Modified Gallego’s Stain as Differential Stain for Oral Hard Tissues in Oral Pathology: A Preliminary Report
Tamgadge Sandhya, Tamgadge Avinash, Chandni Srivastava, Evie Satheesan, Sudhir Bhalerao

Abstract
Objectives: Differential staining of hard tissues seen in tooth, bone and pathological lesions to gain insight into their nature and also to confirm histopathological diagnosis obtained via routine staining procedures. Materials and Methods: Initially, normal (control) tissues were stained to study the staining properties of hard tissue, using hematoxylin and eosin stain (except ground section) which was followed by modified gallego’s staining of soft tissue biopsy, decalcified sections as well as ground sections. Pathological lesions containing hard tissues were also stained similarly to confirm the nature of hard tissues. Results: In a properly stained slide cementum stains red and dentine and bone stains green and enamel stains pink in color. The shade of color differs with the amount of mineralization of the hard tissues. Conclusion: Histopathological diagnosis is confirmed by Gallego’s staining, also the colors of hard tissue components in the control corresponded to those of the pathological lesions. Clinical Relevance: The stain can be successfully used to differentiate various types of oral hard tissues in oral pathology using decalcified section, soft tissue biopsies and as well as ground sections which is rarely used for histochemical stains.

Keywords: Gallego’s stain; Cementum; Dentine; Enamel; Bone; Mineralization.

Introduction
Modified Gallego’s stain is a variant of Lille’s stain that uses basic reagents hematoxylin, carbol fuschin and aniline blue. The purpose of the stain is differential staining of the hard tissues seen in tooth and other pathological lesions. The study is done to obtain a clearer understanding of the histological picture of the hard tissue forming lesions of the oral cavity. When combinations of hard and soft tissues as well as multiple hard tissue components are evident in the same tumor, they pose diagnostic difficulties; also identification of types of calcified structures in their initial phase is crucial for diagnosis of such lesions.

The study was therefore performed with the following aims and objectives:
- To differentiate between the hard and soft tissues.
- To differentiate between various types of hard tissues in the same lesion.
- To correlate the histopathological diagnosis using hematoxylin and eosin (H&E) and Modified Gallego’s stain.

Materials and Methods
The study consists of tissues selected from archives which had calcified components in the form of cementoid, dentinoid, osteoid, enamel, dentin, cementum, dystrophic calcifications, bone etc. Ground sections, decalcified sections and soft tissues were selected for the staining procedure. The sections were stained with H&E followed by Gallego’s stain to correlate the staining character of hard tissues. The following samples were selected for Gallego’s stain.

1. Ground sections
   A) Normal tissue
      a) Bone
      b) Tooth (Figure 1)
   B) Pathologic tissue
      a) Odontome

2. Decalcified sections
   A) Normal tissue
      a) Bone
      b) Tooth
   B) Pathologic tissue
      a) Odontome

3. Soft tissue biopsies of pathological tissues
   A) Calcifying Odontogenic Cyst
   B) Adenomatoid odontogenic tumor
   C) Cemento-ossifying fibroma

Sections were stained with H&E (except ground section) same sections were also stained with Modified Gallego’s by using the following technique:
1. Deparaffinize the sections
2. Stain in hematoxylin for 8-12 minutes
3. Rinse in distilled water
4. Stain in mordant for 2 minutes (Mix 200 ml of distilled water in 1.5 ml of concentrated nitric acid with 1 ml of 40 per cent formaldehyde and 1.5 ml of U.S.P. iron chloride). Rinse in distilled water.
5. Stain with 3 ml of carbol fuschin in 50 ml of 0.2 per cent acetic acid and rinse in distilled water.
6. Wash in mordant for 1-2 minutes
7. Stain with 0.01 per cent aniline blue in saturated picric acid solution for 30 seconds
8. Dehydrate and clear with xylene, and mount in DPX mounting media.

Figure 1: The photomicrograph of Gallego’s stained section at low power view demonstrating the ground section of tooth (a) and bone (c), whereas the decalcified section demonstrating dentin, cementum (b) and bone (d). [Tissues are denoted as dentin (a), enamel (b), cementum (c), bone (d)]

Results
All the tissues, normal and pathologic were stained with H&E and Gallego’s stain. Interpretation was done under microscope and following observations were made. The histopathological diagnosis after H&E staining was correlated with Gallego’s staining. (Table 1 and Table 2)

<table>
<thead>
<tr>
<th>Normal Tissue</th>
<th>Colour</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tooth (ground section)</td>
<td>Green</td>
<td>Dentine</td>
</tr>
<tr>
<td></td>
<td>Dark red</td>
<td>Cementum</td>
</tr>
<tr>
<td></td>
<td>Pink</td>
<td>Enamel</td>
</tr>
<tr>
<td>Tooth (decalcified section)</td>
<td>Green</td>
<td>Dentine</td>
</tr>
<tr>
<td></td>
<td>Dark red</td>
<td>Cementum</td>
</tr>
<tr>
<td>Bone (ground section)</td>
<td>Green</td>
<td>Bone</td>
</tr>
<tr>
<td>Bone (decalcified section)</td>
<td>Green</td>
<td>Bone</td>
</tr>
</tbody>
</table>

Table 1: Interpretation of nature of calcification using Modified Gallego’s stain in Normal Tissues

A. Odontogenic Tumor: In the category of odontogenic tumors the following lesions were studied- Dentineogenic Ghost Cell tumor, Odontoma and Adenomatoid Odontogenic Tumor.

1) Dentineogenic ghost cell tumor (ameloblastomatous proliferating type). Under H&E, section showed proliferation of odontogenic epithelium in ameloblastomatous pattern. Ghost cells along with calcification were clearly evident, suggestive of dentinogenic ghost cell tumor. Two types of deposition were seen one within the stellate reticulum like cells. The other deposition was seen adjacent to the tall columnar cells. Under Gallego’s stain the deposition within the sheets of stellate reticulum was green in colour suggesting that it is dentinoid in nature. The deposition adjacent to the tall columnar cells was pink in color suggesting that it was enamel matrix, which matched the colour of enamel in
the ground section of a normal tooth under Gallego’s stain (Figure 2).

<table>
<thead>
<tr>
<th>Tissue Stained</th>
<th>Calcification</th>
<th>Colour</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Odontome (Decalcified section)</td>
<td>Haphazard arrangement of multiple hard tissues</td>
<td>Green</td>
<td>Dentine like</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Red</td>
<td>Cementum like</td>
</tr>
<tr>
<td>Odontome (Ground Section)</td>
<td>Haphazard arrangement of multiple hard tissues</td>
<td>Green</td>
<td>Dentine like</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dark red</td>
<td>Cementum like</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pink</td>
<td>Enamel like</td>
</tr>
<tr>
<td>Dentinogenic ghost cell tumor (ameloblastomatous proliferating type)</td>
<td>Surrounded by stellate reticulum like cells</td>
<td>Pale green</td>
<td>Dentine like</td>
</tr>
<tr>
<td></td>
<td>Adjacent to tall columnar cells</td>
<td>Pink</td>
<td>Enamel like</td>
</tr>
<tr>
<td>Cemento-ossifying fibroma</td>
<td>Present within the stroma</td>
<td>Green</td>
<td>Bone</td>
</tr>
<tr>
<td></td>
<td>Present in the periphery of lesion</td>
<td>Dark red</td>
<td>Cementum like</td>
</tr>
<tr>
<td>Adenomatoid odontogenic tumour</td>
<td>Present within the stroma</td>
<td>Dark red</td>
<td>Cementum like</td>
</tr>
<tr>
<td></td>
<td>Along the tall columnar cells of ductal pattern intra-luminally</td>
<td>Pink</td>
<td>Enamel matrix</td>
</tr>
</tbody>
</table>

Table 2: Interpretation of nature of calcification using Modified Gallego’s stain in Pathological Tissues

Figure 2: The photomicrograph of Gallego’s stained section of Dentinogenic ghost cell tumor (ameloblastomatous proliferating type): hybrid odontogenic tumor demonstrating green deposition within sheets of odontogenic epithelium indicative of dentinoid (a, 10X) and pink deposition adjacent to the tall columnar cells indicative of enamel matrix (b, 40X), Whereas odontoma in ground section (c, 40X) shows dentin (b) and cementum (a) and decalcified section (d, 10X) demonstrated enamel spaces(c), dentine (a) and cementum(b).

2) Odontoma (Complex): **Ground sections**: A section showed dentine with dentinal tubules running through it and enamel deposition in globules of dark brown. Irregular cementum deposition was also seen. **Under Gallego’s stain**, the deposition of dentine was green in color with dentinal tubules. The enamel was seen in globular depositions in pink and cementum was seen as a dark red deposition in sheets. **Decalcified section: Under H&E**, irregular arrangement of dentine, enamel, enamel spaces, cementum and pulp-like connective tissue forming an irregular mass was evident. **Under Gallego’s stain**, irregular arrangement of hard tissue was observed, with the colour matching the normal components of tooth under this stain. Sheets of green deposition were seen and with tubules running through them suggesting that it was dentine. Dark red deposition was also seen irregularly arranged either in sheet or globules suggesting it was cementum. Also delicate connective tissue was
seen enclosed within the dentine appears pulp-like. Enamel spaces were also seen.

3) Adenomatoid odontogenic tumor (Figure 3): Under H&E, Tumor had a well-defined fibrous capsule. Connective tissue stroma was highly vascular. Duct-like structures with lumina were seen which were lined by a single layer of tall columnar cells, arranged radially with the nuclei placed away from the lumen. Centrally homogenous eosinophilic material was seen. Solid areas were also seen along with whorls or rosettes arrangement. Calcified material was also present in the connective tissue stroma in abundance but was unevenly distributed and irregular in shape. Under Gallego’s stain, the depositions were analyzed, one was associated with the tall columnar cells and the other one was lying in the connective tissue. Irregular dark red depositions were seen within the highly cellular connective tissue stroma suggestive of cementum like deposits. Pink coloured deposition adjacent to the tall columnar cells within the lumen suggests of enamel matrix.

![Figure 3: The photomicrograph of Gallego’s stained section of adenomatoid odontogenic tumor demonstrating red (a, 10X) and pink colored deposits adjacent to the tall columnar cells indicative of cementum and enamel matrix respectively (b, 10X). Inset shows a high power magnification of the same (40X).](image)

B. Cemento-ossifying Fibroma (intra-osseous) (Figure 4): Under H&E, there were globular depositions that were basophilic in nature suggestive of cementum and multiple depositions of trabecular bone were also detected. The connective tissue was highly cellular and composed of delicate fibrillar stroma. Under Gallego’s stain, multiple green colored globular/ trabecular depositions were observed, which suggests that they were bony in nature; several irregular dark red depositions were also seen which were cementum in nature.

![Figure 4: The photomicrograph of Gallego’s stained section of cemento-ossifying fibroma demonstrating multiple green colored globular depositions indicative of osteoid (a) and Irregular dark red deposits indicative of cementoid (b, 10X). Inset shows higher magnification of the same. (40X).](image)

**Discussion**

The amount of calcification of hard tissues such as dentine, cementum, enamel in certain pathologies pose problem in differentiation after staining. Using routine histological procedure like Hematoxylin and Eosin in decalcified section of bone it is difficult to differentiate osteoid from calcified woven or lamellar bone sometimes even with cementum when it shows globular shape. Also presence or absence of calcification in connective tissue tumors, be it central or peripheral, benign or malignant, is a challenge to detect. These commonly encountered pathologies of the oral cavity, identification of organic matrix of calcified structure often create diagnostic difficulties. In the study conducted by Gallego (1954), tooth and bone (decalcified, ground, frozen) sections were stained, the results were as follows, dentine and bone stained green, and cementum stained red. Various stains differentiate between soft and hard tissue components in mixed lesions. But the use of histochemical stain that differentiates between various hard tissues are uncommon in the literature especially in dental literature. Also usually the special stains are performed in soft tissue and decalcified tissue but the study of staining ground sections of hard tissue have not been reported in histo-pathological literature. Ground sections are the easiest and fastest method of histotechnique, thus enabling the
pathologist to reach a quick diagnosis using Gallego’s stain.

In the present study ground sections and decalcified sections of both tooth and bone were stained. Furthermore, the fact that the stain is capable of identifying hard tissue nature was confirmed by selecting certain lesions with calcified components. The result were the same as the original study, but we also determined that enamel stains pink in color and immature hard tissue deposition stains in shades of red and green. Thus, it is evident that the stain is efficient as a diagnostic tool in cases where the diagnosis is hampered as the nature of hard tissue is undetermined.

Conclusion
The Gallego’s stain could be considered as a practical tool in diagnosis using not only soft tissues and decalcified but also 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 an added insight into the character of the lesion and therefore it is advantageous to use it at times to reach a diagnosis or also to confirm analysis of a lesion.

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