

Comparison of Hematoxylin and Eosin Stain with Modified Gallego's Stain for Differentiating Mineralized Components in Ossifying Fibroma, Cemento-ossifying Fibroma, and Cementifying Fibroma

Snehal Dhouskar, Sandhya Tamgadge, Avinash Tamgadge, Treville Periera, Uma Mudaliar, Aswathy Pillai

Department of Oral and Maxillofacial Pathology and Microbiology, D Y Patil Deemed to be University School of Dentistry, Nerul, Navi Mumbai, Maharashtra, India

Abstract

Objective: The nature of calcifications in fibro-osseous lesions is difficult to differentiate under hematoxylin and eosin (H and E) stain and could be misleading. Special stains could be used. Modified Gallego's stain is a differential stain for hard tissues, which has been discussed recently in the literature. **Methods:** Retrospective study was done from June to December 2015 to differentiate various types of mineralized tissues in ossifying fibroma (OF), cemento-OF (COF), and cementifying fibroma (CF), using modified Gallego's stain and its correlation with H and E stain. Control group comprised of decalcified section of bone, tooth, and odontoma, stained with modified Gallego's stain. Study group comprised of 30 lesions (10 OF, 10 COF, and 10 CF) stained with both modified Gallego's stain and H&E stain. This study did not have any numerical data; therefore, no appropriate statistical test could be performed. Hence, cross tabulation of the categorical data was used followed by descriptive statistical analyses. Results were presented on continuous measurements using mean \pm standard deviation, and results on categorical measurements were presented in number (%). **Results:** Modified Gallego's staining showed that, out of 10 cases of OF, 9 cases were interpreted as OF; one case of juvenile psammomatoid OF was interpreted as juvenile psammomatoid COF. Out of 10 cases of COF, 4 cases were interpreted as OF. Out of 10 cases of CF, 2 cases were interpreted as COF and 3 cases as OF. **Conclusions:** Fibro-osseous lesions are difficult to diagnose using H and E staining alone. Modified Gallego's stain could be a best adjunct.

Keywords: Bone, cementum, dentin, fibro-osseous lesions, mineralization, modified Gallego's stain

INTRODUCTION

Fibro-osseous lesions of the jaws are challenging group of pathologies as they pose a diagnostic challenge because they show variations in type of stroma and nature of mineralized tissue (ranging from bone: woven/lamellar bone, cementum, or dystrophic calcifications).^[1,2] They share a wide overlapping histomorphologic spectrum and have difficulty in assessing the origin and pathogenesis, rendering a confirmed diagnosis. The nature of the organic matrix too poses diagnostic difficulties many times.^[1,3] The components of hard tissues of various lesions have dentin, enamel, cementum, bone and other calcified deposits, and mature and immature collagen which can pose problems for identification in routine "Haematoxylin and Eosin (H and E)" and eosin-stained sections.^[4]

However, there is not a single specific stain that could describe the nature of such hard tissue in terms of composition and genesis.^[4] Over the years, few stains have been employed to study cementum to confirm histologically.^[3,5] Sometimes, lesions consist of multiple tissues, and use of combinations of stains can demonstrate such components of hard tissues and soft tissues distinctly.^[5] Many special stains have been used in literature to determine the varying types of calcified tissues

Address for correspondence: Dr. Sandhya Tamgadge, Department of Oral and Maxillofacial Pathology and Microbiology, D Y Patil Deemed to be University School of Dentistry, Nerul, Navi Mumbai - 400 706, Maharashtra, India. E-mail: sandhya.tamgadge@gmail.com

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

How to cite this article: Dhouskar S, Tamgadge S, Tamgadge A, Periera T, Mudaliar U, Pillai A. Comparison of hematoxylin and eosin stain with modified Gallego's Stain for differentiating mineralized components in ossifying fibroma, cemento-ossifying fibroma, and cementifying fibroma. *J Microsc Ultrastruct* 2019;7:124-9.

Access this article online

Quick Response Code:



Website:
<http://www.jmau.org/>

DOI:
10.4103/JMAU.JMAU_2_19

such as Verde Luz-orange G-acid fuchsin stain, modified tetra chrome method, methylene blue/acid fuchsin stain, and modified Attwood's stain.^[6,7] Modified Gallego's stain is a variant of Lille's stain that consists of basic reagents "H and E", carbol fuchsin, and aniline blue.^[5] The cementum stain as deep red as compared to the green stain of the dentin and bone.^[8]

In addition, identification of all types of calcified structures in their initial phase of mineralization is crucial for diagnosis of such lesions.^[5] Keeping this in view, a study was conducted to differentiate various types of oral hard tissues in oral pathology using decalcified sections, soft tissue biopsies, and its correlation with the histopathological diagnosis. True nature of mineralizing component of fibro-osseous lesions cannot be determined using H and E stain. Therefore, authors have used modified Gallego's stains which have never been used till date. Fibro-osseous lesions have high rate of recurrence and have aggressive potential; therefore, there is a need of an hour to identify the exact histochemical nature of mineralized deposits because "H and E" and eosin stain would give diagnosis only on morphological characteristics which could be misleading.^[9,10] In addition, there are not any proven immunohistochemical methods for decalcified and ground sections of tissues containing cementum and bone. This could be a preliminary study of the same.

Modified Gallego's stain had been tried only in normal dental hard tissues, but Sandhya *et al.* did a preliminary study in oral lesions containing hard tissues using the same stain.^[5] Therefore, this study was planned to determine the exact histochemical identification of fibro-osseous lesions containing cementum and bone, which has not been done before, to reduce recurrences and to plan treatment.

Lesions with osseous components include ossifying fibroma (OF), cemento-OF (COF), and cementifying fibroma (CF) which are considered to be a spectrum of events arising from cells in the periodontal ligament and having the potential to form bone, cementum, and fibrous tissue in varying proportions and combinations.^[11]

The fibrous connective tissue of the periodontal ligament have the capacity to produce bone, cementum, and fibrous tissue under pathological conditions.^[12]

In decalcified "H and E" stained sections, the distinction between dentine and cementum is exceedingly ill defined; so much so that, it is difficult to distinguish between them. This may be because both are mesodermal in origin with similar histologic character and physiological function.^[13]

Fibro-osseous lesions contain varying amount of mineralized deposits, and diagnosis using H and E often poses challenges for pathologists because it is often difficult to differentiate the organic matrix of osteoid in the initial phase of osteogenesis from cementum-like depositions.^[14-17] Thus, differential staining of cementum can be of profound importance in highlighting the nature and biological behavior of such lesions.^[15]

CF has a high recurrence rate; so, exact definite diagnosis is mandatory to prevent its recurrence.^[12] Various stains have been implemented for cementum but most are not economical and available easily. An inexpensive and distinctive stain for cementum can be extremely useful in investigation, observation, diagnosis, and teaching. A search of the published literature has shown that, at present, there are only few techniques which will differentially stain the cementum of human teeth such as cresyl violet, Alcian blue, and Nuclear fast red stains. However, modified Gallego's stain has promising results as it gives deferential staining to various hard tissues present in lesions,^[14] which is uncommon in the literature especially in dental literature.

METHODS

The retrospective study was done from June to December 2015 at DY Patil University School of Dentistry, Nerul, Navi Mumbai. Institutional ethical approval was not required as patient's identity was not disclosed. Study comprised of total 30 cases in study group, among which 10 cases of each were OF, COF, and CF, respectively. As this stain could be used in decalcified, ground, and soft tissues, control group (total 10) consisted of ground sections of bone and teeth, decalcified sections of bone and teeth, and odontoma which had both cementum and soft tissues [Figure 1].

Two paraffin-embedded sections of each sample were obtained and stained with H and E and Modified Gallego's stain^[8] as follows:

1. Deparaffinize the sections
2. Stain with "H and E" for 8–12 min
3. Rinse in distilled water
4. Stain in mordant for 2 min (Mix 200 ml of distilled water in 1.5 ml of concentrated nitric acid with 1 ml of 40% formaldehyde and 1.5 ml of USP iron chloride). Rinse in distilled water

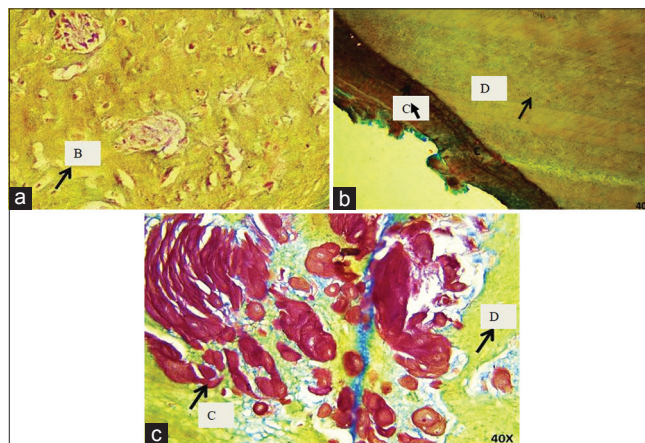


Figure 1: Photomicrograph showing (a) decalcified section of bone showing bone (B) in green ($\times 40$), (b) decalcified section of tooth showing dentin (D) in green and cementum (C) in red color ($\times 40$), (c) odontome showing irregularly arranged cementum (C) depositions in red color and dentin (D) in green ($\times 40$) (modified Gallego's stain)

5. Stain with 3 ml of carbol Fuchsin in 50 ml of 0.2% acetic acid and rinse in distilled water
6. Wash in mordant for 1–2 min
7. Stain with 0.01% aniline blue in saturated picric acid solution for 30 s
8. Dehydrate and clear with xylene and mount in dibutyl phthalate in xylene mounting media.

The sections were examined under microscope at each magnification, and nature of calcification was determined as per criteria given by Gallego.^[8] Three separate microscopic fields were randomly selected to observe and analyze the mineralized deposits using ×40 objective under a light microscope Leica research microscope (Leica application suite [LES] core version 3.8) of Leica research microscope (Model No. DM1000 LED, Leica Microsystems GmbH Ernst-Leitz-Straße 17–37 | 35,578 Wetzlar [Germany]) by 2 pathologists who were blindfolded. Care was taken to visualize same fields in both sections. Images were captured using LES software of image analysis. The nature of calcification was determined, and histopathological diagnosis was compared and correlated in both stains to confirm whether the diagnosis remained same or changed. Since this study did not have any numerical data, no appropriate statistical test could be performed using the statistical software. Hence, cross tabulation of the categorical data was done, and further descriptive statistical analyses were carried out. Results on continuous measurements were presented on mean ± standard deviation, and results on categorical measurements were presented in Number (%).

The statistical software IBM SPSS statistics 20.0 (IBM Corporation, Armonk, NY, USA) was used for the analyses of the data, and Microsoft word and Excel were used to generate graphs and tables.

RESULTS

Tissues in control group were interpreted as green as bone and dentin and red as cementum^[5] [Figure 1]. When cross tabulation of hematoxylin staining and modified Gallego's staining for OF group was done, in the modified Gallego's staining, 9 (90%) samples displayed bony tissue which was the same as in "H and E" stain; therefore, the diagnosis did not change but 1 (10%) sample showed both bone and cementum which was only bone in the hematoxylin stain, suggestive of change in final diagnosis as COF [Figure 2 and Table 1].

When cross tabulation of hematoxylin staining and modified Gallego's staining for COF group was done, in the modified

Gallego's staining, 6 (60%) samples displayed bone and cementum. Therefore, there was no change of diagnosis. However, 4 (40%) cases showed green-colored mineralized deposits suggestive of bone. Thus, there was change in diagnosis from COF to OF [Figure 3 and Table 1].

When cross tabulation of "H and E" staining and modified Gallego's staining for CF group was done, it was found that in modified Gallego's staining, 5 (50%) samples displayed cementum which was the same in "H and E" stain but 3 (30%) samples displayed bone and 2 (20%) samples displayed both bone and cementum in the modified Gallego's staining which was only cementum in the "H and E" stain suggestive of change in diagnosis from CF to OF and COF, respectively [Figure 4 and Table 1].

In one case of CF, a combination of deposits of bone and cementum in different colors were seen. One case of juvenile psammomatoid OF was interpreted as juvenile psammomatoid cement-ossifying fibroma under modified Gallego's staining [Figures 5 and 6].

DISCUSSION

Gallego (1954) conducted a preliminary study to identify mineralized components with the help of modified Gallego stain in decalcified, ground sections of tooth in which dentin and bone stained green and cementum stained red.^[8] In addition, special stains are performed in soft tissue and decalcified tissue but not in ground sections which can only be done by modified Gallego stain.^[5]

In 1956, Robert J. Levey performed a differential staining of cementum using modified Gallego's Iron Fuchsin Stain, where they concluded that the cementum is deep red in contrast to the green stain of the dentin and bone.^[8] In the present study, authors tried ground sections, decalcified sections of both tooth and bone, and even soft pathological tissues, still results were same as the original study.

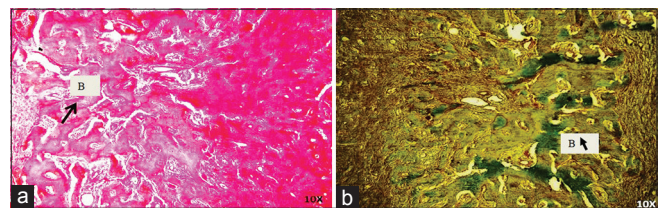


Figure 2: Photomicrograph (a) ossifying fibroma showing numerous bony deposits (B) suggestive of osteoid (H and E, ×10). Photomicrograph (b) same section of ossifying fibroma stained with modified Gallego's stain demonstrating bone (B) in green (×10)

Table 1: The cross tabulation of hematoxylin and eosin staining and modified Gallego's staining for study group

Hematoxylin and eosin staining	Modified Gallego's staining showing various calcifications			Total, n (%)
	Bone, n (%)	Bone and cementum, n (%)	Cementum, n (%)	
Ossifying fibroma	9 (90)	1 (10)	Nil	10 (100)
Cemento-ossifying fibroma	4 (40)	6 (60)	Nil	10 (100)
Cementifying fibroma	3 (30)	5 (50)	2 (20)	10 (100)

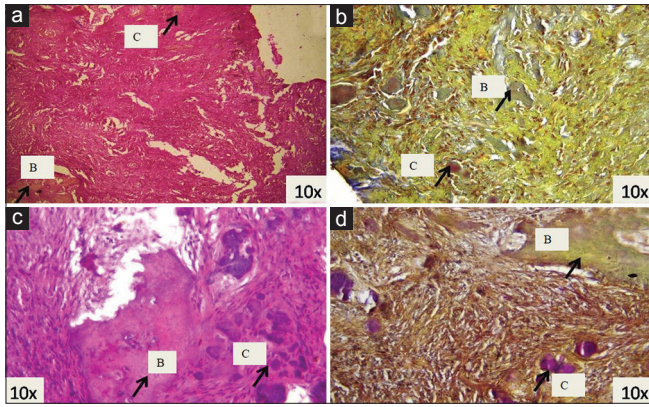


Figure 3: Photomicrograph (a) cemento-ossifying fibroma showing bone (B) and cementum (C) like deposits (H and E, $\times 40$). (b) Same section of cemento-ossifying fibroma stained with modified Gallego's stain showing bone (B) in green and cementum (C) like deposits in red ($\times 40$). (c) cemento-ossifying fibroma showing globular masses of cementum (C) like deposition and bony (B) deposits ($\times 40$). (d) Same section of cemento-ossifying fibroma stained with modified Gallego's stain showing bone (B) in green and cementum (C) like deposits in red ($\times 40$)

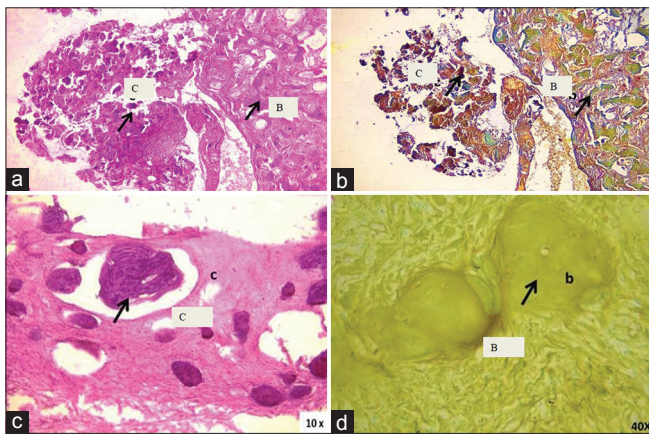


Figure 5: Photomicrograph (a) juvenile psammomatoid ossifying fibroma showing bone (B) and cementum (C) (H and E, $\times 10$). (b) Same section of juvenile psammomatoid ossifying fibroma stained with modified Gallego's stain demonstrating bone (B) in green and cementum (C) like deposits in red ($\times 10$). (c) Cementifying fibroma (Haematoxylin and Eosin stain) showing globular deposits without lacunae suggestive of cementum (C) (10X). (d) Same section of Cementifying fibroma stained with Modified Gallego's stain showing globular deposits in green as bone (B) suggestive of Ossifying Fibroma (40X).

In 2015, Sandhya *et al.* conducted a differential staining of hard tissues of tooth, bone, and pathological lesions such as calcifying odontogenic cyst, adenomatoid odontogenic tumor, and COF using the modified Gallego's stain to know the nature of calcifications. They concluded that enamel stains were pink in color and immature hard tissue deposition stains were in shades of red and green. In pathological lesions such as cement-ossifying fibroma, the bony deposits stained green and the cementum-like deposits stained red.^[5]

In the present study, three different pathological lesions such as OF, COF, and CF were studied using modified Gallego's

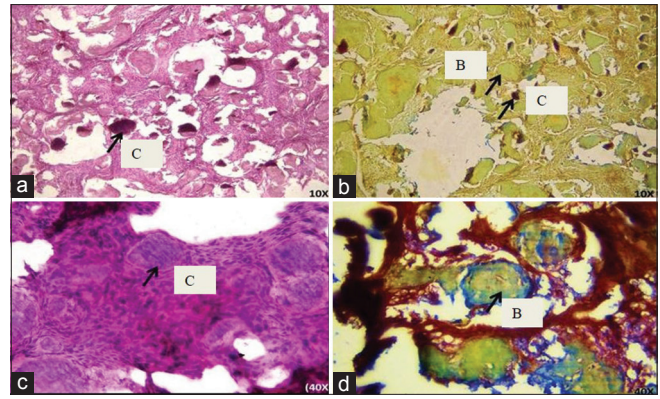


Figure 4: (a) Cementifying fibroma demonstrated numerous calcifications suggestive of cementum (C) (H and E, $\times 10$). (b) Same section (modified Gallego's stain) showing globular deposits in green as bone (B) and cementum (C) like deposits in red suggestive of cemento-ossifying fibroma ($\times 10$). (c) Cementifying fibroma showing globular concentric deposits suggestive of cementum (C) like depositions (H and E, $\times 40$). (d) Same section (modified Gallego's stain) showing globular concentric deposits of bone (B) without lacunae in green suggestive of ossifying fibroma ($\times 10$)

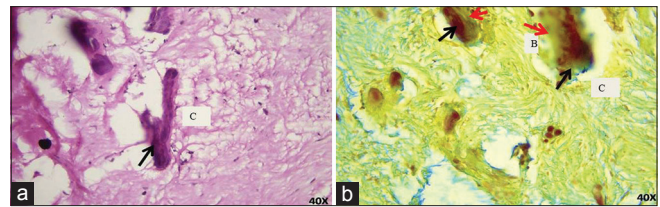


Figure 6: Photomicrograph (a) cementifying fibroma showing calcifications suggestive of cementum (C) (H and E, $\times 40$). (b) Same section of cementifying fibroma stained with modified Gallego's stain showing combination of deposits of bone (B) and cementum (C) (black arrow) in different colors ($\times 40$)

stain and "H and E" stain which showed similar results as the above study. Although some criteria, namely the presence of bone and cementum have been laid down for the diagnosis of these three lesions, their diagnostic significance has been questioned.^[16] The bony tissues can be identified as irregular depositions with osteoblasts rimming and osteoblasts in the lacunae.^[17] Larger masses and sheets of secondary and matured cementum are virtually indistinguishable from bone most of the times under light microscopy. In cementum typical osteoblasts like rimming of cells is not seen. In comparison with bone, the lacunae in cementum contain fewer recognizable cellular elements.^[17]

Initial phases of osteoid might show cementum-like features which could be confused with cementum depositions. However, under modified Gallego's stain, these globular concentric deposits appeared green in color representing bone and suggestive of OF. This is supported by the review of Bhaskar and Cutright who suggested that CFs are in reality OFs, in which the bone tissue appears basophilic and resembles cementum superficially. CFs and OFs are two distinct benign neoplasms representing two facets of the same tumor.^[18]

In the present study, it was observed that the section thickness should be uniform to visualize the lacunae for differentiation of bone from cementum which was seen in three cases of this study.

In one case of CF, a bone deposition was covered by a layer of cementum deposits at paces under modified Gallego's stain. We hypothesize that this could be possibly due to similar tissue of origin in these tissues. Mesenchymal blast cells of periodontal ligament are capable of being stimulated to produce tumors composed of cementum, lamellar bone, fibrous tissue, or any combination of these tissues^[1,3] It could also be hypothesized that it could be attributed to the degree of mineralization. The color of the mineralized components depends on the degree of mineralization, highly calcified enamel shows light pink, bone and dentin are almost equally mineralized showing green color, and the less mineralized cementum shows red color. The bone is green in color and covered by osteoid tissue which is less mineralized and has the composition similar to cementum. Therefore, osteoid might appear red as cementum which was observed in few cases as combination of both tissues.

Another unique finding in this study was that, two cases of CF, demonstrated both, green calcifications representing bone as well as red calcifications representing cementum like depositions, under modified Gallego's stain, suggestive of COF.

An admixture of the two types of calcifications in COFs is extremely variable in appearance and represents various stages of bone and cementum deposition. The hard tissue portions consist of trabeculae of osteoid and bone or basophilic and poorly cellular spherules that bear a resemblance to cementum. The bony trabeculae vary in size and often demonstrate a mixture of woven and lamellar patterns. Peripheral osteoid and osteoblastic rimming are usually present. The spherules of cementum-like material often demonstrate peripheral brush borders that blend into the adjacent connective tissue.^[12] Similar findings were observed in our cases.

In the present study, spherical calcifications or spherules were observed in the OF cases which were acellular round to oval structures without lacunae of various sizes under modified Gallego's stain. This could be due to the initial stages of osteogenesis.

In few cases, the globular green cementum-like deposits showed lacunae under modified Gallego's stain. Thus, bony areas cannot be identified only on the basis of trabecular shape.

Boysen *et al.* have also reported the presence of spheroid calcifications more in cases of OF.^[19] However, Sisson *et al.* reported the presence of calcified spherules in cases of extra gnathic fibrous dysplasia and suggested that the presence of calcified spherules is not suggestive of diagnosis of CF or of odontogenic origin.^[20]

Satheesan *et al.* examined dental follicle tissues associated with impacted teeth and evaluated types of mineralized tissue deposits in it, using modified Gallego's stain and Panthula Veenila Mudhiraj *et al.* studied in oral lesions.^[21,22]

The true nature of these calcifications cannot be determined by light microscopy; therefore, it is difficult to say whether they are bone, cementum, metaplastic bone, or dentin.^[16] However, using modified Gallego's stain, this could be confirmed in this study.

In one case of Juvenile Psammomatoid OF, deposits of bone were seen under "H and E" and eosin-stained sections. However, under modified Gallego's stain, same sections were seen demonstrating bone in green and cementum-like deposits in red. This could be due to the presence of typical psammoma bodies. Therefore, psammoma bodies consist of cementum-like deposition, proved under Gallego in this study.

Since, psammoma-like ossicles seen in psammomatoid juvenile OF resembles cementicles in COF, it has been argued that psammomatoid juvenile OF is a type of COF. However, the marked cellularity of JOF is in sharp contrast to the usually stroma-rich appearance of the latter group of lesions.^[23] However, in literature, few cases have been reported in which a large number of spheroidal cementum-like calcifications have been termed as psammomatoid bodies.^[24] Thus, in this study, this case of juvenile psammomatoid OF was suggestive of juvenile psammomatoid COF under modified Gallego's stain. Thus, it is obvious that the modified Gallego's stain is efficient as a diagnostic tool in cases where the diagnosis is questionable as the nature of hard tissue is challenging.

As the duration was small for this short study and to manage equal number of patients in each group, sample size was small for this original research.

CONCLUSIONS

The calcifications can be misdiagnosed using routine "H and E" and eosin stain. Hence, to overcome this problem, modified Gallego's stain was used in this study.

The modified Gallego's stain could be considered as a practical tool in diagnosis not only for soft tissues and decalcified tissues but also for ground sections which has not been reported so far. Because of the ease of staining, it should be considered as an alternative before the pathologist moves on to more advanced methods such as immunohistochemistry. The stain gives a proper insight into character of the mineralized deposits, and therefore, it is helpful to arrive at a final diagnosis or also to confirm analysis of a lesion.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Kulkarni RR, Sarvade SD, Boaz K, Srikant N, Nandita KP, Lewis AJ. Polarizing and light microscopic analysis of mineralized components and stromal elements in fibrous ossifying lesions. *J Clin Diag Res* 2014;8:ZC42-5.
2. Lasisi TJ, Adisa AO, Olusanya AA. Fibro-osseous lesions of the jaws in

- Ibadan, Nigeria. *Oral Health Dent Manag* 2014;13:41-4.
3. Cawson RA, Evenson JW. *Cawson's Essentials of Oral Pathology and Oral Medicine*. New York; Saunders: 2008.
 4. Belaldavar C, Hallikerimath S, Angadi PV, Kale AD. Comparison of tetrachromic VOF stain to other histochemical staining techniques for characterizing stromal soft and hard tissue components. *Biotech Histochem* 2014;89:545-51.
 5. Sandhya T, Avinash T, Srivastava C, Satheesan E, Bhalerao S. Modified Gallego's stain as differential stain for oral hard tissues in oral pathology: A preliminary report *Int J Oral Maxillofac Pathol* 2014;5:2-6.
 6. Gupta K, Kale AD, Hallikeremath SR, Kotrashetti VS. A histochemical comparison of methylene-blue/acid fuchsin with hematoxylin and eosin for differentiating calcification of stromal tissue. *Biotech Histochem* 2012;87:249-56.
 7. Putns S, Desa DJ. Application of modified Atwood's stain to study the decalcified section of the bone sections. *Tech Methods* 1977;30:900-3.
 8. Levey RJ. A modified Gallego's iron fuchsin stain as a differential stain for cementum. *J Dent Res* 1956;35:491-3.
 9. Rama Raju D. Cementifying fibroma – A case report. *Ann Essences Dent* 2010;2:1-4.
 10. Mergoni G, Meleti M, Magnolo S, Giovannacci I, Corcione L, Vescovi P, *et al.* Peripheral ossifying fibroma: A clinicopathologic study of 27 cases and review of the literature with emphasis on histomorphologic features. *J Indian Soc Periodontol* 2015;19:83-7.
 11. Waldron CA, Giansanti JS. Benign fibro-osseous lesions of the jaws: A clinical-radiologic-histologic review of sixty-five cases. II. Benign fibro-osseous lesions of periodontal ligament origin. *Oral Surg Oral Med Oral Pathol* 1973;35:340-50.
 12. Sheikhi M, Mosavat F, Jalalian F, Rashidipoor R. Central cementifying fibroma of maxilla. *Dent Res J (Isfahan)*. 2013;10(1):122-5. doi: 10.4103/1735-3327.111814.
 13. Shukla D, Vinuth DP, Sowmya SW, Jeevan MB, Kale AD, Hallikerimath S, *et al.* Cementum made more visual. *J Forensic Odontostomatol* 2012;30:29-37.
 14. Kvaal SI, Solheim T, Bjerketvedt D. Evaluation of preparation, staining and microscopic techniques for counting incremental lines in cementum of human teeth. *Biotech Histochem* 1996;71:165-72.
 15. MacDonald-Jankowski DS. Fibro-osseous lesions of the face and jaws. *Clin Radiol* 2004;59:11-25.
 16. Maheshwari P, Rao NN, Radhakrishna R. Histochemical evaluation of stromal components in fibrous dysplasia, central ossifying fibroma and juvenile ossifying fibroma affecting the jaw bones. *Int J Dent Res* 2014;2:3-7.
 17. Kumar GS, editor. *Orban's Oral Histology and Embryology*. 12th ed. India: Elsevier India; 2008. p. 128-9.
 18. Bhaskar SN, Cutright DE. Multiple enostosis: Report of 16 cases. *J Oral Surg* 1968;26:321-6.
 19. Boysen ME, Olving JH, Vatne K, Koppang HS. Fibro-osseous lesions of the cranio-facial bones. *J Laryngol Otol* 1979;93:793-807.
 20. Sissons HA, Steiner GC, Dorfman HD. Calcified spherules in fibro-osseous lesions of bone. *Arch Pathol Lab Med* 1993;117:284-90.
 21. Satheesan E, Tamgadge S, Tamgadge A, Bhalerao S, Periera T. Histopathological and radiographic analysis of dental follicle of impacted teeth using modified Gallego's stain. *J Clin Diagn Res* 2016;10:ZC106-11.
 22. Mudhiraj PV, Vanje MM, Reddy BN, Ahmed SA, Suri C, Taveer S, *et al.* Nature of hard tissues in oral pathological lesions -using modified Gallego's stain. *J Clin Diagn Res* 2017;11:ZC13-5.
 23. Thankappan S, Nair S, Thomas V, Sharafudeen KP. Psammomatoid and trabecular variants of juvenile ossifying fibroma-two case reports. *Indian J Radiol Imaging* 2009;19:116-9.
 24. Tamgadge S, Avinash T, Bhalerao S, Rajhans S. Juvenile psammomatoid ossifying fibroma with aneurysmal bone cyst in the posterior mandible. *Ecancermedalscience* 2014;8:471. doi:10.3332/ecancer.2014.471.