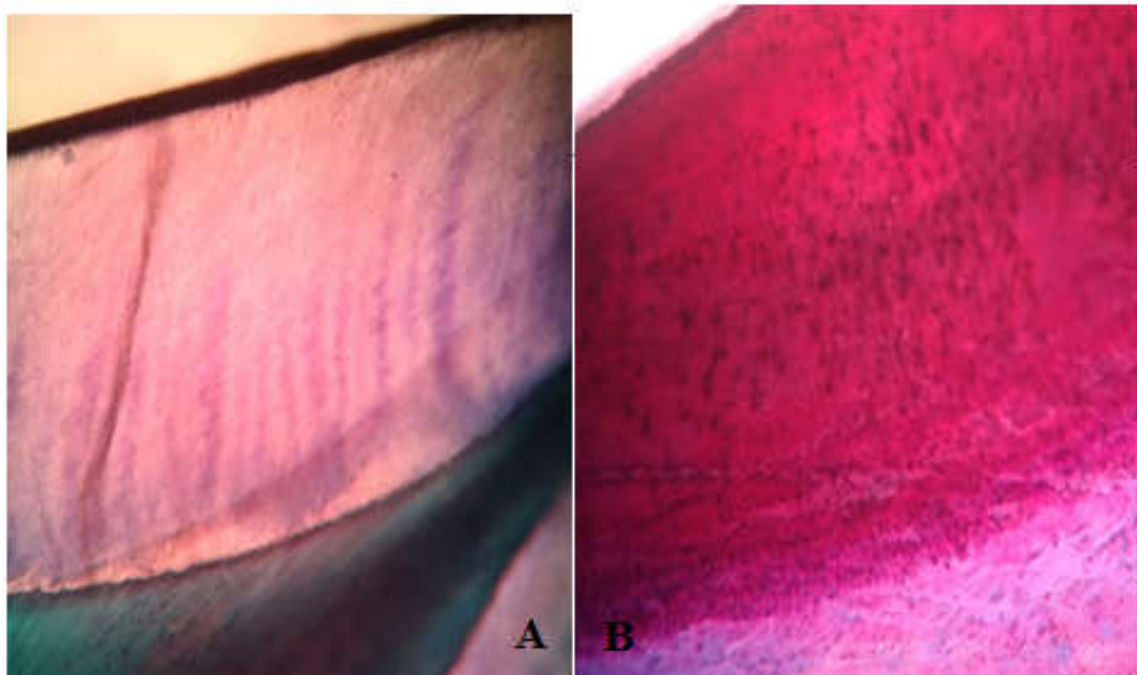
Odontogenic tumours are a diverse group of lesions with disparities in the histopathology. Some of them produce secretory material which may exhibit calcification.

**Table 1. Interpretation of nature of secretion using Modified Gallego's stain in normal tissues**

Normal Tissue	Colour	Interpretation
Tooth	Pink	Enamel
(Fetal tooth germ and Ground section)	Green	Dentin
	Dark red	Cementum
	Green	Bone

**Table 2. Interpretation of nature of secretion using Modified Gallego's stain in pathological tissues**

Tissue stained	Secretion	Colour	Interpretation
Adenomatoid Odontogenic Tumour	Along the tall columnar cells of ductal pattern intra-luminally	Green	Dentin like
Calcifying Epithelial Odontogenic	Between Polyhedral tumour cells	Green	Dentin like
Calcifying Odontogenic Cyst	In and around the Ghost cells	Varying shades of green	Dentin like
Cemento- ossifying fibroma	Present within the stroma	Green	Bone like
		Dark red	Cementum like



**Figure 1. (A) Ground section showing pink coloured enamel, green coloured dentin and (B) dark red stained cementum. (Modified Gallego's stain, 20X)**

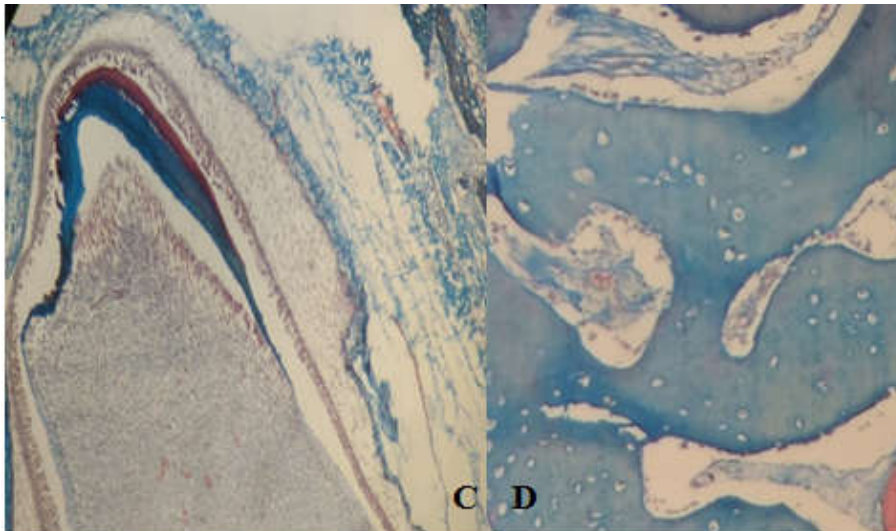


Figure 2. (C) Fetal Tooth Germ showing pink coloured enamel, green coloured dentin and (D) bone with osteocytes. (Modified Gallego's stain, 40X)

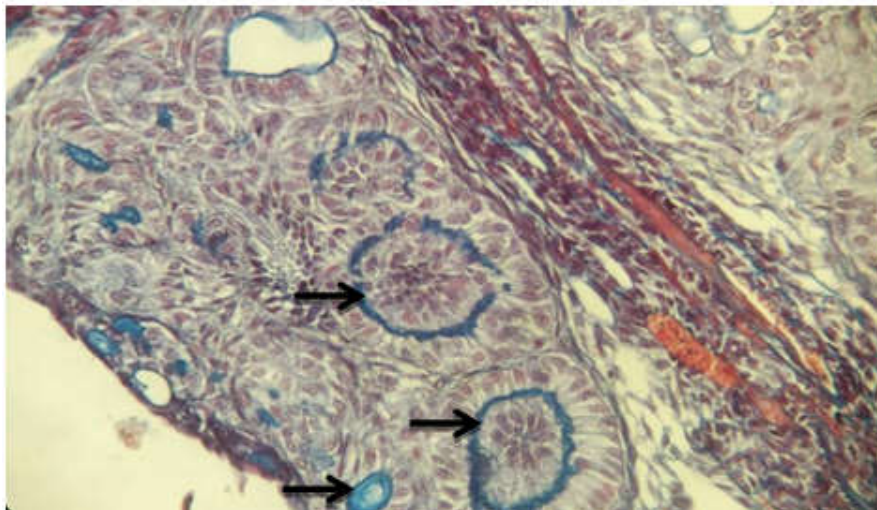


Figure 3. Green coloured secretory material seen in ductal & rosette pattern (arrows) of Adenomatoid Odontogenic Tumor. (Modified Gallego's stain, 20X)

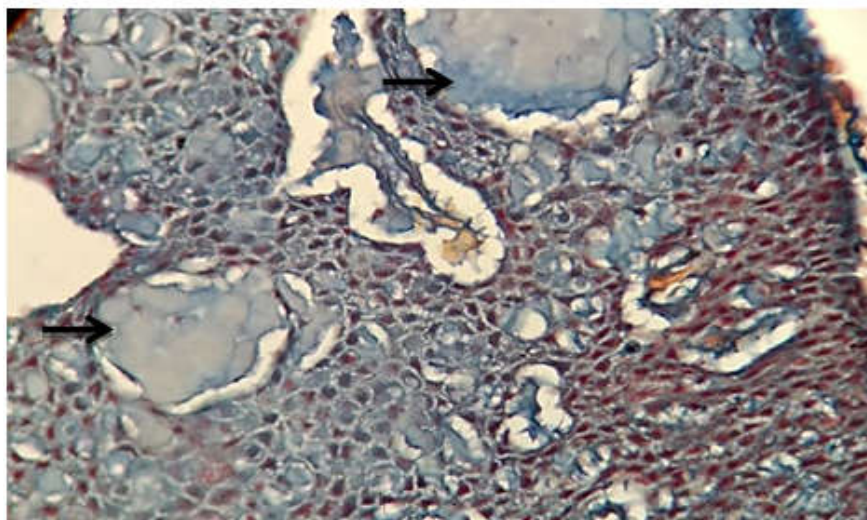
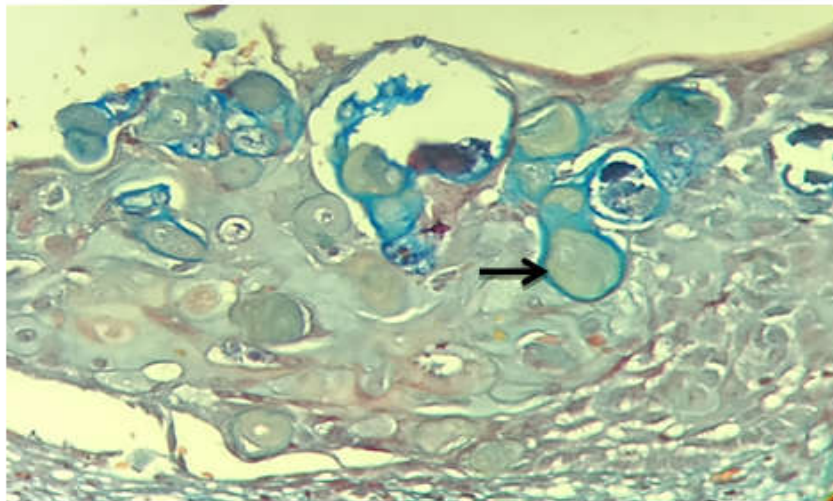
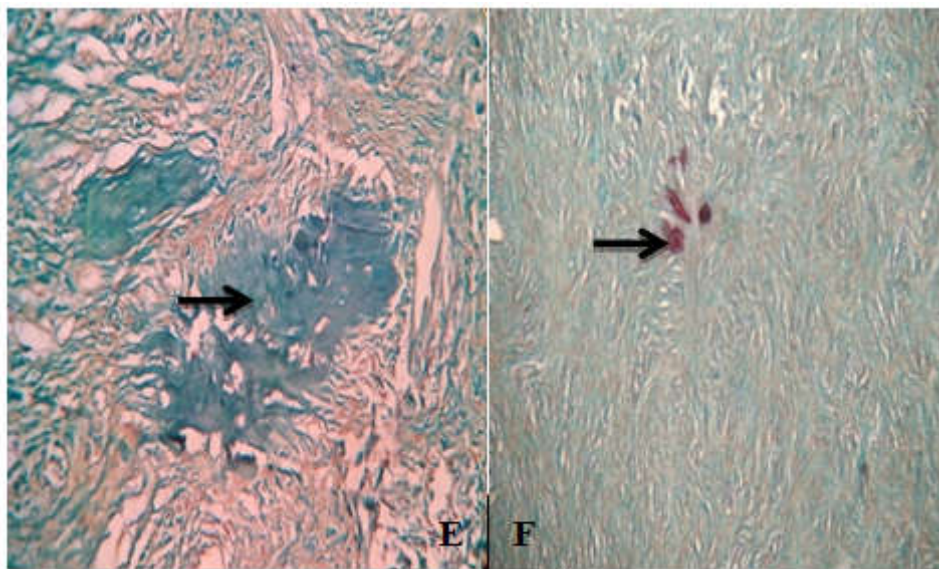


Figure 4. Green coloured secretory material between tumour cells in Calcifying Epithelial Odontogenic Tumor. (Modified Gallego's stain, 20X)





**Figure 5. Varying shades of green colour secretory material, prominent especially around the ghost cells in Calcifying Odontogenic Cyst. (Modified Gallego's stain, 20X)**



**Figure 6. (F) Green coloured secretory material & (G) dark red coloured secretory material present focally in cemento-ossifying fibroma. (Modified Gallego's stain, 20X)**

However, the nature of these secretory materials in routine haematoxylin and eosin stains remains an enigma. Numerous studies including special stains, electron microscopy and immunohistochemistry have been carried out to know the nature of these secretory materials. These secretory materials have been variably interpreted as enamel, dentin, cementum, bone, basement membrane component, laminin etc. However, the true nature of the component is still a paradox (Sandhya *et al.*, 2014) A study on odontogenic tumours observed a large inorganic crystals in the lumen of duct like structures in AOT which was attributed to the enamel crystals on electron microscopy based on the size of the crystals observed. (Yamamoto *et al.*, 1981). However, these methods of analysis have several limitations as they are expensive and time consuming. Equally important observation in this study was the nature of the secretory material was identified based on the inorganic component and not the organic component. To overcome this, immunohistochemistry has been used to target the secretory element by several authors using various biomarkers in odontogenic tumours. In a study of AOT with cytokeratin, laminin and vimentin, laminin positivity at the

luminal cell bordering the ductal component of the tumor was observed.(Crivelini *et al.*, 2005).While, enamel in luminal spaces and amelogenin cytoplasm of the odontogenic tumour epithelial cells was found using Immunoperoxidase technique(Murata *et al.*, 2000). In our study shades of green colour were observed only in the luminal portion adjacent to the tall columnar cells and in the rosette like area in Adenomatoid odontogenic tumour. Though green colour as seen in the control tissue is exhibited both by dentin and bone, we attributed this secretory material as dentin like material due to the distinctive absence of cells within its constituent. Various special stains have been used to demonstrate secretory material in odontogenic tumours till date. Special stains like PAS, Mucicarmine, Congo Red were used to detect the nature of the secretory material in CEOT. Amyloid was noted in the connective tissue close to the epithelial cell membrane as stained by Congo Red (Aviel-Ronen *et al.*, 2000). Similar to our study, Modified Gallego's stain was used and categorized the secretory material observed between tumour cells in CEOT as dentinoid like material (Mudhiraj *et al.*, 2017). Likewise, even in our study, secretory material in CEOT was observed

between the polygonal epithelial cells and was attributed to dentin like material based on the colouration and the absence of cells within it. A study of COC with CK's 10 and 14, EGFR, Tenascin, Ki-67 showed positive staining of CK14 in the connective tissue stroma adjacent to the odontogenic epithelium and accredited it as dentin like material (Ramadoss *et al.*, 2015). Though IHC is very specific, the above staining was not conclusive in detecting the nature of the secretory material. Thus, special stains were used & demonstrate Van Gieson stain positive ghost cells in COC (Pereira *et al.*, 2014). Similar to the above study in which ghost cells in COC were positive for Ayoub-Shklar stain, authors have attributed the ghost cells to keratin based on the positivity for the above mentioned stain (Jose *et al.*, 2016). Pereira *et al.*, used Modified Gallegos' stain and observed dentinoid-like material adjacent to odontogenic epithelium. However when Methylene Blue-Acid fuchsin stain was used strong positive staining was noted in connective tissue stroma beneath the odontogenic epithelium which was considered as dentin like material by Jose D. In our study, varying shades of green colour secretory material was observed in COC. Ghost cells exhibited a pale green colour where as a prominent green colour attributed to dentinoid like material, especially around the ghost cells was noted. Sandhya *et al.*, used Modified Gallego's stain to know the nature of the secretory material in AOT, odontome, dentinogenic ghost cell tumours and cemento-ossifying fibroma and inferred it to be enamel matrix in AOT; enamel, dentin and cementum in odontome; dentin and enamel in dentinogenic ghost cell tumours and bone and cementum in cemento-ossifying fibroma based on the comparison of the secretion of odontogenic tumour with that of the control tissue.

In this study, green colour was observed along with entrapped cells which was attributed to osteocytes within the lacunae of the bone while, at focal areas dark red coloured cementum like material was observed in cemento-ossifying fibroma cases. Von Kossa, Alizarin red for the bone, Picrothionin for dentin, Toulidine blue & Alcian blue for cementum are available but a single histochemical stain that differentiates between the diverse hard tissues of the tooth are rare. (Mudhiraj *et al.*, 2017) Therefore, the present study was done to identify the nature of secretory material in the pathological lesions using Modified Gallego's stain. Modified Gallego's- a differential stain, stains different tissues with a specific colour which makes it possible for easy identification especially when normal tooth and fetal tissues are used as control. The staining procedure is easy that can be done in routine laboratories, is inexpensive and not very technique sensitive. (Satheesan *et al.*, 2016) In our study, the green colour taken up by the secretory material in AOT, CEOT and COC was attributed to dentin while in Cemento-ossifying fibroma was designated as bone because of the presence of trapped osteocytes within the lacunae of the bone. Though green is taken up by both dentin and bone, they can be still set apart based on their cellular inclusion as seen in bone.

## Conclusion

Modified Gallego's stain offers a simple inexpensive method to detect the secretory material in odontogenic tumors.

This technique may serve as a diagnostic tool, an alternative to immunohistochemistry and provide better insight into the characteristic of lesion by increasing the sample size and comparing the results to that of immunohistochemistry.

## REFERENCES

- A Modified Gallego's Iron Fuschin Stain as a Differential stain for Cementum. *J Dent Res.* 1956; 35(3):491-93.
- Aviel-Ronen, S., Liokumovich, P., Rahima, D., Polak-Charcon S., Goldberg, I. and Horowitz A. 2000. The amyloid deposit in calcifying epithelial odontogenic tumour is immunoreactive for cytokeratins. *Arch Pathol Lab Med*, 124(6):872-6.
- Cawson, RA. and Evenson JW. 2008. Oral pathology and Diagnosis. New York; Saunders:p.130-63.
- Crivelini, MM., Soubhia, AMP. and Felipini RC. 2005. Study on the origin and nature of the adenomatoid odontogenic by immunohistochemistry. *J appl oral sci*, 13(4):406-12.
- Jose, D., Desai, K., Manne, DR. and Kale, A. 2016. Calcifying Cystic odontogenic tumour with Ameloblastoma: A Case Report with Review of the Literature. *IJPCDR.*, 2016;3(1): 38-41.
- Mudhiraj, PV., Vanje, MM., Reddy, BN., Ahmed, SA., Suri, C., Taveer, S. and Kumar, S. 2017. Nature of Hard Tissues in Oral Pathological Lesions-Using Modified Gallego's Stain. *J Clin Diagn Res, JCDR.*, 11(4):ZC13. Levey RJ.
- Murata, M., Cheng, J., Horino, K., Hara, K., Shimokawa, H. and Saku T. 2000. Enamel proteins and extracellular matrix molecules are co-localized in the pseudocystic stromal space of adenomatoid odontogenic tumour. *J Oral Pathol Med*, 1;29(10):483-90.
- Neville, Damm, Allen and Bouquot. 2002. Oral and Maxillofacial pathology. Philadelphia: Saunders; p.589-620.
- Pereira, T., Tamgadge, AP., Bhalerao, S. and Tamgadge, S. 2014. Hybrid odontogenic with COC and multiple variants of ameloblastoma-a rare case report. *Oral Hyg Health*, 2:126.
- Ramadoss, R., Krishnan, R., Peddanna, SK., Cherian, E., Gunasekaran, N. and Thayalan D. 2015. Immunohistochemical analysis of cytokeratin 10, cytokeratin 14, epidermal growth factor receptor, tenascin, and Ki-67 in selected odontogenic cysts. *SRM J Res Dent Sci*, 1;6(2):96.
- Sandhya, T., Avinash, T., Srivastava, C., Satheesan, E. and Bhalerao S. 2014. Modified Gallego's Stain as Differential Stain for Oral Hard Tissues in Oral Pathology: A Preliminary Report. *Int J Maxillofac Pathol*, 5(2):02-06.
- Satheesan, E., Tamgadge, S., Tamgadge, A., Bhalerao, S. and Periera T. 2016. Histopathological and radiographic analysis of dental follicle of impacted teeth using modified Gallego's stain. *J Clin Diagn Res: JCDR.* 2016 May;10(5):ZC106.
- Yamamoto, H., Kozawa, Y., Hirai, G., Hagiwara, T. and Nakamura, T. 1981. Adenomatoid odontogenic tumour: light and electron microscopic study. *Int J Oral Surg*, 1;10(4):272-8.