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Cementum Made More Visual

Deepika Shukla¹, Vinuth D P², Sowmya S. V³, Jeevan M.B⁴, Alka D Kale⁵, Seema Hallikerimath⁵

¹ Department of Oral Pathology and Microbiology, Faculty of Dentistry, Jamia Millia Islamia, Delhi, INDIA.

² Department of Oral Pathology and Microbiology, Hitkarni Dental College & Hospital, Jabalpur, INDIA

³ Department of Oral Pathology and Microbiology, M.S.Ramaiah Dental College and Hospital, Bangalore 560054, Karnataka, INDIA.

⁴ Department of Oral Pathology and Microbiology, Vydehi Institute of Dental Sciences and Research Centre, Bangalore, INDIA.

⁵ Department of Oral Pathology and Microbiology, KLE VK Institute of Dental Sciences and Hospital, Belgaum, Karnataka, INDIA.

Corresponding author: deepika_shukla06@yahoo.com

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ABSTRACT

Dental cementum is a specialized calcified structure covering the root of a tooth. This study aims to investigate cementum using various stains which can be exceedingly useful in investigation, observation and diagnosis. 4µm sections of 25 extracted normal teeth, 25 cases of various cemental pathologies and 25 ground sections were stained using cresyl violet, H&E, toluidine blue and periodic acid Schiff and were observed under light and fluorescence microscopes. Cresyl violet showed best contrast amongst all stains in decalcified and ground sections under light and fluorescence microscopy. Under the fluorescence microscope, cementum fluoresced more distinctly than dentin and enamel. Among the cemental pathologies examined, osteoid and cementoid exhibited fluorescence but cementum and bone did not fluoresce. Incremental lines were prominently visualised with cresyl violet under fluorescent microscopy, which may aid in forensic determination of age. The present results demonstrate that cementum in normal decalcified teeth and cemento-osseous lesions, could be observed best using cresyl violet stain under fluorescence microscopy.

KEYWORDS: dental cementum; microscopy; pathology

INTRODUCTION

Cementum is an avascular, inorganic, dense, inert, extremely narrow layer of tissue whose sole purpose is that of affording attachment for a tooth to its articulation. Various authors have suggested the possibility of age estimation from acellular cementum incremental lines.¹⁻⁶ Human cementum has been shown in studies to exhibit a strong propensity for fluorescence.⁷

The dentino-enamel junction is well defined in light microscopy. In contrast, the distinction between root dentine and cementum is exceedingly ill defined; so much so that, in decalcified haematoxylin and eosin [H&E] stained sections, it is difficult to distinguish between them. This may be because both are mesodermic in origin with similar histologic character and physiological function. Various stains have been used for cementum but not many are easily available and economical. An inexpensive and distinctive stain for cementum can be extremely useful in investigation, observation, diagnosis & teaching. A search of the published literature has shown that, at present, there are only few techniques⁸ which will differentially stain the cementum of human teeth.

Various controversies exist about the cemento-osseous lesions affecting oral cavity. This group of lesions are characterized microscopically by fibrous stroma containing various combinations of bone and cementum-like material. Histologically, they may be indistinguishable from other fibro-osseous lesions, except by the clinical and radiographic findings.⁹ Thus differential staining of cementum can be of great importance in highlighting the nature and biological behaviour of such lesions.

The present study concerns a successful differential staining technique for human cementum and aims to investigate cementum of decalcified and ground

sections using various stains like Cresyl Violet, Hematoxylin and Eosin, Toluidine

Blue and PAS under conventional light & fluorescent microscopy. We have also studied the differential staining of cementum in various pathological cemento-osseous lesions.

MATERIALS AND METHODS

25 normal human teeth which were extracted for orthodontic reasons from KLE VK Institute of Dental Sciences, Belgaum were used for the study. Institutional Review Board and Ethical Committee approval was obtained prior to the start of the study. None of the teeth were extracted because of periodontal disease, which destroys the periodontal fibres and stops the formation of cementum in the affected areas. These normal human teeth were decalcified, formalin fixed & paraffin embedded. 25 cases of cemental pathologies which included histopathologically diagnosed cases of peripheral cemento-ossifying fibroma, central cemento-ossifying fibroma and cementoma were retrieved from the archives of the Department of Oral and Maxillofacial Pathology. 4µm sections taken from these tissues and 25 longitudinally cut ground sections were stained using Hematoxylin and eosin,¹⁰ PAS,¹¹ toluidine blue¹² and cresyl violet.⁸ The stained sections were examined using conventional light microscopy (Magnus MLX-Bi) and fluorescent microscopy (LeicaTM DM2500) independently by five experienced oral pathologists. The sections were graded from 1 to 4 according to the contrast of cementum in various stains (Table 1).

RESULTS

When sections were viewed under light microscope and approximate average of the grades by five oral pathologists were calculated, cementum was best

distinguished in cresyl violet stained decalcified and ground sections (Table 2). Toluidine blue also enabled better differentiation than PAS and hematoxylin and eosin under light microscope (Table 2;

Fig.1). In ground sections differentiation between cementum and dentin was more appreciable in cresyl violet than the other three stains (Table 2; Fig. 1).

Table 1: Grading for distinguishing cementum from dentin and periodontal ligament in decalcified and ground sections; in cemento-osseous lesions differentiation of cementum from cementoid.

GRADE 1	Cementum distinguishable with difficulty
GRADE 2	Moderately distinguishable
GRADE 3	Differentiation of cementum easy
GRADE 4	Cementum brightly contrasted

Table 2: Differentiation of cementum from dentin in decalcified and ground sections under various stains as observed under light and fluorescence microscope (approximate average of the grades of the intensity of staining by five oral pathologists for 20 tooth sections)

Grade	Cresyl violet stain		Periodic acid Schiff (PAS) stain		Toluidine blue		Hematoxylin and eosin (H&E) stain	
	Decal.	Gr.	Decal.	Gr.	Decal.	Gr.	Decal.	Gr.
Light microscopy								
1	0	0	4(20)	1(5)	1(5)	3(15)	19(95)	17 (85)
2	0	5(25)	15(75)	16(80)	2(10)	15(75)	1(5)	2(10)
3	1(5)	15(75)	1(5)	3(15)	17(85)	2(10)	0	1(5)
4	19 (95)	0	0		0	0	0	0
Fluorescence microscopy (green light)								
1	0	0	0	0	0	0	1(5)	2(10)
2	0	0	4(20)	17(85)	0	0	3(15)	12(60)
3	2(10)	12(60)	14(70)	3(15)	0	0	13(65)	4(20)
4	18(90)	8(35)	2(10)	0	0	0	3(15)	2(10)

Decal= % Decalcified sections, Gr=% Ground sections

Under fluorescence microscope maximum contrast was seen in all cresyl violet stained sections using green light excitation of wavelength 590 nm. Cementum showed red fluorescence as compared to enamel, dentin and PDL (Fig.2). This fluorescence was brighter than that seen in PAS and hematoxylin and eosin stained sections. Incremental lines were prominently seen as they did not show fluorescence under fluorescence microscopy (Fig. 2). These incremental lines were more noticeable at approximately apical third of the root and

furcation area where cementum is more prominent.

Under light microscope maximum contrast between cementum and cementoid, and bone and osteoid was seen in cresyl violet and toluidine blue. PAS showed better contrast of cementum than H&E stained sections (Table 3). Hematoxylin and eosin stained sections of cemental pathologies showed cementum as basophilic spheroidal lobules and cementoid as eosinophilic deposit around it. Bone was seen as trabeculae with eosinophilic osteoid deposit around it. In PAS stained sections cementum was stained magenta in color.

Cementum was stained blue surrounded by purple cementoid in toluidine blue and in cresyl violet cementum was stained purple. The most appreciable contrast was observed in cemental pathologies stained with cresyl

violet, where osteoid and cementoid showed brilliant red fluorescence under fluorescence microscope with green light excitation but cementum and bone did not fluoresce (Fig.3; Table 3).

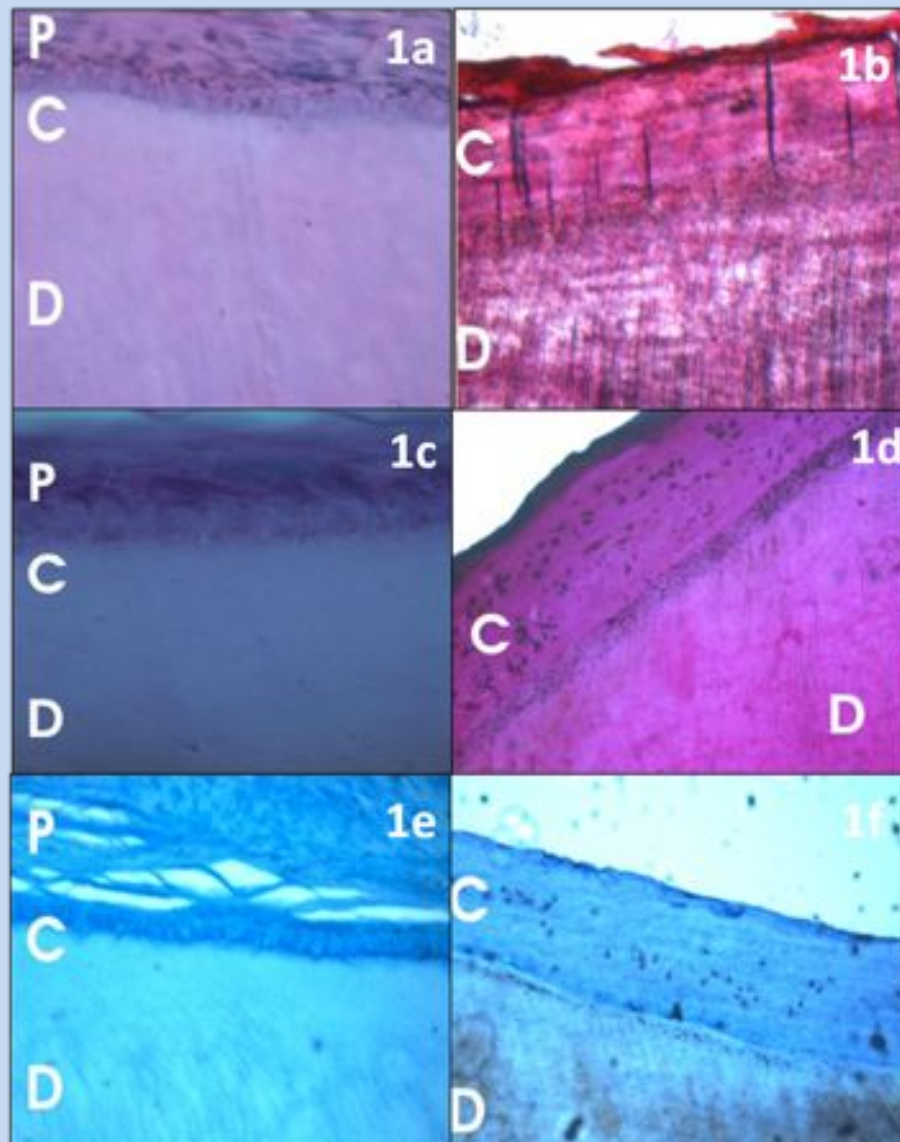


Figure 1: Normal cementum in various stains as observed under light microscope. (1a) Hematoxylin and eosin stained (H&E) decalcified section showing periodontal ligament (P), cementum (C) and dentin (D) (magnification 40 X). (1b) H & E stained (magnification 40 X) ground section showing cementum (C) and dentin (D). (1c) Periodic acid Schiff's stained (PAS; magnification 40 X) decalcified section showing better contrast of cementum (C) from dentin (D) and periodontal ligament (P) than H&E. (1d) PAS (magnification 40 X) of a ground section showing better contrast of cementum (C) from dentin (D) than H&E. (1e) Toluidine blue stain (magnification 40 X) of a decalcified section showing better contrast of cementum (C) from dentin (D) and periodontal ligament (P) than PAS and H&E. (1f) Toluidine blue stain (magnification 40 X) of a ground section showing better contrast of cementum (C) from dentin (D) than PAS and H& E.

DISCUSSION

In the present study cresyl violet stain exhibits better contrast of cementum in decalcified and ground sections than other stains under light microscopy with a clear and even background. Toluidine blue stain

showed better contrast than PAS and H & E (Fig. 1). The contrast was more appreciable in PAS stained sections than H & E. This suggests that cresyl violet staining affords the best appreciation of cementum in decalcified and ground sections.

Further, contrast was better using fluorescence microscopy than light microscopy since the stained cemental bands, but not the incremental lines, fluoresced after staining with cresyl violet, PAS and hematoxylin and eosin. Incremental lines in cementum are most prominently seen in sections stained with cresyl violet excited by green light observed under fluorescent microscopy, a property which may be exploited in forensic studies (Fig. 2d). Since incremental lines are not destroyed by acids and stain differently than the remaining cementum, it is likely that they possess an organic structure which differs from the cementum⁸. It was observed that the width of incremental lines varied significantly from one region to another. The lines seemed to spread and were better distinguished in apical third of root. Incremental lines are considered to represent periods of varying activity in matrix formation and mineralization during cementogenesis.¹³ Apposition of cementum occurs in phases resulting in two types of layers with different optical and staining properties. Narrow, dark staining incremental lines are separated by wider bands of pale

staining cementum. The distance from one line to the next represents a yearly increment deposit of cementum in many mammals, and counting these lines has been used routinely to estimate the age of the human beings.⁸

In the present study very distinct demarcation between dentin and cementum can be made as the cementum fluoresced more than dentin and enamel using cresyl violet seen under fluorescence microscopy (Fig. 2). Cresyl violet shows red fluorescence with green light of wavelength 590 nm in contrast to dentin and PDL. This staining is easy to perform with good shelf life. Cresyl violet was resistant to bleaching and fluorescence persisted for several days with the background fluorescence being minimal. Another interesting finding was the fluorescence of cementum seen in H & E stained sections. This could be probably due to staining of collagen fibres with eosin Y as occurs in other types of connective tissue. This study demonstrates the successful differential staining of the human cementum, in decalcified sections of teeth, showing the deeply stained layer of cementum (C), contrasting markedly with the dentin (D) and the periodontal membrane (P) (Fig. 2). This was consistent with Sigrid I. Kvaal et al. who concluded that human cementum could be best observed using decalcified sections stained with cresyl violet.⁸ The possible explanation for the findings in the present study could be due to the fact that human cementum contains much more organic material & hence fluoresced strongly than dentin & enamel. It is probable that the fluorescing substances in cementum are associated with the organic fraction.

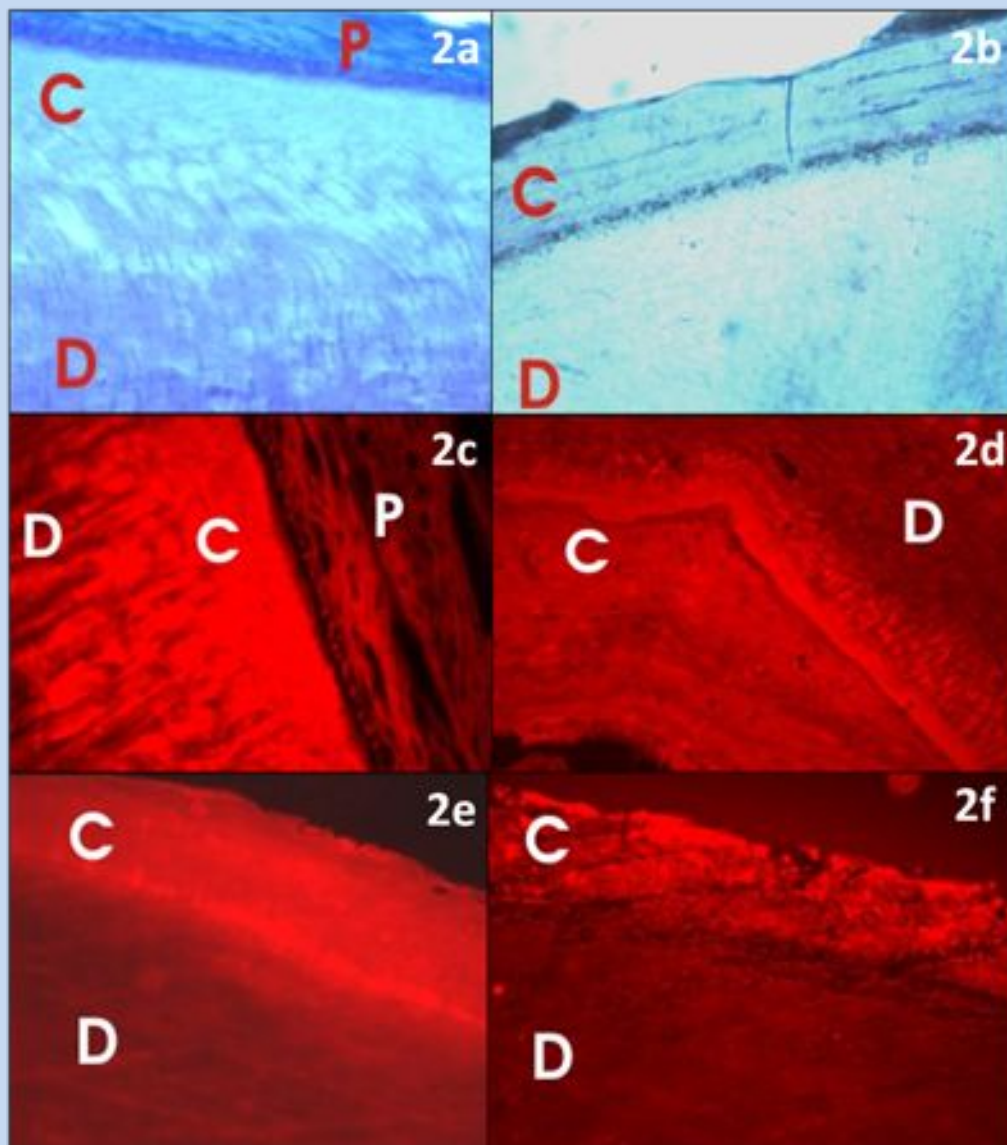


Figure 2: Normal cementum in cresyl violet stain as observed under light and fluorescence microscope. (2a) Cresyl violet stained decalcified section (magnification 40 X) showing best contrast of cementum (C) from dentin (D) and periodontal ligament (P) than other stains under light microscopy. (2b) Cresyl violet stained ground section showing best contrast of cementum (C) than other stains under light microscopy (magnification 40X). (2c) Cresyl violet stained cementum shows red fluorescence with green light of wavelength 590 nm in contrast to dentin and PDL as seen under fluorescence microscopy (magnification 40X). (2d) At the bifurcation of a mandibular molar incremental lines in cementum are prominently seen as they do not show fluorescence with cresyl violet under fluorescent microscopy (magnification 40X). (2e & 2f) Cresyl violet stained ground sections showing distinctly demarcated cementum (C) from dentin (D) as cementum show red fluorescence under fluorescence microscopy (magnification 40X).

In the present study the sections from cemental pathologies showed calcified material which is more appropriately considered to be cementum. This belief is based upon a number of criteria. A globular accretion pattern with basophilic tendency is

evident in these lesions under plain light. In a trabecular form the trabeculae are usually “molded” and do not have the rather sharp angles that are seen in bone. With plain light microscopy, solid masses of secondary cementum are virtually indistinguishable

from bone. In cementum typical osteoblasts and rimming of cells is not seen. In comparison with bone, the lacunae in cementum contain fewer recognizable cellular elements. The use of these criteria for the distinction between bone and cementum was valid in a high percentage of cases observed in the present study. Cementum, while is undoubtedly a phylogenetic derivation of bone, is at the same time different in a number of respects. Cementum appears to be destined to form dense, sclerotic masses without organization that are incapable of remodelling and appears to be resistant to osteoclasts.¹⁴

Another interesting finding in the present study was that under fluorescence microscopy, sections of cemental pathologies with osteoid and cementoid showed fluorescence but cementum and bone did not fluoresce at all (Fig. 3), though in decalcified and ground sections cementum showed red fluorescence when excited by green light. This could possibly be due to alteration in structure and biochemical composition of cementum which is affected by several diseases.⁵ This could make cementum lose its property to fluoresce in cemental pathologies, thus suggesting that when cementum is uncalcified (cementoid) it

shows fluorescence probably due to increased organic proportion, whereas when calcified it loses its fluorescent properties. We hypothesise that the inorganic portion is abundant in pathologic cementum which replaces the organic portion making the cementum non fluorescent. Alternatively, the fluorescing substances may be lost in the cementum formed in these pathologies. Further studies are required to investigate the biochemical composition of cementum in cemental pathologies.

CONCLUSION

Cresyl violet showed better contrast of cementum than toluidine blue, PAS and H&E in decalcified and ground sections under light and fluorescence microscopy. Cementum fluoresced appreciably more than dentin and enamel under fluorescence microscope when stained with cresyl violet and excited with green light. In cemental pathologies, osteoid and cementoid showed fluorescence but cementum and bone did not show fluorescence. Incremental lines are clearly seen with cresyl violet under fluorescence microscopy, which could play an important role in forensic sciences

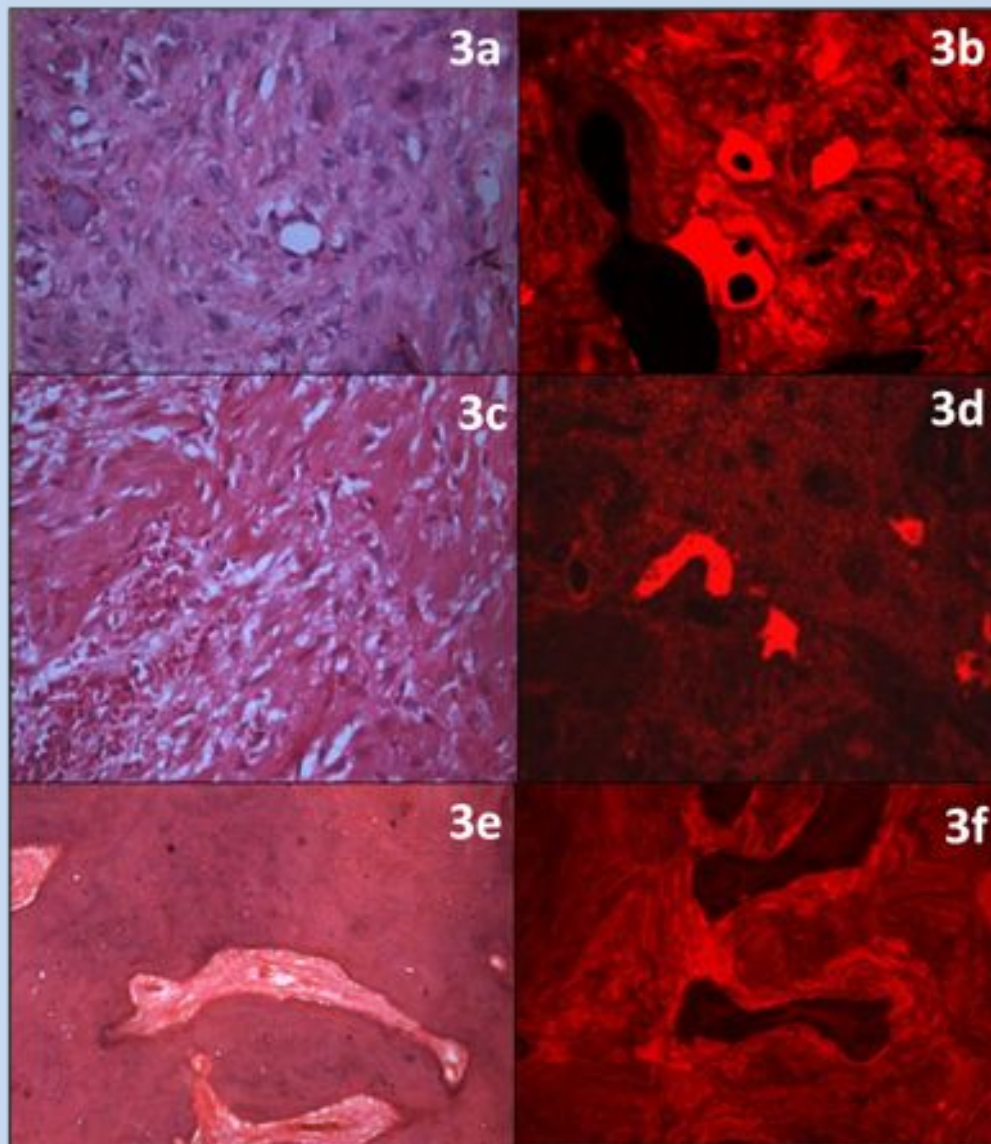


Figure 3: Various cemento-osseous lesions stained with H & E and cresyl violet observed under light and fluorescence microscopy. (3a) Photomicrograph showing H&E stained peripheral cemento ossifying fibroma with small round basophilic globular masses having calcific foci in the centre resembling cementum surrounded by eosinophilic cementoid in fibrocellular stroma (magnification 40 X). (3b) Cresyl violet stained section of peripheral cemento ossifying fibroma showing red fluorescing cementoid with non fluorescing calcified centre resembling cementum. Bony trabeculae seen on left side shows no fluorescence (magnification 40X). (3c) H&E stained central cement-osseous fibroma showing irregular bony trabeculae with interlacing collagen fibers interspersed by active, proliferating fibroblasts (magnification 40X). (3d) Cresyl violet stained section of central cemento-ossifying fibroma showing fluorescing osteoid in the centre. Few oval to round fluorescing cementoid like globules with non fluorescing calcified centre are seen on right side (magnification 40X). (3e) H&E stained section of cementoma showing sheets of cementum like tissue with scattered reversal lines. (3f) Cresyl violet stained section of cementoma showing calcified cemental trabeculae with cementoblasts at their borders, surrounded by fluorescing cementoid (magnification 40X).

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