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Differential Stains For Histological Analysis (Enamel,Dentin,Cementum) Of Teeth And Bone Using Van Gieson And Masson's Trichrome Stain.

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ABSTRACT:

Aim: To analyse the histology of teeth(enamel,dentin,cementum) and bone using van gieson and masson's trichrome stain.

Introduction: Identification of hard tissues is a challenging task,various methods have been done and demonstrated. Identification of calcified mass and pathological lesions is very difficult. Staining is an additional technique which shows contrasting images under a microscope. It provides the histological features of pathological tissues.

Materials and methods: 5 teeth samples were taken from dental clinics. Prepared slides of sections of teeth using van gieson and masson trichrome to identify siaolith ,osteomyelitis and odontome and observed under electron microscope.

Result: Tissues stained with Van gieson showed collagen-blue, muscle and cornified epithelium-yellow, nuclei-blue-black. Tissue stained with masson trichrome showed
collagen-blue, nuclei-black, muscle,cytoplasm and keratin-red.H&E stained tissue showed Nucleus-purple hematoxylin, cytoplasm-pink eosin.

Conclusion : H and E and Masson's trichrome stain are cost-effective. It identifies hard tissue components such as bone, cementum, and dystrophic deposits from stromal components by staining them separately.

Keywords : pathological lesions, siaolith, fibro-osseous lesions, mineralization, Massons trichrome, Van gieson, innovative techniques.

INTRODUCTION:

Identification of hard tissues is a challenging task, various methods have been done and demonstrated. Identification of calcified mass and pathological lesions in hard tissues is very difficult. Staining is an additional technique which is used to contrast images under a microscope. Differential staining is the process where more than one chemical is used for staining and diagnosis. (1).One of

the techniques are van gieson and masson trichrome stain. Masson trichrome is used for identifying pathological lesions whereas van gieson stain is used for identifying collagen fibres.The main principle of masson trichrome staining is it pulls acidophilic tissue components first then basophilic at last.(2) The main principle of van gieson stain is to differentiate between collagen and smooth muscle.

Siaolith is a formation of calcified mass in the salivary gland. (3)Osteomyelitis is a bone infection where it causes flow of blood through bone.(1) Odontoma is a benign odontogenic tumour which resembles teeth structures.(4) Various connective tissue stains have been used to distinguish between hard and soft tissue components over time. Von Kossa, Masson's Trichrome, and silver staining before decalcification are a few stains that preserve the ability to differentiate stromal hard tissues.The purpose of stain is to differentiate the hard tissues found in tooth and other pathological lesions. The goal of the research is to gain a better knowledge of the histological image of oral hard tissue producing lesions. When different hard tissue components, as well as combinations of hard and soft tissues, are present in the same tumour, diagnostic challenges arise; therefore, identifying the types of calcified structures in their early stages is critical for the diagnosis of such lesions.

Other advantages of staining is that it is a cost efficient and simple method compared to advanced diagnostic aids like immunohistochemistry. The goal of the current study is to gain a better knowledge of the histological image of oral hard tissue producing lesions. When different hard tissue components, as well as combinations of hard and soft tissues, are present in the same tumour, identifying the types of calcified structures in their early stages is critical for diagnosing such lesions. Our team has extensive knowledge and research experience that has translated into high quality publications. (5),(6),(7),(8),(9),(10),(11),(12),(13),(14),(15),(16),(17),(18),(19),(20),(21),(22),(23),(24). This present study aimed to analyze the histology of teeth (enamel, cementum,dentine) and bone using van gieson and masson trichrome differential stains.

MATERIALS AND METHODS:

Masson trichrome stain:

Bouin solution is microwaved and allowed to stand for 15 minutes. Picric acid has been removed and placed for 5 minutes. Weigert solution is added and placed for 5 minutes and has been rinsed in distilled water for 5 minutes. Bieberch scarlet is added and placed for 5 minutes until exposed and has been rinsed in distilled water for 5 minutes. Phospotungstenic acid/phospomolybic acid is added and placed for 10 minutes. Aniline blue is transferred directly and has been placed for 10 minutes . 1% of acetic acid solution is added and placed for 10 minutes. Slide has been observed under a microscope.(2)

Van gieson:

Nuclei has been stained with Schiff's reagent and placed for 5 minutes. Then the slide has been stained with hematoxylin and placed for 5 minutes. Dehydrate rapidly in alcohol , mount and observe it under a microscope.(25)

H&E:

Hematoxylin has been mixed with metallic salt followed by the slide has been rinsed with weak acid to remove excess staining , and then with mild alkaline water. The tissue is counterstained with eosin.(1)

RESULTS:

fig-1:Photomicrograph showing decalcified teeth staying using H&E and masson trichrome stain respectively.

fig-2:Photomicrograph showing siaolith stained using masson trichome stain

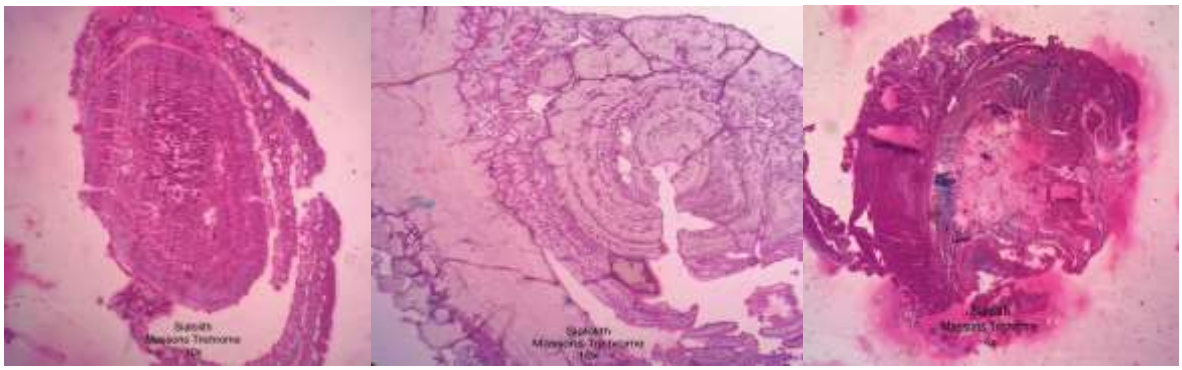

Fig-3: photomicrograph showing siaolith stained using van gieson stain

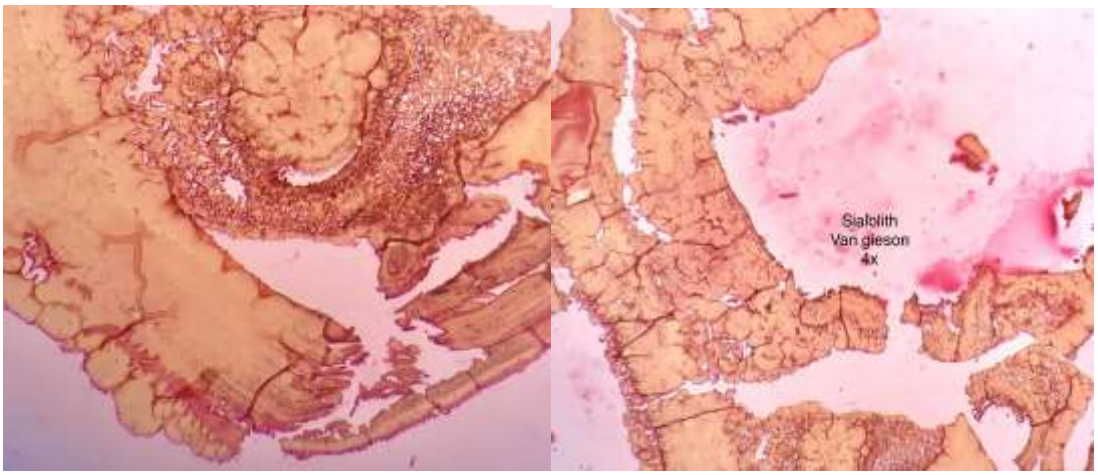

Fig-4 : photomicrograph showing osteomyelitis stained using masson trichrome stain.

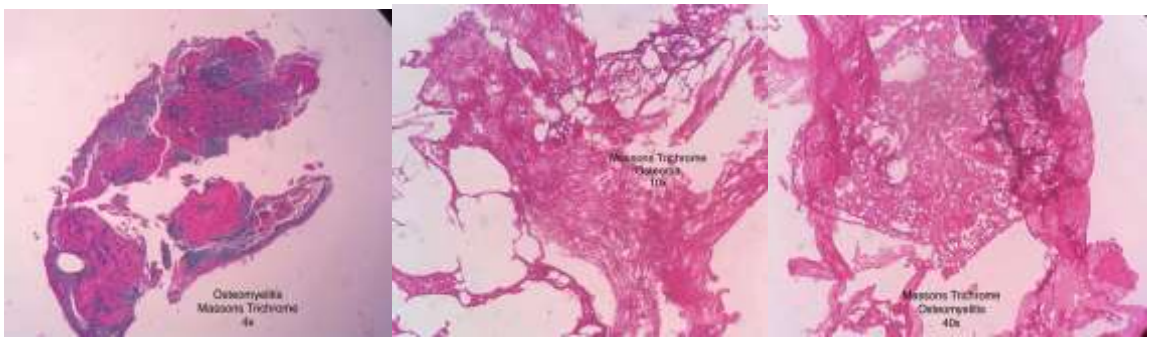

Fig-5: photomicrograph showing osteomyelitis stained using van gieson stain.

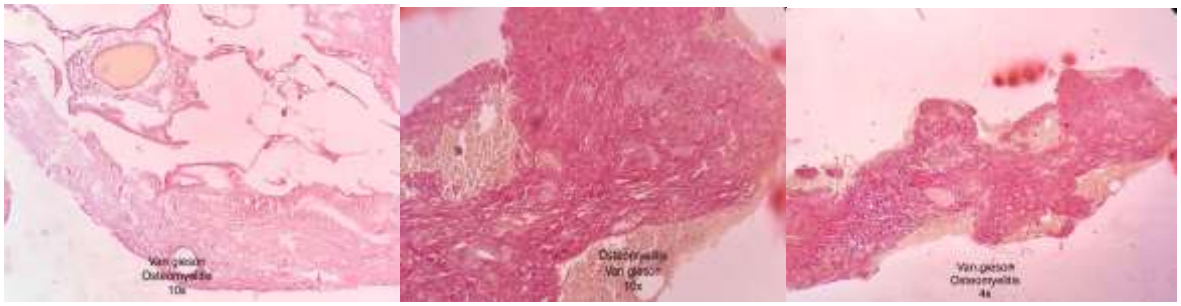

Fig-6:photomicrograph showing odontoma stained using van gieson

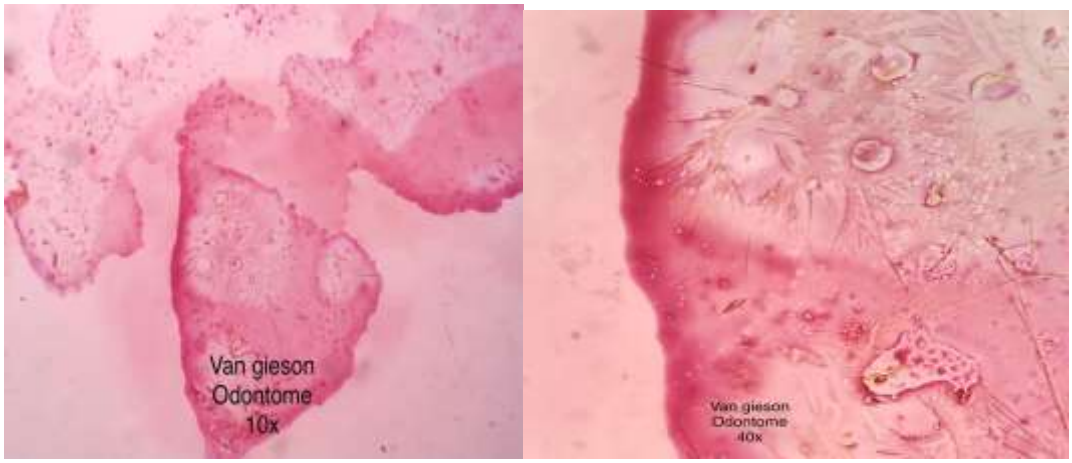

Fig-7: photomicrograph shows osteoma stained using van gieson and masson trichrome respectively

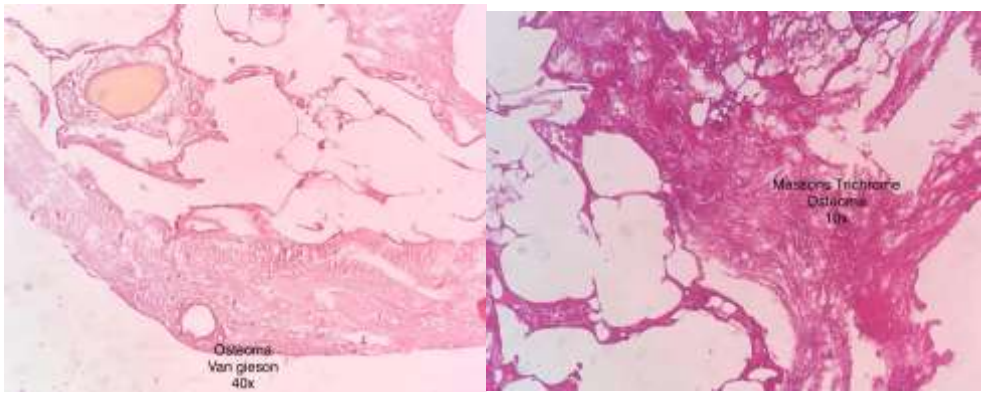

Table-1:Result table shows differential stains of histological analysis of various pathological lesions

	Van gieson	H&E	Massons Trichrome
Tooth	Orange	Purple and pink	Blue and pink
Osteomyelitis	Orange and red	Purple and pink	Pink
Osteome	Orange	Pink	Pink
Odontome	Orange and yellow	Pink	Blue and pink
Sialolith	Orange and yellow	Purple and pink	Blue and pink

DISCUSSION:

The result shows all of the stromal components in our investigation were stained in various shades of pink and purple, as shown in H and E. The colours of calcifications, however distinct over H and E, frequently blend with soft tissue stroma in Masson's trichrome because it stains in the same hues of slightly varying hues of blue and red. In contrast, all of the stromal components in the van gieson display are stained in various shades of orange, yellow, and red.

There are three main deficiencies which can be identified: the calcified and pathological lesions of hard tissues by masson trichrome and van gieson stain. They are siaolith, osteomyelitis and odontome. Siaolith are calcified structures found within the ducts of salivary glands. They form calcified mass around stenson’s duct. Siaolith causes swelling and pain. This siaolith can be prevented by conservation and surgical ultrasonography. Ultrasonography is considered as a most preferable technique. Our patients met the diagnostic criteria for sialolithiasis histopathologically, which is thought to be caused by an initial central core of organic material that expands progressively through layered deposition of organic and inorganic elements, resulting in a lamellar pattern or a globular calcified zone. It's also been suggested that mitochondria and lysosome bodies from the ductal system have a role in the aetiology of salivary gland stones. Similarly, the majority of the lesions in our investigation were generated by calcified nodular formations arranged in a lamellar pattern. Lipids

have recently been discovered to be the primary constituents of the sialolith's first central core. Furthermore, whitlockite crystallites are involved in the initial mineralization process as well as the progression of transformation into more stable hydroxyapatite crystallites.

Osteomyelitis is an infectious disease which affects bone marrow and bone. Osteomyelitis can be prevented by amputation, limited resection and antibiotic therapy. Surgeries of osteomyelitis is a risk developing factor at the site of skin which causes bone destruction. Due to biopsy it can't be ignored in accordance with previous studies.In diabetic patients, acute osteomyelitis is frequently caused by infection spreading from a nearby skin ulcer. By breaching the periosteum or the joint capsule, inflammation reaches the bone. The most reliable technique to diagnose osteomyelitis is to take a bone biopsy for histologic evaluation and culture. The presence of neutrophils next to necrotic bone characterises acute osteomyelitis. Marrow fibrosis and plasma cells are hallmarks of chronic osteomyelitis. In chronic osteomyelitis, malignancy is a rare complication..(26). We also noticed that, in contrast to the reddish purple center, the osteoid seams in osteosarcomas took on a deep blue tint. In H and E stain, degenerating bone (sequestrum), as seen in osteomyelitis, can be difficult to distinguish.

Odontome are also called as a false tooth that resembles tooth-like structures which causes the growth of ameloblast and odontoblast. They are small and rarely exceed tooth diameter. They can be identified through radiographs like orthopantomogram. Early diagnosis and treatments are less expensive and ensure better diagnosis. The presence of all hard tissues in succession, representing enamel, dentine, and cementum, is frequently visible in a ground section. Both enamel and dentine have hypocalcified regions. In our case, the microscopy revealed some interesting characteristics that prompted further discussion. Odontomas are usually treated with cautious surgical excision and preservation of any impacted or lodged teeth that are present.(4)

It is difficult to distinguish osteoid from calcified braided or lamellar bone using normal histological procedures such as Hematoxylin and Eosin in decalcified sections of bone, and occasionally even with cementum when it has a globular shape. In addition, detecting the presence or absence of calcification in connective tissue tumours, whether central or peripheral, benign or malignant, is difficult.

This type of staining is advantageous to use at times for diagnosis or also analysis of lesions. As a result, we advocate its usage in all suspected situations of normal/abnormal calcifications in order to determine the nature of the calcific deposit. This will undoubtedly aid in improved pathology therapies.

CONCLUSION:

Differential stains are cost-effective, they appear to be reliable. Therefore it can be recommended to identify the abnormal calcified mass and pathological lesions.This type of staining is advantageous to use at times for diagnosis or also analysis of lesions. As a result, we advocate its usage in all suspected situations of normal/abnormal calcifications in order to determine the nature of the calcific deposit. This will undoubtedly aid in improved pathology therapies.

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CONFLICT OF INTEREST: Nil

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