DOI: 10.7860/JCDR/2025/74081.20725

Original Article

Dentistry Section

Painting the Portrait of Fibro-osseous Lesions: An In-vitro Study on Staining Duel of Modified Gallego versus Hematoxylin and Eosin (H&E) Staining

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ABSTRACT

Introduction: Oral cavity tumours involve a mix of soft and hard tissues, with varying levels of calcification posing diagnostic challenges. Detecting the presence or absence of calcification in connective tissue tumours, whether central or peripheral, benign or malignant, is particularly challenging. Routine stains like Haematoxylin and Eosin (H&E) fail to adequately reveal the specific features of these hard tissues. Alternative histochemical staining procedures, such as Modified Gallego's Stain (MGS), offer better visualisation of the hard-tissue components in the decalcified sections.

Aim: To evaluate and compare the staining efficacy of H&E and MGS to differentiate bone and cemental tissue in histopathologically diagnosed oral and maxillofacial pathologies.

Materials and Methods: An in-vitro histochemical retrospective study was conducted at the Department of Oral and Maxillofacial Pathology at MGM Dental College, Navi Mumbai, Maharashtra, India, from October 2018 to January 2021. A total sample size of 60 was chosen using a convenient sampling technique, comprising decalcified tissue sections of normal teeth with periapical bone (n=10), normal bone (n=10) and normal teeth (n=10) as the control group. The study group included pathologies of bone and cemental tissues (cemento-ossifying fibroma, odontome, focal cemento-osseous dysplasia) (n=10), pathological bony tissue (juvenile ossifying fibroma, fibrous dysplasia, aneurysmal bone cyst, peripheral ossifying fibroma, juxtacortical osteosarcoma) (n=10) and pathological cemental tissue (odontome, cementoblastoma) (n=10). Serial sections from each sample were taken. One section was stained with H&E stain and another with MGS. Stained sections were viewed using routine light microscopy and evaluated under 40X, 100X and 400X magnification. Histochemical evaluation was performed for the intensity of stain and its tissue differentiation capability for osteoid, immature bone, mature bone, cementoid and cementum. All statistical analysis were performed using the Statistical Package for Social Sciences (SPSS v 26.0, IBM). The data obtained were presented using descriptive statistics. The Chi-square test was used and statistical significance was set at a p-value of less than or equal to 0.05.

Results: The MGS had better efficacy than routine H&E stain in differentiating bone and cemental tissues of decalcified tissue sections from various oral and maxillofacial pathologies. A comparison of the H&E and MGS between various study and control groups showed statistically significant results ($p<0.05$) in differentiation and intensity.

Conclusion: The MGS is a better histochemical stain than the routine H&E stain, with increased differentiation and clarity in identifying bone and cemental tissues; hence, it can be considered a reliable method to differentiate pathological tissues.

Keywords: Bone, Collagen, Connective tissue tumours

INTRODUCTION

Bone is a vascular, mineralised connective tissue. It comprises approximately 5% non collagenous structural proteins, including bone sialoprotein, osteocalcin, osteonectin, osteopontin and proteoglycans. In addition, an organic matrix known as osteoid is present, accounting for 28% of its weight and primarily comprising type I collagen [1]. In contrast, cementum is a hard and avascular connective tissue that covers the roots of teeth. Type I collagen, referred to as cementoid, predominates in cementum, making up to 90% of its organic composition [2]. Despite sharing several properties with dentin and bone, cementum exhibits distinct characteristics, underscoring its specialised nature compared to bone tissue [3].

Tumours of the oral cavity often involve a combination of both soft and hard tissues [4]. The involved hard tissues exhibit varying degrees of calcification. Detecting the presence or absence of calcification in connective tissue tumours, whether central or peripheral, benign or malignant, poses a significant diagnostic challenge. These are frequently encountered pathologies in the oral cavity, where identifying the organic matrix of calcified structures can often be diagnostically challenging, such as in odontomas and osteomas [5]. Routine H&E staining often fails to reveal the specific features of these hard tissues. Consequently, alternative histochemical staining procedures play a crucial role in demonstrating these structures. These staining techniques serve as adjuncts in predicting histopathological diagnosis, offering valuable insights. Various combinations of histochemical stains can effectively highlight different types of hard tissues [6]. The colour variations observed in combined stains occur due to differences in molecular size and dye permeability [4]. Examples of such combination stains include Masson's trichrome, Periodic acid Schiff, Alcian blue-nuclear fast red, Verdeluz orange and MGS [7-9].

MGS offers a valuable tool for differentiating the components of hard tissue within the oral cavity [4]. In particular, this staining technique is effective in highlighting the hard-tissue components of decalcified and ground sections, thus assisting in the identification of the nature of calcified structures present in various oral and maxillofacial pathological lesions [5]. Specifically, cementum is stained a distinct red colour when MGS is used, while bone and dentin exhibit a green hue [10]. Given the challenges in differentiation with routine H&E stain,

exploring alternative staining methods becomes imperative. The present study was conducted to better comprehend the histological picture of the hard tissues forming oral lesions. Identifying the calcified structures in their initial phase is crucial for diagnosing such lesions.

The present study aimed to evaluate and compare the efficacy of MGS and H&E stain for the differentiation of bone and cemental tissue associated with histopathologically diagnosed oral and maxillofacial pathologies.

MATERIALS AND METHODS

An in-vitro histochemical retrospective study was conducted at the Department of Oral and Maxillofacial Pathology at MGM Dental College, Navi Mumbai, Maharashtra, India, from October 2018 to January 2021. The institutional ethical committee approved the study (approval no: MGMDSH/Opath/18/200).

Sample size calculation: A total sample size of 60 was selected using a convenient sampling technique. The sample size was based on a comparison of differentiation scores using the Mann-Whitney 'U' test. A sample size of 11 achieved 81.19% power to detect a mean of paired differences of 0.2, with an estimated standard deviation of differences of 0.2 and a significance level (alpha) of 0.050 using a two-sided paired test (Wilcoxon) [4]. Thus, a total of 11 samples were needed for the study; however, 60 samples were included.

Inclusion criteria: Inclusion criteria were as follows: Only intraosseous oral and maxillofacial pathologies displaying both bone and cemental tissue were included in the study group. Decalcified tissue sections of normal bone and teeth without any pathology were included as controls.

Exclusion criteria: Peripheral pathologies and dystrophic calcifications were excluded from the study group.

Study Procedure

The present study consisted of two groups: the control group and the study group. The control group (n=30) was composed of three subgroups:

Group A: Decalcified sections of normal teeth with periapical bone (n=10);

Group B: Decalcified sections of normal bone (n=10);

Group C: Decalcified sections of normal teeth (n=10);

The study group (n=30) comprised three subgroups:

Group D: Oral and maxillofacial lesions showing both pathological bone and cemental tissues (cemento-ossifying fibroma, odontoma, focal cemento-osseous dysplasia) (n=10);

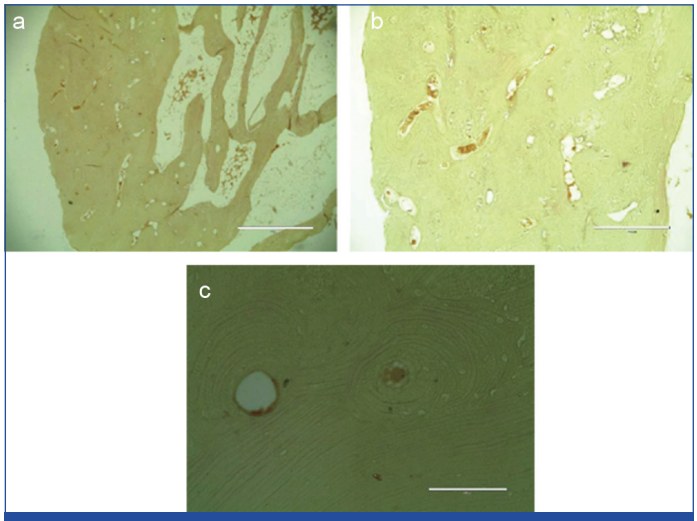
Group E: Oral and maxillofacial lesions displaying pathological bony tissue (juvenile ossifying fibroma, fibrous dysplasia, aneurysmal bone cyst, peripheral ossifying fibroma, juxtacortical osteosarcoma) (n=10);

Group F: Oral and maxillofacial lesions demonstrating pathological cemental tissue (odontoma, cementoblastoma) (n=10).

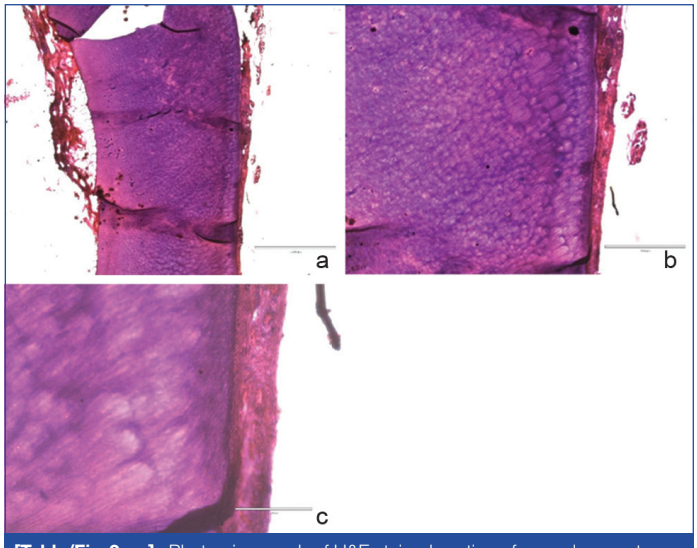
The paraffin-embedded blocks were arranged on a cooling device to ease the sectioning. The blocks were dissected into sections of 5 µm thickness using a soft tissue microtome. Sections were floated on a water bath for approximately 30 seconds. Individual sections/ribbons were then floated onto the slide. For mordant preparation, 200 mL of distilled water was mixed with 1.5 mL of concentrated nitric acid, 1 mL of 40% formaldehyde and 1.5 mL of iron chloride.

For H&E staining, sections were then deparaffinised in xylene, rehydrated through descending grades of alcohol and then brought into the water. The sections were then stained using Harris haematoxylin for 10-20 minutes and washed in tap water for five minutes to remove excess stain. Sections were then differentiated with 1% acid alcohol (1% HCl in 70% alcohol) and washed in tap water for 15 minutes. The sections were then stained using 1% eosin Y for 10 minutes and washed in tap water for five minutes.

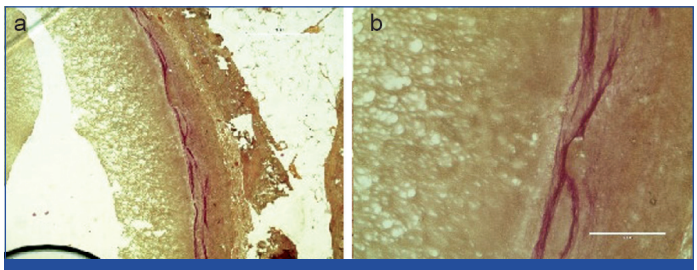
The sections were then dehydrated through ascending grades of alcohol, cleared using xylene and finally mounted with Dibutylphthalate Polystyrene Xylene (DPX) [11]. For MGS, the sections were initially deparaffinised, stained in haematoxylin for 10 minutes and rinsed in distilled water. They were then sensitised in mordant for two minutes and rinsed in distilled water. Later, they were stained using carbol fuchsin solution for five minutes, rinsed in distilled water and washed in mordant for two minutes. Finally, these sections were stained with aniline blue solution for 10 minutes, dehydrated, cleared with xylene and mounted in DPX mounting media [5]. The stained sections were viewed using routine light microscopy and were interpreted at 40X, 100X and 400X magnifications. Various parameters, including osteoid, immature bone, mature bone, cementoid and cementum, were evaluated for the intensity of staining and tissue differentiation [Table/Fig-1-3]. All the samples were evaluated histopathologically and histochemically to obtain data. Histopathological evaluation was conducted by two



[Table/Fig-1a-c]: Photomicrograph of modified Gallego stained section of normal bone. (Magnification: 40X, 100X and 400X, respectively).



[Table/Fig-2a-c]: Photomicrograph of H&E stained section of normal cementum. (Magnification: 40X, 100X and 400X, respectively).



[Table/Fig-3a,b]: Photomicrograph of modified Gallego stained section of normal cementum. (Magnification: 100X, 400X), respectively.

pathologists along with one moderator for the stained slides of bone and cemental tissues using H&E and MGS stains in the control and study groups. Scoring was performed semi-quantitatively using a 3-degree scale for intensity and differentiation as follows: absent=0, mild=1, moderate=2 and high=3 [4].

STATISTICAL ANALYSIS

The data obtained were entered into a Microsoft Excel worksheet (v 2019, Microsoft Redmond Campus, Redmond, Washington, United States) and presented using descriptive statistics. Descriptive statistics were expressed as numbers for each group. The staining efficacy in terms of intensity and differentiation of H&E stain and MGS to differentiate bone and cemental tissues was assessed using the Chi-square test, with a p-value less than or equal to 0.05 considered statistically significant. All analysis were performed using SPSS v 26.0, IBM.

RESULTS

The study included 60 samples. Upon evaluation of control groups A and B, mature bone and cementum were observed. In control group C, cementum was noted. In study group D, both mature bone and cementum were present. In study group E, osteoid, immature bone and mature bone were evident, while in study group F, cementum was observed. Therefore, only these parameters were included in further statistical analysis.

Statistical comparison of the H&E stain and MGS between study group D and control group A was conducted to evaluate parameters like mature bone and cementum [Table/Fig-4]. For mature bone, statistically significant results were observed in study group D in terms of intensity (p=0.002) and differentiation (p=0.002). In control group A, statistically significant results were noted in terms of intensity (p=0.011) and differentiation (p=0.021). For cementum, statistically significant results were found in study group D in terms of intensity (p=0.004) and differentiation (p=0.002). In contrast, in control group A, statistically significant results were obtained in terms of intensity only (p=0.049). Statistical comparison of the H&E stain and MGS between study group E and control group B was made to evaluate parameters such as osteoid, immature bone and mature bone. For osteoid, statistically significant results were observed in study group E in terms of intensity (p=0.001) and differentiation (p=0.012). However, it was absent in the control group, thus not compared. For immature bone, statistically significant results were observed in study group E in terms of intensity (p=0.004), but no other groups showed statistical significance in terms of both intensity and differentiation. For mature bone, statistically significant results were noted in study group E in terms of intensity (p=0.007) and differentiation (p=0.004). In contrast, control group B showed statistically significant results in terms of intensity only (p=0.002). For cementum, statistically significant results were observed in study group F in terms of intensity (p=0.002) and in control group C in terms of differentiation (p=0.019).

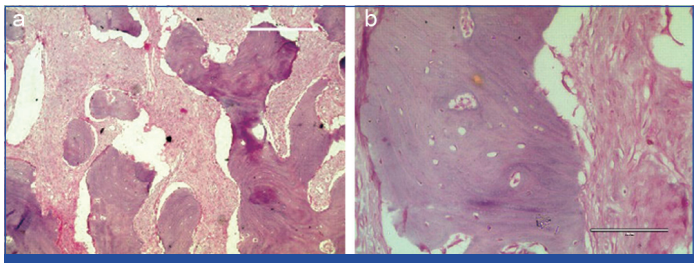
In the present study, various pathologies of different tissue origins, such as bone and cementum, including histopathologically diagnosed cases of juvenile ossifying fibroma (n=4), focal cement-osseous dysplasia (n=3), cemento-ossifying fibroma (n=3) and odontoma (n=4), were examined. When cases of juvenile ossifying fibroma (n=4) were evaluated in MGS sections, hard tissues in shades of green suggestive of bone were noted; however, red-coloured hard tissue suggestive

Variables		(H&E)				(MGS)				χ^2	p-value
		Ab*	Mild	Moderate	High	Ab	Mild	Moderate	High		
Mature bone	Study group (Intensity)	0	7	3	0	0	5	2	3	10.00	0.002
	Control group (Intensity)	0	6	4	0	0	3	4	3	6.875	0.011
	Study group (Differentiation)	0	6	4	0	0	3	4	3	10.00	0.002
	Control group (Differentiation)	0	7	3	0	0	3	3	4	6.429	0.021
Cementum	Study group (Intensity)	0	7	3	0	0	7	3	0	10.00	0.004
	Control group (Intensity)	0	6	4	0	0	2	6	2	6.032	0.049
	Study group (Differentiation)	0	0	7	3	0	0	7	3	10.00	0.002
	Control group (Differentiation)	0	7	3	0	0	2	3	5	4.286	0.117
Osteoid	Study group (Intensity)	4	4	2	0	4	6	0	0	10.00	0.001
	Control group (Intensity)	10	0	0	0	10	0	0	0	-	-
	Study group (Differentiation)	4	6	0	0	4	6	0	0	6.26	0.012
	Control group (Differentiation)	10	0	0	0	10	0	0	0	-	-
Immature bone	Study group (Intensity)	0	6	4	0	0	5	5	0	6.667	0.004
	Control group (Intensity)	0	7	3	0	0	1	9	0	0.476	0.490
	Study group (Differentiation)	0	7	3	0	0	6	4	0	6.429	0.067
	Control group (Differentiation)	0	8	2	0	0	1	9	0	0.278	0.598
Mature bone	Study group (Intensity)	3		6	1	3	0	4	3	12.22	0.007
	Control group (Intensity)	0	2	8	0	0	0	2	8	10.00	0.002
	Study group (Differentiation)	3	4	3	0	3	0	2	5	13.00	0.004
	Control group (Differentiation)	0	5	5	0	0	0	2	8	2.50	0.429
Cementum**	Study group (Intensity)	0	3	7	0	0	0	3	7	10.00	0.002
	Control group (Intensity)	0	6	4	0	0	0	4	6	0.741	0.295
	Study group (Differentiation)	0	5	5	0	0	0	3	7	4.28	0.168
	Control group (Differentiation)	0	7	3	0	0	0	5	5	1.90	0.019

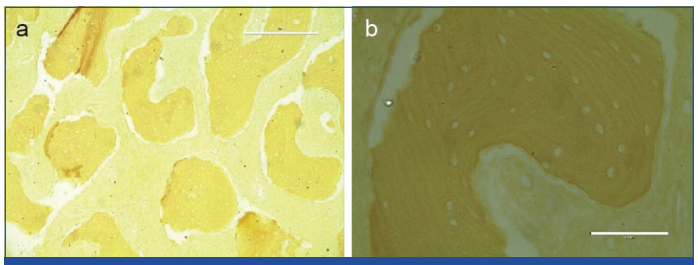
[Table/Fig-4]: Descriptive comparison of intensity and differentiation of Haematoxylin and Eosin (H&E) stain and Modified Gallego's Stain (MGS) for mature bone, cementum, immature bone and osteoid using Chi-square test.
(p-value=≤0.05 is statistically significant)
Chi-square test was used
*Ab denotes negative staining or staining absent
**Cementoid was expected to be present in the samples but unfortunately the authors didn't get the same and saw only cementum that's why the proposed methodology was different from what was observed in the results

of cementum was not found. Thus, the previous histopathological diagnosis of juvenile ossifying fibroma in H&E was confirmed.

In the present study, fibrous dysplasia (n=2) showed immature bone and mature bone in varying shades of green in MGS. Mature bone was stained in a darker shade of green compared to immature bone due to its higher mineral content [Table/Fig-5,6].

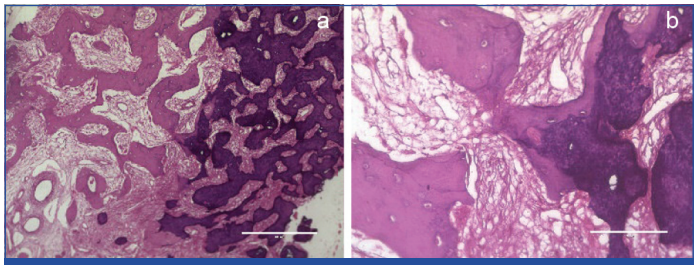


[Table/Fig-5a,b]: Photomicrograph of H&E stained section of bone pathology: Fibrous Dysplasia. (Magnification: 100X, 400X), respectively.

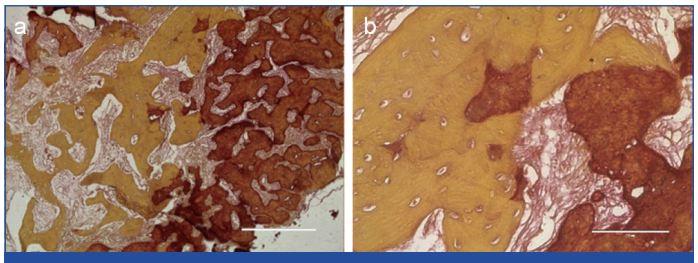


[Table/Fig-6a,b]: Photomicrograph of modified Gallego stained section of bone pathology: Fibrous Dysplasia. (Magnification: 100X, 400X), respectively.

In cases of cemento-ossifying fibroma, a benign odontogenic tumour with membranous ossification (n=3), there was difficulty in differentiating cementum from the basophilic immature bone in the H&E stain. This differentiation was made easier by MGS due to the colour-specific differentiation of hard tissues [Table/Fig-7,8].



[Table/Fig-7a,b]: Photomicrograph of Haematoxylin and Eosin (H&E) stained section of bone and cementum combined pathology: Cemento-ossifying Fibroma. (Magnification: 100X, 400X), respectively.



[Table/Fig-8a,b]: Photomicrograph of modified Gallego stained section of bone and cementum combined pathology: Cemento-ossifying Fibroma. (Magnification:100X and 400X, respectively).

Focal cemento-osseous dysplasia, a benign fibro-osseous lesion of bone characterised by the replacement of normal bone with fibrous tissue, followed by its calcification with osseous and cementum-like material (n=3), demonstrated differential staining for bone (light green) and cemental tissue (red) under MGS stain, which confirmed the previously given histopathological diagnosis under H&E stain.

The histopathological diagnosis of odontoma cases (n=4) was based on the tooth-resembling structures found in the specimen and clinicoradiological correlation, but not on histopathologically identifiable hard tissues. Under MGS, each dental hard tissue was specifically identified: bone appeared green and cementum appeared red. Dentin was also evident with its characteristic dentinal tubules in green, similar to that of bone, but lacking trabeculae. Immature bone showed a light green colour compared to mature bone. The statistical comparison of the H&E stain and MGS between various study groups and control groups showed significant results regarding differentiation and intensity.

DISCUSSION

The MGS had better efficacy than the routine H&E stain in differentiating bone and cemental tissues from decalcified tissue sections of various oral and maxillofacial pathologies. While H&E stain is the standard stain used in histopathology, it presents diagnostic difficulties in distinguishing between different mineralised structures within oral cavity pathologies. Differential staining, a process employing multiple chemical stains, offers improved differentiation of various structures, addressing the limitations of H&E staining [7,11]. One such differential stain is MGS, a variant of Lille's stain. This stain not only effectively colours decalcified sections but also provides differential staining of hard tissues in teeth and calcified structures present in pathological lesions [12].

As MGS is a trichrome stain, it involves multiple solutions, including haematoxylin, carbol fuchsin and aniline blue. PanthalaMudhiraj PV et al., have standardised the laboratory preparation of these solutions for MGS [5]. However, the standardisation of staining procedures requires continuous repetition to minimise errors. In the present retrospective study, technical difficulties were encountered during the staining process. To address these challenges and ensure staining efficacy, certain modifications were implemented.

For the preparation of the mordant solution using ferric chloride, a minimum concentration of iron chloride, specifically 37.2 g in 100 mL of distilled water, was used. It is crucial to ensure that the standardised mordant solution remains clear, as any turbidity can affect the staining results. Achieving the appropriate colour of cementum under microscopy, such as a dark red hue, was facilitated by using a freshly prepared carbol fuchsin solution. Old stock solutions of carbol fuchsin should be avoided, as they may not efficiently produce the required colour. When preparing the aniline blue solution, using commercially available saturated picric acid solution was found to yield better results compared to laboratory-made solutions. Additionally, commercially available aniline blue liquid was more efficient than aniline blue powder. Consistent with the suggestion by Singh P et al., the addition of 2 mL of light green to the solution enhanced staining intensity and contrast [12].

The procedure followed the staining protocol outlined by PanthalaMudhiraj PV et al., but this study observed a lack of staining intensity and contrast [5]. To address this issue, a modified standardised staining protocol was developed by adjusting the staining time using different intervals for various steps. Freshly decalcified samples yielded more promising results compared to samples stored in departmental archives, as older samples resisted serial sectioning and lacked stain-retaining properties. Additionally, the use of xylene after MGS, which was already used for H&E staining purposes, affected the colour obtained by MGS. Therefore, a separate xylene glass jar for MGS is mandatory. These modifications in the preparation and staining with MGS led to improved staining efficacy.

In the present study, the authors investigated various pathologies originating from different tissues, such as bone and cementum. The true nature of pathologic calcifications posed a challenge during H&E staining under light microscopy, as it was difficult to differentiate between dentin, cementum and bone. Moreover, the transient phase of osteoid to immature bone could be mistaken for cementum deposits. However, these challenges were effectively addressed using MGS. The authors found that identification,

intensity and contrast were more appreciable in sections stained with MGS compared to H&E stain. This highlights the superiority of MGS in accurately differentiating between various pathologies.

When cases of juvenile ossifying fibroma (n=4) were evaluated in MGS sections, hard tissues in shades of green suggestive of bone were noted, while red-coloured hard tissue suggestive of cementum was not found; thus, the previous histopathological diagnosis of juvenile ossifying fibroma in H&E was confirmed. This was unlike the study by Dhouskar S et al., in which a case of juvenile psammomatoid ossifying fibroma with spheroidal calcifications in H&E was diagnosed as juvenile psammomatoid cemento-ossifying fibroma on MGS, as the spheroidal calcifications stained red, signifying the presence of cementum-like material [13]. Notable changes in the diagnosis of this fibrous lesion upon modified Gallego staining suggest that pathologic calcifications can be misdiagnosed under routine H&E staining.

Limitation(s)

The study had a small sample size, which may limit the generalisability of the findings. Additionally, MGS was highly technique-sensitive, requiring precise execution for reliable results.

CONCLUSION(S)

While H&E stain remains the gold standard, its limitations in identifying hard tissues arise from all hard structures being stained in various shades of pink and blue. Histochemical staining with MGS presents a new horizon in the identification of these hard tissues. Thus, MGS emerges as a superior histochemical stain compared to routine H&E stain, offering enhanced differentiation and clarity in identifying bone and cemental tissues.

Acknowledgement

The authors would like to express their sincere gratitude to Mrs. Shalaka Borkar and Mrs. Meenakshi Bangar for their exceptional technical assistance in the histopathology laboratory. Their expertise and dedication were invaluable to the successful completion of this work.

REFERENCES

[1] Kumar GS. Orban's Textbook of Oral Histology & Embryology, 13th Edition, 2011.
[2] Nanci A. Ten Cate's Textbook of Oral Histology. Development, Structure and Function, 8th Edition, 2013.
[3] Foster BL. Methods for studying tooth root cementum by light microscopy. Int J Oral Sci. 2012;4(3):119-28.
[4] Kunche A, Kiresur MA, Ananthaneni A, Guduru VS, Puneeth HK, Bagalad B. Comparison of verdeluz orange G and modified Gallego stains. Biotech Histochem. 2017;92(8):547-51.
[5] PanthalaMudhiraj 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(4):ZC13-ZC15.
[6] 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. International Journal of Oral & Maxillofacial Pathology. 2014;5(2):02-06.
[7] 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(8):545-51.
[8] Shukla D, Vinuth DP, Sowmya SW, Jeevan MB, Kale AD, Hallikerimath S. Cementum made more visual. J Forensic Odontostomatol. 2012;30(1):29-37.
[9] Levey RJ. A modified Gallego's iron fuschin stain as a differential stain for cementum. J Dent Res. 1956;35(3):491-93.
[10] Bancroft JD, Gamble M.Theory and practice of histological technique. 5th ed. Churchill Livingstone, New York.1996.
[11] Suvarna SK, Layton C, Bancroft JD. Bancroft's Theory and Practice of Histological Technique. 7th edition.
[12] Singh P, Nambiar KS, Haragannavar VC, Augustine D, Sowmya SV, Rao RS. Differential stains for identification of normal and pathological hard-tissues using hematoxylin and eosin, masson's trichrome, and modified gallego's stains: A comparative study. World J Dent. 2018;9(5):372-76.
[13] 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 mineralised components in ossifying fibroma, cemento-ossifying fibroma, and cementifying fibroma. J MicroscUltrastruct. 2019;7(3):124-29.

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PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Jul 08, 2024
- Manual Googling: Dec 23, 2024
- iThenticate Software: Dec 25, 2024 (10%)

ETYMOLOGY: Author Origin

EMENDATIONS: 5

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? No
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **Jul 07, 2024**
Date of Peer Review: **Nov 26, 2024**
Date of Acceptance: **Dec 27, 2024**
Date of Publishing: **Mar 01, 2025**