

# Demystifying the nature of hard tissues in odontogenic tumors using Modified Gallego's stain: A preliminary study

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## Abstract

**Introduction:** Histological stains are dyes that bind to a variety of tissues. Modified Gallego's (MG) stain is a modification of Lille's stain that can be used as a differential stain for identification of hard tissues in oral pathological lesions.

**Objectives:** The objective of this study was to identify the presence of hard tissues such as enamel, dentin and cementum in normal extracted teeth and odontogenic tumors using MG stain and to compare the efficacy of MG stain with hematoxylin and eosin (H&E) stain.

**Methods:** A total of fifty samples, twenty decalcified sections of teeth and thirty cases of odontogenic tumors, were included in the present study. Two sections were cut from the above cases and stained with H&E stain and MG stain, respectively, and assessed for the nature of hard tissue.

**Results:** In H&E staining, enamel, dentine, cementum and bone stained pink. Whereas, in MG stain, enamel stained pink, dentin and bone stained green, while cementum stained red. The shade of color differs with the degree of mineralization of the hard tissues in MG stain.

**Conclusion:** MG stain can be used as a differential stain for different hard-tissue structures when compared to routine H and E staining.

**Keywords:** Hematoxylin and eosin stain, Modified Gallego's stain, odontogenic tumors

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## INTRODUCTION

Histological staining is a string of technique undertaken in the preparation of tissue samples using histological stains to aid in the microscopic study.<sup>[1]</sup> It is the interaction between a colored dye and a tissue substrate which resists simple washing.<sup>[2]</sup> The process involves multiphase interaction of the tissues with the staining reagents, resulting in staining of tissues based on the composition, affinity and interaction between stain and the dye.<sup>[3]</sup>

Hematoxylin and eosin (H&E) is the most widely used stain in the histopathological laboratory. Under H&E staining, hard-tissue structures such as bone, dentin and cementum and soft-tissue structures such as collagen, amyloid, muscle and keratin stain eosinophilic in nature where differentiating one from other is challenging.<sup>[4]</sup>

Apart from conventional H&E stain, there are alternative staining procedures which aid in highlighting the specific

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features of the tissues that are not appreciated with routine H&E. Such alternative staining techniques can be used as an adjunct for diagnosing the histopathological lesions.<sup>[1]</sup>

Various special stains such as von Kossa, Alizarin red for the bone, picrothionin for dentin, toluidine blue and Alcian blue for cementum are available, but the use of single histochemical stain that differentiates between the hard tissues of tooth is rare.<sup>[5]</sup>

Modified Gallego's (MG) stain is one such stain which not only stains the decalcified sections, but also differentially stains the calcified structures present in the pathological lesions that helps in obtaining a clear histological picture. MG stain was first introduced by Gallego in 1954 and has been derived from the modification of Lille's stain which uses the basic reagents such as hematoxylin, carbol fuchsin and aniline blue to stain the hard-tissue components.<sup>[6]</sup>

Thus, the present study was designed to stain the calcified tissues of teeth and to differentiate and identify the presence of hard tissue-like components in various odontogenic tumors using MG stain. The efficacy of MG stain was compared with routine H&E stain to identify the nature of hard tissue and to devise an optimal staining technique that would be specific, easy and cost-effective.

## MATERIALS AND METHODS

The study was planned to stain calcified components in tissue sections and hence, H&E-stained sections were reviewed for the presence of calcified structures in known odontogenic tumors such as odontoma, adenomatoid odontogenic tumor (AOT), calcifying epithelial odontogenic tumor (CEOT), dentinogenic ghost cell tumor (DGCT), odontoameloblastoma (OA) and ameloblastic fibrodentinoma (AFD).

A total of thirty paraffin-embedded tissue blocks were retrieved from the archives of the department of oral pathology. The control group included twenty freshly extracted teeth which were decalcified using 10% nitric acid for 3 days. Two sections of 4  $\mu$ -thickness paraffin-embedded sections were taken. Each section was stained with H&E and MG stains, respectively.

### Procedure for hematoxylin and eosin stain

1. The deparaffinized sections in xylene were dehydrated in various grades of alcohol for 5 min each
2. After water wash for 10 min, the slides were stained with Harris's hematoxylin stain for 10 min
3. After water washed for 10 min and differentiation in acid alcohol, the slides were dipped in lithium

carbonate for bluing for 5 min and were stained with eosin for 15 s

4. Then the sections were dehydrated with graded alcohol, cleared in xylene and mounted.

The staining time was 20 min.

### Procedure for Modified Gallego's stain<sup>[5]</sup>

#### Staining solutions

1. Carbol fuchsin
2. 0.01% aniline blue in saturated picric acid.

#### Staining procedure

1. Deparaffinize the sections
2. Stain in hematoxylin 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
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 DPX mounting media.

The staining time was 15 min.

#### Color interpretation

- Dentin – green color
- Cementum – red color
- Bone – green color
- Enamel – red color.

The stained tissue sections were evaluated and results were tabulated.

## RESULTS

In H&E staining technique, enamel, dentin, cementum and bone were stained in varying shades of pink in normal decalcified section and various odontogenic tumors [Table 1].

In the MG staining technique, of decalcified teeth, enamel stained pink, dentin stained green and cementum stained red [Table 1 and Figure 1]. In odontoma, both red and green color deposits were seen suggesting dentin- and cementum-like materials, respectively. In DGCT, haphazard arrangement of ghost cells adjacent to odontogenic epithelium stained green, suggesting it to be derived

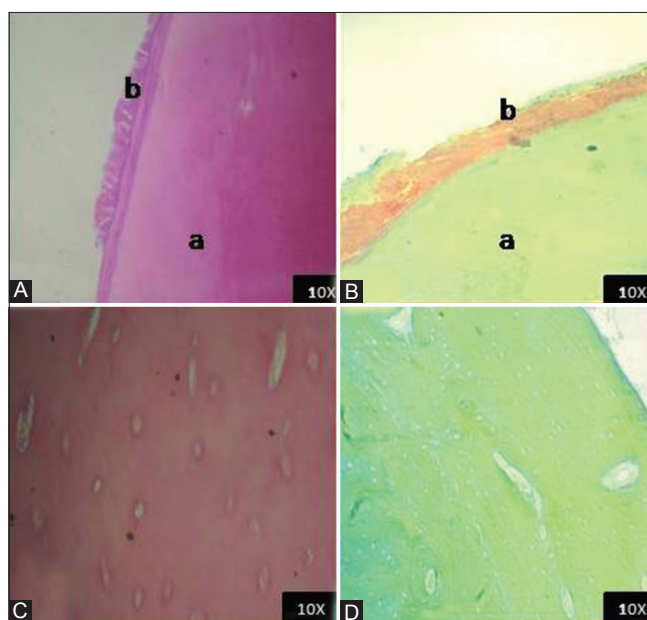
from dentin. In AFD, haphazardly arranged hard tissue deposits stained green, suggesting it to be dentin/bone-like component. In CEOT, amyloid-like material stained pink and Liesegang rings stained green, suggesting to be derived from enamel and dentin/bone, respectively. In OA, calcifications stained pink, suggesting it to be enamel-like material. In AOT, pink color deposits were seen, suggesting it to be enamel-like material in nature [Table 2].

**DISCUSSION**

The hard-tissue components of teeth such as enamel, dentin and cementum and soft tissues such as amyloid, keratin and collagen stained eosinophilic in routine H&E staining and thus are difficult to differentiate.<sup>[7]</sup> In several odontogenic tumors, the deposition of dental hard tissues such as enamel, dentin and cementum may occur and an accurate diagnosis depends on the identification of these tissue types.<sup>[8]</sup> In connective tissue tumors, be it central or peripheral, benign or malignant, the presence or absence of calcification is sometimes challenging to detect, posing diagnostic difficulties.<sup>[5]</sup>

On reviewing the literature, there are very few studies that have used special stains to identify the nature of hard structures of teeth. Special stains contain combinations of dyes that interact in a complex manner and differentially stain tissue components and thus aid in the easier identification of tissue properly.<sup>[7]</sup> Color variations for the combined stains are due to molecular size and permeability differences. Basic dyes have affinity toward acidic components and acidic dyes have affinity toward basic components.<sup>[2]</sup>

MG stain is one such stain that differentially stains hard-tissue components. The present study was an attempt to stain the dental hard structures such as enamel, dentin and cementum with MG stain and compare the efficacy with that of H&E stain. MG stain was developed to overcome the disadvantages of other stains. It was introduced by Gallego in 1954. It is a combination of dyes such as carbol fuchsin and aniline blue that are used consequently in staining procedure to stain calcified structures.<sup>[5]</sup> The dyes in MG stain compete for binding sites in the tissue; the low-molecular-weight dyes generally are displaced by larger dye molecules. The larger dye then reacts by Van der Waals forces with the tissue. However,



**Figure 1:** Photomicrograph of decalcified section in hematoxylin and eosin and Modified Gallego's stains. (A) Decalcified section of teeth in hematoxylin and eosin ([a] Dentin – pink, [b] cementum – pink). (B) Decalcified section of teeth in Modified Gallego's stain ([a] Dentin – green, [b] Cementum – red). (C) Decalcified section of bone in hematoxylin and eosin. (D) Decalcified section of bone in Modified Gallego's stain (bone – green)

**Table 1: Interpretation of color in teeth using hematoxylin and eosin staining and Modified Gallego's staining**

Structure	H and E Staining	Modified Gallego's stain
Dentin	Pink	Green
Cementum	Pink	Red
Bone	Pink	Green

**Table 2: Interpretation of color in odontogenic tumors using hematoxylin and eosin and Modified Gallego's stain**

Odontogenic tumor	Calcifications	H&E stain	Modified Gallego color and interpretation
Odontome	Haphazard arrangement of multiple hard tissue	Pink	Green - Dentin-like material, Red - cementum-like material
DGCT	Haphazard arrangement of ghost cells adjacent to odontogenic epithelium	Pink	Green - Dentin-like material
AFD	Haphazardly arranged hard tissue adjacent to odontogenic epithelium	Pink	Green - Dentin-like material
CEOT	Orderly arranged calcification adjacent to polyhedral sheet of cells	Pink	Pink - Amyloid-like material
OA	Haphazardly arranged calcification surrounding proliferating odontogenic epithelium in mature connective tissue stroma	Pink	Pink - dysplastic enamel
AOT	Orderly arranged calcification adjacent to rosettes of cells	Pink	Red - Cementum-like material Green - Dentin-like material

DGCT: Dentinogenic ghost cell tumor, AFD: Ameloblastic fibro dentinoma, CEOT: Calcifying epithelial odontogenic tumor, OA: Odontoameloblastoma, AOT: Adenomatoid odontogenic tumor, H&E: Hematoxylin and Eosin

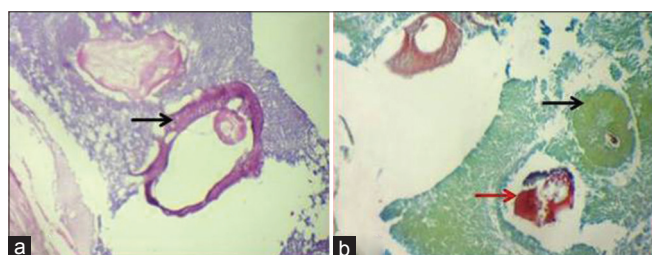
uncertainty remains regarding the exact mechanism because the binding of dye molecules appears to depend on the permeability of the tissue.<sup>[9]</sup>

Thirty paraffin-embedded tissue sections were studied including odontogenic tumors such as odontoma, AOT, CEOT, DGCT, OA and AFD. H&E staining of odontogenic tumor revealed dentin-, enamel-, bone-, cementum- and amyloid-like materials in different shades of pink, whereas in MG staining technique, enamel-like material stained pink, dentin- and bone-like material stained green and cementum-like material stained red.

Odontome is a hamartomatous lesion which under H&E stain consists primarily of a disordered mixture of dental tissues, often of spherical shape structures stain pink. Occasionally, the calcified masses may include tooth-like structures, indicating that the degree of morphodifferentiation varies greatly. Cementum or cementum-like structures often admixed with the dentinoid substance may be observed within the calcified/mineralized masses of dentin.<sup>[10]</sup> In odontoma under MG stain, irregular arrangement of hard tissues was noticed. Sheets of green deposition were seen suggesting it as dentin/bone-like material. Focal areas show dark red masses suggesting cementum-like material [Figure 2]. The results of the present study were in accordance with the study conducted by Tamgadge *et al.*<sup>[5]</sup> and Mudhiraj *et al.*<sup>[6]</sup>

Cementum is composed of type 1 collagen (90% organic content and noncollagenous matrix proteins [NCPs]). NCPs include osteopontin, bone sialoprotein, osteocalcin, fibronectin and several species of proteoglycans, such as decorin, biglycan, lumican and fibromodulin,<sup>[11]</sup> making it slightly acidic in nature.<sup>[12]</sup> The strong retention of basic dye (carbol fuchsin) by cementum in MG staining technique could be attributed to the acidic nature of cementum, thus staining cementum red.

Histopathologically, DGCT is characterized by sheets and rounded islands of odontogenic epithelial cells seen



**Figure 2:** Photomicrograph of odontome: (a) Hematoxylin and eosin stain showing pink-colored deposits. (b) Modified Gallego's stain showing dentin/bone-like material in green (black arrow) and cementum-like material in red color (red arrow)

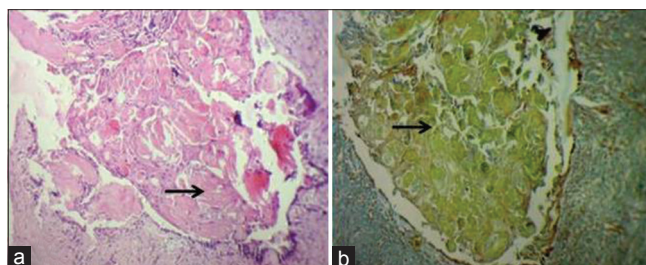
in a mature connective tissue. Numerous round-to-ovoid homogeneously basophilic globules of calcification are also evident. The formation of dentinoid or osteoid material which is frequently seen with the masses of ghost cells is a characteristic finding of the lesion and stains pink in routine H&E staining.<sup>[10]</sup> Under MG stain, haphazard arrangement of calcified ghost cells adjacent to odontogenic epithelium stained green suggesting it to be dentin/bone-like material [Figure 3]. The results of the present study were in accordance with the study conducted by Tamgadge *et al.*<sup>[5]</sup>

Under H&E stain, the AFD is composed of strands and islands of odontogenic epithelium in a cell-rich primitive ectomesenchyme. Dentinoid or osteodentin is often seen deposited.<sup>[10]</sup> The calcifications were stained pink. In MG stain, adjacent to odontogenic epithelium haphazard arrangement of hard tissue stained green in color suggesting dentin/bone-like material [Figure 4].

CEOT, also known as Pindborg tumor, is classified as uncommon, benign odontogenic neoplasm of epithelial origin. Microscopically, it is characterized by the presence of polyhedral epithelial cells arranged in cords and sheets, amyloid like material and calcifications in the form of Liesegang rings which stain eosinophilic in H&E staining.<sup>[13]</sup> Under MG stain the amyloid like material stains pink in color suggesting it could be probably derived from enamel and Liesegang ring stain green in color suggesting it to be derived from dentin/bone-like material [Figure 5]. The result of our study was in consistent with the study conducted by Mudhiraj *et al.*<sup>[6]</sup>

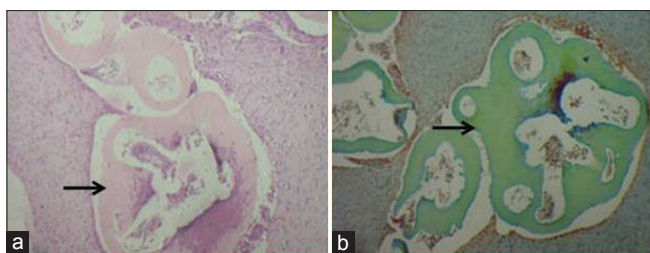
In MG staining technique, dentin and bone stain green in color. The mechanism of staining could be attributed to the fact that both dentin and bone are acidophilic in nature as they contain type I collagen, which has affinity for anionic aniline blue dye present in the staining solution.<sup>[7]</sup>

Odontoameloblastoma in H&E staining reveals proliferating odontogenic epithelium in a mature connective tissue

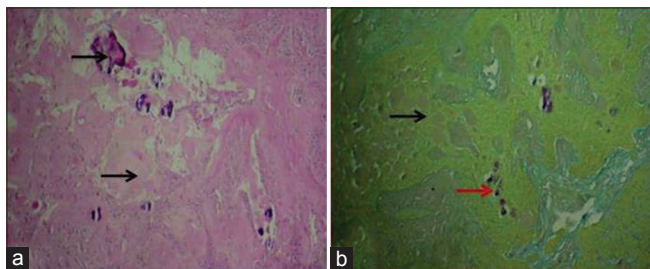


**Figure 3:** Photomicrograph of dentinogenic ghost cell tumor: (a) Hematoxylin and eosin stain showing pink-colored deposits. (b) Modified Gallego's stain showing dentin/bone-like material in green (black arrow)





**Figure 4:** Photomicrograph of ameloblastic fibro dentinoma: (a) Hematoxylin and eosin stain showing pink-colored deposits. (b) Modified Gallego's stain showing dentin/bone-like material in green (black arrow)



**Figure 6:** Photomicrograph of odontoameloblastoma: (a) Hematoxylin and eosin stain showing pink-colored deposits. (b) Modified Gallego's stain showing dentin/bone-like material in green color (black arrow) and enamel-like material in pink (red arrow)

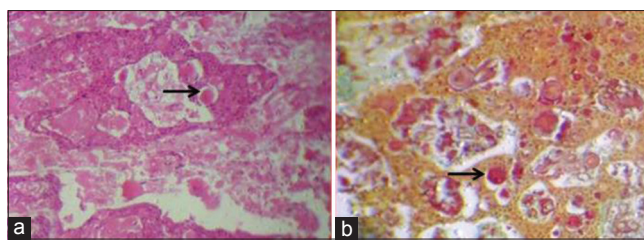
stroma. The neoplastic odontogenic epithelium forms islands and cords between dysplastic dentinoid substances and enamel and stain eosinophilic in nature.<sup>[10]</sup> In MG stain haphazardly arranged calcification surrounding proliferating odontogenic epithelium in mature connective stroma is seen. Pink color deposition suggests dysplastic enamel formation, whereas green colored deposits suggest dentin/bone-like material [Figure 6].

In AOT, under H&E staining, the most striking pattern is that of multisized solid nodules of cuboidal or columnar epithelial cells forming nests or rosette-like structures with duct-like appearance. Calcified material in varying amounts occurs in most lesions which stains pink in color.<sup>[10]</sup> Under MG stain, pink color calcifications were observed suggesting enamel-like material [Figure 7]. The result of our study was in accordance with Tamgadge *et al.*<sup>[5]</sup>

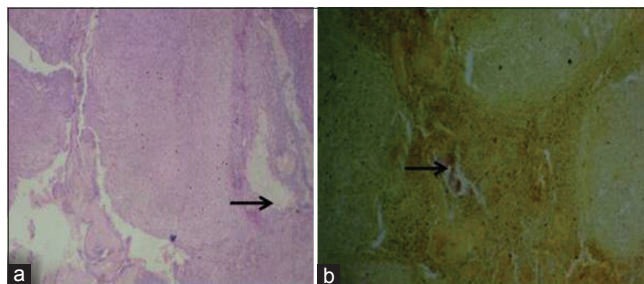
The main advantage of MG staining is that it stains various types of hard tissues such as enamel, dentin and cementum in different shades of color compared to the routine H&E staining. The readily available dyes make it a superior differential stain.

## CONCLUSION

MG stain can be used as one of the efficient differential stains to identify the nature of hard-tissue components in



**Figure 5:** Photomicrograph of calcifying epithelial odontogenic tumor: (a) Hematoxylin and eosin stain showing pink-colored deposits. (b) Modified Gallego's stain showing amyloid-like material in pink (black arrow)



**Figure 7:** Photomicrograph of adenomatoid odontogenic tumor: (a) Hematoxylin and eosin stain showing pink-colored deposits. (b) Modified Gallego's stain showing enamel-like material in pink (black arrow)

odontogenic tumors and other connective tissue tumors and can be used as an adjunct to routine H&E staining technique for more appropriate and definitive diagnosis.

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Nil.

## Conflicts of interest

There are no conflicts of interest.

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