

EFFICACY OF H AND E, PAS AND MUCICARMINE IN STAINING MUCOCELE

Running Title: EFFICACY OF STAINING MUCOCELE

Type Of Article: ORIGINAL STUDY

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Abstract:

Introduction:

Mucoceleles are benign lesions related to the minor salivary glands and their respective ducts frequently affecting oral structures which are generally asymptomatic. Mucoceleles are generally characterized by swollen nodular lesions preferentially located on the lower lip and differ from the so-called ranulas, which are lesions located on the floor of the mouth and related to the sublingual or submandibular glands. The prevalence of mucoceleles is 2.5 lesions per 1000 population. The objective of the present study is compare to the efficacy of special staining techniques such as Mucicarmine , PAS and hematoxylin and eosin in the assessment of mucus extravasation lesion. In this study, four cases which is investigated as mucocele said were selected and stained using H and E, pas and mucicarmine. The stained slides are scored by the observers. From the study, we found that the H and E is good in staining the mucocele, pas and mucicarmine is good in staining the mucin collected in the connective tissues.

Keyword: Mucocele, mucin extravasated lesion, periodic acid shiff, mucicarmine, Hematoxylin and eosin.

I. Introduction:

Oral mucocele refer to two terms namely mucus extravasated lesion , mucous retention cyst. The former is a swelling of connective tissue due to the accumulation of fluid called mucin. This occurs because of rupture of salivary gland duct usually caused by trauma in case of mucus extravasated lesion, and an obstructed or ruptured salivary duct in case of mucous retention cystic[1]. The mucus extravasated lesion most commonly found (92.45%); however , mucous retention cyst were also observed (7.54%). The mucocele as a bluish translucent colour and is more commonly seen in children and young adult. Mucoceleles are usually formed secondary to rupture of an excretory duct of a salivary gland, which leads to an outpouring of saliva into the surrounding tissues. The resulting pool of glandular secretion is first surrounded by inflammatory cells and later by reactive granulation tissue consisting of fibroblasts[2]. This granulation tissue reflects an immune response (i.e., to wall off the mucin). Although there is no epithelial lining surrounding the mucin, it becomes well encapsulated by this granulation tissue and is therefore categorized as a false cyst or pseudocyst. In contrast, a mucus retention cyst is a true cyst, lined with epithelium. This type of cyst appears to be caused by epithelial proliferation of a partially obstructed salivary duct. Complete obstruction of a salivary duct by a calcified mass is called a sialolith, also known as a salivary calculus or stone[3].

Mucoceleles are generally formed after traumatic rupture of an excretory duct leading to the outpouring of saliva into the surrounding tissue[4]. The glandular secretion is then surrounded by the cells of inflammatory origin and later by the fibroblasts. There is absence of any epithelial lining around the mucin. However, encapsulation by the granulation tissue causes its inclusion into the category of pseudocyst[5]. Lesion has no definitive sex predilection. It occurs more commonly in children as well as in young adults. Clinically it presents as a discrete, translucent, soft, fluctuant nodular mass. The size of the lesion may vary from a few millimeters and may reach upto few centimetres[6,7].

Treatment includes surgical removal of the lesion along with the surrounding mucosa and glandular tissue down to the muscle layer. Cryosurgery has also been used for the treatment with encouraging result. Parafunctional habits such as lip biting may contribute to the lower lip being the most commonly described location of mucoceleles[8,9]. Cohen and others observed that, of 63 mucoceleles, 82% were found on the lower lip,

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8% on the buccal mucosa, 3% on the retromolar area, and 1% on the palate. The Armed Forces Institute of Pathology collected data on 2,339 cases of mucocele and found that 33.0% occurred on the lower lip, 7.7% on the buccal mucosa, 6.3% on the floor of the mouth, 6.1% on the tongue and only 0.4% on the upper lip[9]. Curtis and Hutchinson documented a single case of mucus extravasation phenomenon of the posterior hard palate after a periodontal free gingival graft procedure[10,11] A potential source of trauma to the upper lip is surgery, such as plastic surgery for lip reduction or augmentation, but no documented cases of mucocele in conjunction with surgery have been identified[12,13].

The most common location of mucocele is inner surface of lower lip and also seen in buccal mucosa. Though it is a most commonly occurring salivary gland disorder[15], there is no definite stains for investigation. Hence, in this study, the efficacy of stains such as Mucicarmin, PAS, H and e in staining the mucocele is revealed.

II. Materials And Methods:

In the study, four sample which is previously diagnosed as mucus extravasated lesion was chosen from the oral pathology department in Saveetha dental college and selected for the study. The selected cases were chosen with serious care by producing sample from the wax block and stained it and also selected by viewing the previous slides which were used for diagnosis of the case. The selected sample were latest sample, new samples were selected due to distortion can occur due to longer storage time. From each, three slides were prepared. The stains such as Hematoxylin and eosin, PAS and mucicarmin were prepared for study. The slides prepared from the selected cases were stained carefully, because the staining technique and the concentration and PH of the stain can affect the originality of the study and perfectly stained slide were only selected for the study. These stained slides were examined by the two observers by interobserver agreement and scoring was given by two observer separately. The slides were viewed at 4x which shows mucous glands and 40x, shows mucus pooling. The slides were scored by four categories, based on cytoplasmic staining, nuclear staining, staining specificity and back ground staining. Each slides were observed and scored very accordingly. The scorings were given as very good, good, fair and poor.

III. Result:

In this study, All the stains are good at staining the structures, on the basis of staining specificity, which defines that the efficacy of stains to stain a particular structure by discriminating other structure. H&e shows poor staining specificity. H&e was scored as poor by both the observers, all four sample shows poor staining specificity for H&e. In case of pas and mucicarmin, both observers scored good staining specificity for three slides and scored one of the four sample as very good staining specificity, thus comparing Hand e with pas and mucicarmin, Hand e has poor efficacy in staining specific structures.

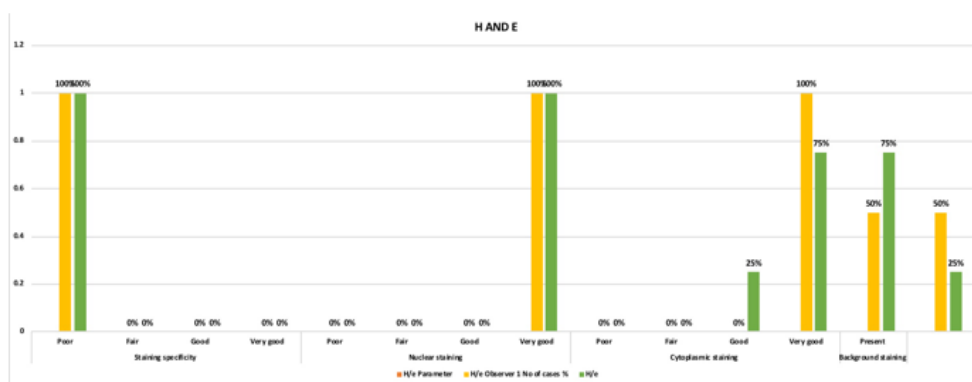
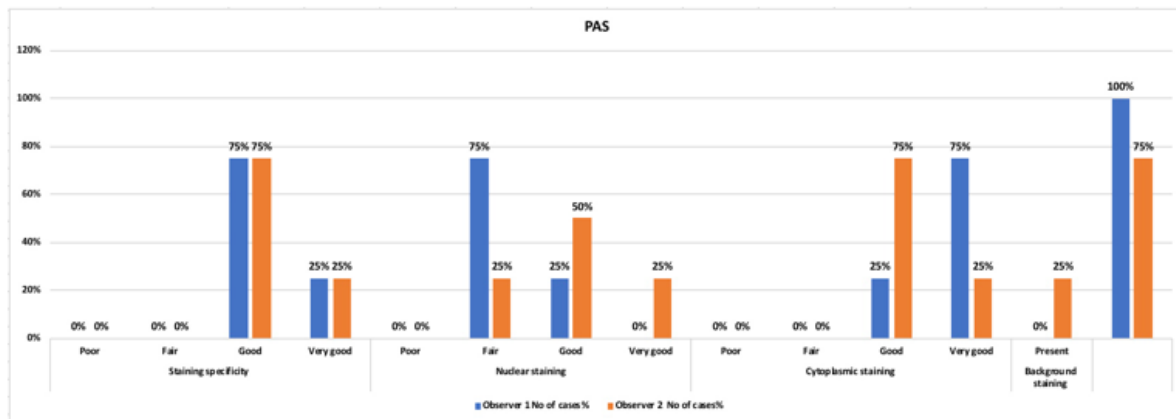


Figure 1: Efficiency of H and E in Staining the Mucocele

On the basis of nuclear staining, H and e was scored as very good for all slides by both observers. It has very good efficacy of staining the nucleus of the acini. Observer 2 has scored one slide shows very good nuclear staining, two slide shows good nuclear staining, one side shows fair nuclear staining for pas. Observer 1 scored fair for three slides and good for one side for the efficacy of pas staining the nucleus. For mucicarmin staining, both the observer scored good for three slides stained by mucicarmin. One slide was scored as fair by both observer. Hand E was very good in nuclear staining, pas is good in nuclear staining than the mucicarmin.

In cytoplasmic staining, H and E was scored as very good in all four slide by observer 1, but observer 2 has given very good for three slides, good for one slide for H and E. In pas staining. observer 1 had scored very good for three slides and scored good for one slide, observer 2 scored good for three slides and very good for only one slide. Similarly observer 1 has scored very good for three slides stained by mucicarmin, good for one slide, observer 2 scored fair for one slide, good for one slide and very good for two slides stained by mucicarmin .

Diagram showing the efficacy of pas in staining the mucocele



On basis of background staining, in H and E, observer 1 scored that background staining was present in two slides and absent in two slides, observer 2 scored that background staining present in three slides and absent in one slides. In pas, observer 1 said that background staining was present in one slide and absent in three slides, observer 2 scored background staining was absent in all slides. In case of mucicarmine, similarly observer 1 scored presence of background staining in only one slide, absent in rest of the slides, observer 2 scored they was a absence of background staining in all slides.

Examination of slides reveals that mucin staining is well discernible in mucicarmine than PAS staining. H and E is scored as very good in nuclear staining by both the observers. Cytoplasmic staining is good in all the 3 stains. Only PAS and mucicarmine discriminates between mucin and other structures. Nuclear staining is well discernible in mucicarmine than pas staining. 50% of background staining is seen in H/ E and 25% of background staining is seen in mucicarmine. Background staining is completely absent in PAS.

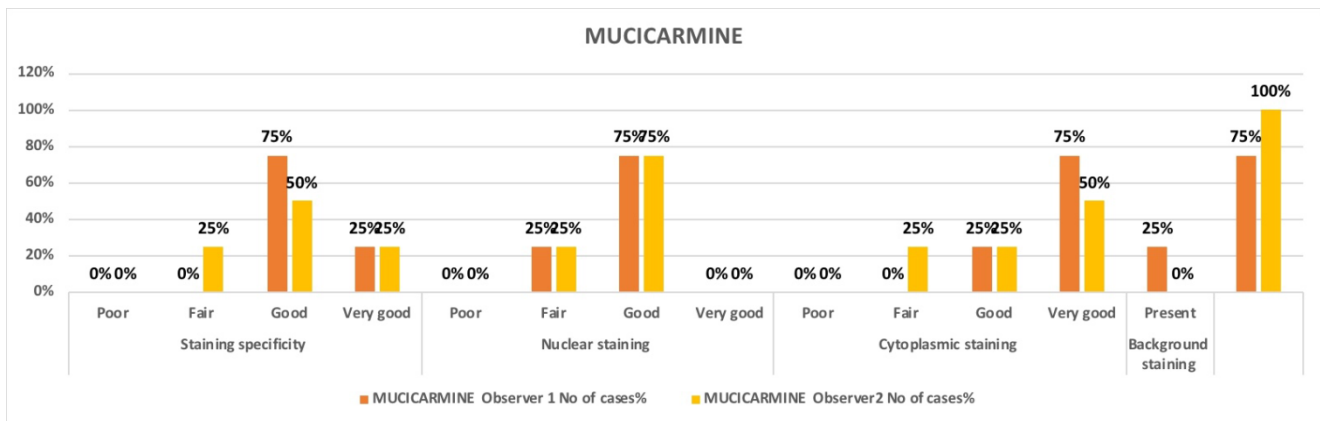


Figure3:Efficacy of Mucicarmine in staining the mucocele

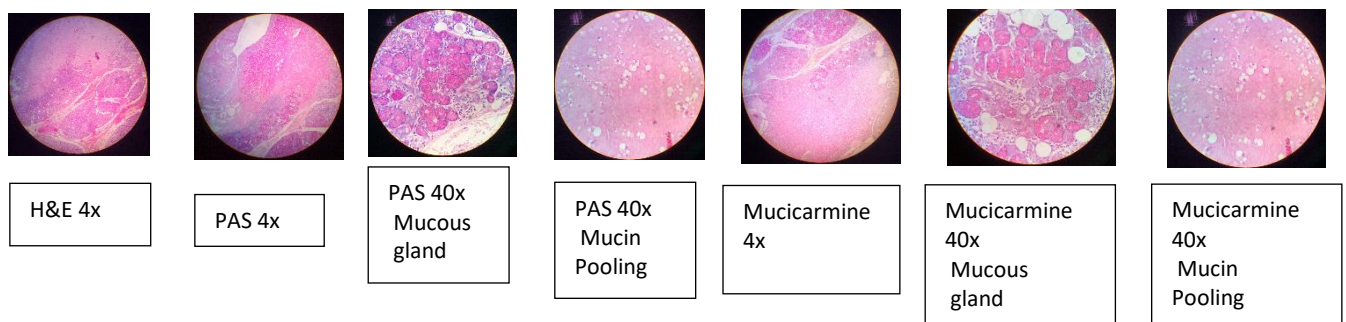


Figure 4:Microscopicimageofstainedmucocele

IV. Discussion:

From this study, it reveals that the H and E is good in staining the mucocele but it does not have staining specificity. It was scored as poor in staining specificity for all slides by both the observers. In pas, 80% has good efficacy in staining the specific structures, 20% has very good in staining specificity. In mucicarmine, the efficacy is less good compared to pas.

Thus, pas shows good staining specificity than mucicarmine, but H and E has poor staining specificity. In nuclear staining, H and e has very good nuclear staining which was described by both observer. Pas also good nuclear staining than mucicarmine. Comparing all three stains, mucicarmine has poor nuclear staining than others. In case of cytoplasmic staining, all three stains shows good cytoplasmic stains, but, H and e shows very good cytoplasmic staining, pas has good cytoplasmic staining compared to mucicarmine. In background staining, H and E have very good back ground staining, pas and mucicarmine, has same grade of background staining.

Only pas and mucicarmine discriminates between mucin and other structures. nuclear staining is well discernible in mucicarmine than pas staining. Cytoplasmic staining is good in all the 3 stains studied. The major factor affecting the study, involving the intensity of stain and staining technique. The concentration and ph of the stain differs from preparation done by people, which affect the study. The staining technique also affect the study, errors can occur during the staining method which may differ every time. PH of stain play major role in staining the slide, when preparation of stains goes wrong, then the required PH cannot be attain leads to failure of stain, this may sometimes taken as false results.

H and e is scored as very good stainer in nuclear staining, mucicarmine is scored as good in three cases, pas is scored as fair in three cases. Similarly H and e is very good in staining the cytoplasm. The mucin were stained good by pas and mucicarmine. In previous study, H and e stains the mucin in pink to pale blue but in our study h and e stains the mucin in pink colour[15,16]. But in previous study, done by M. Costa et al, shows that the H and E is a very good stain in staining the mucocele[17,18]. In present study, H and E has poor staining specificity, but in previous study, M. Costa et al shown that H and E has good staining specificity[19,20].

Examination of slides reveals that mucin staining is well discernible in mucicarmine than PAS staining. H and E is scored as very good in nuclear staining by both the observers. Cytoplasmic staining is good in all the 3 stains. Only PAS and mucicarmine discriminates between mucin and other structures. Nuclear staining is well discernible in mucicarmine than pas staining. 50% of background staining is seen in H/ E and 25% of background staining is seen in mucicarmine. Background staining is completely absent in PAS

V. Conclusion:

In the present study, among all the stains, H and e is good in overall stains except that it has poor specific staining. Pas and mucicarmine is good in specific staining, it stains mucin very well. Also, all the special stains were able to successfully identify the mucin and distinguishing it from the other connective tissue components with a higher intensity, however, H and E showed a higher nuclear staining on an overall basis. All the stains have good cytoplasmic staining. Good background staining is seen in H and E. this study reveals that mucin was well stained by pas and mucicarmine. It also discriminates between mucin and other structures. 50% of background staining is seen in H/ E and 25% of background staining is seen in mucicarmine. Background staining is completely absent in PAS. Cytoplasmic staining was good in all stains. Hence H and e is very good in nuclear and cytoplasmic staining. For some extent, H and E is good in background staining. For specific stains pas and mucicarmine is essential. Hence this would help in reveal the pathology evolving from salivary gland, which helps in differentiating from malignancy and benign tumours.

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