Innovations

Alcian Blue and Safranin O Stains to Reveal Mast Cells in Tissue Sections- A Short Study

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Abstract

Aim: *To evaluate and compare the staining intensity of Alcian blue – safranin O, toluidine blue and Hematoxylin & Eosin in the identification of mast cells in tissue sections.* ***Objectives:*** *To assess the staining effectiveness of Alcian Blue-Safranin O stain, toluidine blue, and H&E stains for identifying mast cells. To compare the efficacy ofalcian blue, toluidine blue, and H&E stains in identifying mast cells within inflammatory / pathological lesions.* ***Methods:*** *The sections were fixed with 50% ethanol for 5 minuteand stained with Safranin O for 1 minute, rinsed in distilled water counterstained with Alcian Blue and washed again with distilled water. Differentiation was carried out using 96% ethanol until the sections appeared pale blue, followed by treatment with 100% ethanol and then xylene for 1 minute each.* ***Result:*** *Staining with Alcian Blue and Safranin O effectively differentiated mast cell subtypes. Alcian Blue highlighted acidic mucopolysaccharides, while Safranin O marked heparin-rich granules. This combined approach enhanced visualization of mast cell heterogeneity and provided a reliable histochemical tool for assessing mast cell maturation in pathological specimen.* ***Conclusion:*** *The Alcian Blue–Safranin O staining technique provides a simple, cost-effective, and reliable method for the demonstration of mast cells. By exploiting the differential glycosaminoglycan composition of mast cell granules, this dual stain allows clear distinction between mucosal (Alcian Blue–positive) and connective tissue (Safranin O–positive) mast cells. Given its accessibility and reproducibility, Alcian Blue–Safranin O can be recommended as a valuable alternative to Toluidine Blue in routine histopathology as well as in research settings.*

Keywords: *Alcianblue-Safranin O stain, H&E stain, mature mast cells, metachromatic granules, Toluidine blue stain*

Introduction:

In 1879, Paul Ehrlich first discovered mast cells and named them "Mastzellan" (meaning well-fed cells). Mast cells are widely distributed throughout the body and play a crucial role in maintaining normal physiological functions, while also contributing to the pathogenesis of many diseases. In the oral cavity, most lesions such as periapical granulomas, periapical cysts, and fibrous inflammatory hyperplasia arise as inflammatory responses to microbial or chemical stimuli. The presence of mast cells in these lesions highlights their active role in inflammation. Mast cells—also known as tissue basophils, mastocytes, or labrocytes—originate from hematopoietic stem cells and are found in nearly all organs. (1) They are especially abundant at sites of host-environment interaction, such as the skin and intestinal mucosa, where they help maintain organ homeostasis against environmental changes.

These immune cells are rich in cytoplasmic granules and are commonly found in connective tissue and mucosal tissue, areas where bacteria typically colonize. Traditionally recognized for their role in IgE-mediated allergic reactions like anaphylaxis, mast cells are now also understood to function as part of the innate immune system, helping fight infections and initiate defensive immune responses. (2) Since mast cells are often difficult to identify with routine haematoxylin and eosin (H&E) staining, they are best demonstrated using special stains such as Toluidine Blue and Alcian Blue–Safranin 0, which highlight their granules inmaturation stages. (3)

Inclusion & Exclusion Criteria

A total of thirty paraffin-embedded tissue samples were obtained from the archival collection of the Department of Oral Pathology and Microbiology at Adhiparasakthi Dental College and Hospital. The University ethical committee approval was obtained. Peripheral reactive proliferations, inflammatory mucosal lesions, inflamed odontogenic cyst and allergic mucosal manifestations were included and Odontogenic neoplasms and benign tumor's without secondary inflammation were not included in the study.

Sample Grouping

- For the present study a total number of 30 samples were considered.

Group	N
I-Alcian blue – safranin O	10
II- Toludine blue	10
III- H & E	10

Materials

Fig:1 Archieval blocks, Microtome, Microscopic slides, Xylene

FIG:2 Iso prophyl alcohol, Alcian blue, Safranin O

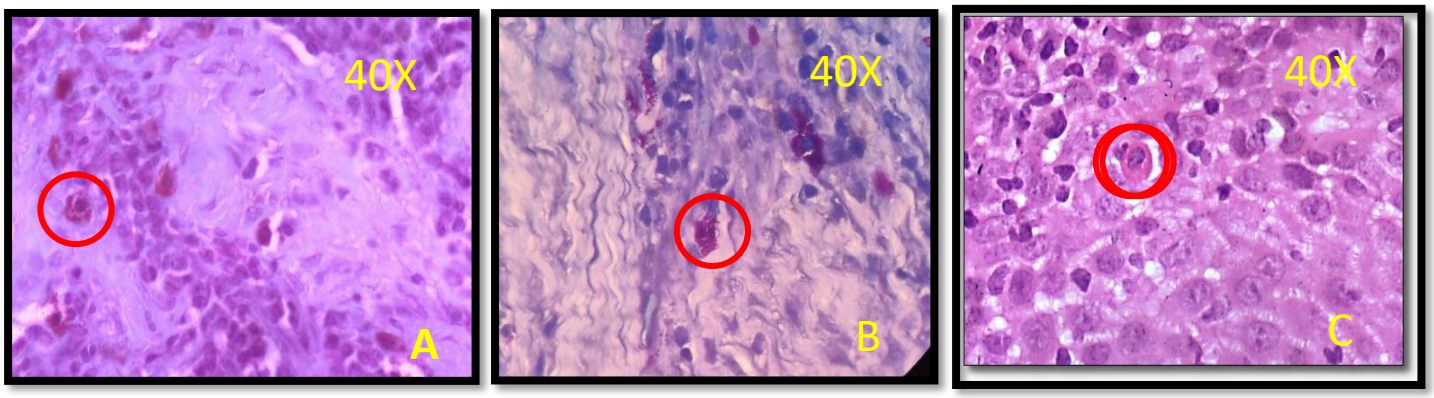
Methodology

Paraffin-embedded tissue sections were first fixed in 50% ethanol for 5 minutes. Subsequently, the sections were stained with Safranin(diluted 1:1 with 50% ethanol) for 1 minute, followed by rinsing in distilled water four times, each for 20 seconds. Thereafter, the sections were stained with Alcian Blue (1%, pH 2.5) for 3–5 minutes. The slides were then rinsed in distilled water for 1 minute and differentiated with 96% ethanol until a pale blue coloration was obtained. The sections were immersed in 100% ethanol for 1 minute, cleared with xylene for 1 minute, and finally mounted with a synthetic mounting medium and cover-slipped for microscopic examination.

Alcian Blue preparation (1%, pH 2.5)
Alcian Blue (C.I. 74240): 1 g Distilled Water: 97 ml Glacial Acetic Acid: 3 ml
Mix and agitate for 1 h, then filter. The solution is stable and usable for several years.

Safranin O preparation
Safranin (C.I. 75100): 1 g 95% Ethanol: 15.5 ml Distilled Water: 14.5 ml
Dilute the stock solution 1:1 with 50% ethanol immediately before use.

Histopathology Pictures Alcian Blue– Safranin O, Toludine Blue and Hematoxylin & Eosin



(A) Alcian blue safranin O stain showing mast cell (Reddish pink colour granules) (40x),
(B) Toluidine blue stain showing mast cells (Magenta pink granules) (40x),
(C) H & E stain showing mast cells (Pink granules) (40x)

Result

Out of 30 cases that were analysed, intensity of staining was observed and scored according to Allred scoring system for alcian blue- safranin O, toluidine blue, H & E stains in all the cases with the P value of 0.10 (table 2). The staining intensity was found to be intense in all the cases for Alcian blue – safranin O and toluidine blue stains.

Table 1: Represents the Descriptive Analysis of the Study Samples

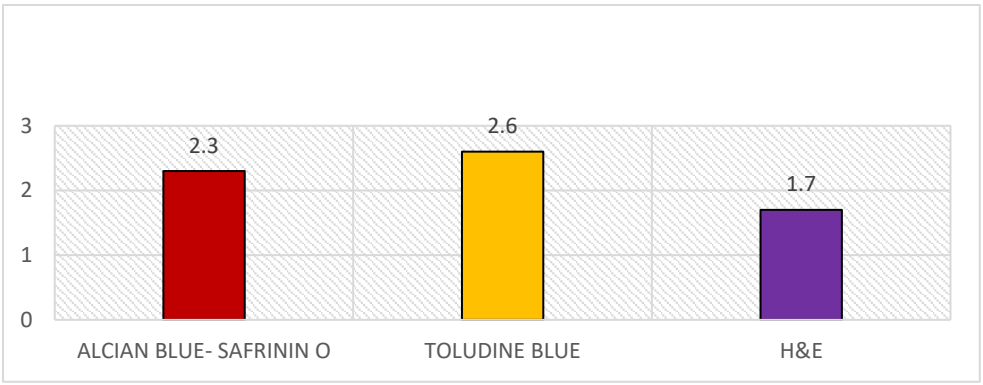
Study Group	Scoring	Frequency N=10	Percentage
Alcian Blue- Safrinin O	1	2	20.0
	2	3	30.0
	3	**5**	**50.0**
Toluidine Blue	1	1	10.0
	2	2	20.0
	3	**7**	**70.0**
H&E	0	2	20.0
	1	2	20.0
	2	3	30.0
	3	**3**	**30.0**
Based on Allred scoring system, staining intensity was evaluated			

Table 2 Represents the Intergroup Comparison between the Study Groups

Study Group	N	Mean	S. D	95% Confidence Interval		P Value
				Lower	Upper	
Alcian Blue-Safrinin O	10	2.30	.823	1.71	2.89	
Toludine Blue	10	2.60	.699	2.10	3.10	
H&E	10	1.70	1.160	.87	2.53	0.100

Toludine blue >Alcian Blue-safranin O >H and E

Graph 1: Mean comparison between Alcian blue – safranin O, Toluidine blue and H&E stains



Discussion

Mast cells, being central players in inflammation and immune regulation, require accurate histochemical demonstration for both research and diagnostic purposes. As an essential component of the defence mechanism in inflammation, mast cells release cytoplasmic granules that exhibit metachromasia with special stains. Owing to their secretory capacity, Singh et al (2016) as described mast cells as "unicellular endocrine glands." (4) Morphologically, mast cells measure 5–15 μm in diameter and appear ovoid, spindle-shaped, or tadpole-shaped in histological sections. Their cytoplasm contains numerous 0.2–0.5 μm granules, which are responsible for their staining characteristics. Although present in small numbers within connective tissue of most organs, they are particularly concentrated around blood vessels and nerves

in the dermis, highlighting their strategic localization at sites of host-environment interaction. (5)

Functionally, mast cells are highly interactive immune cells. They communicate with other immune system components, thereby amplifying inflammatory responses and contributing to processes such as bone resorption. (6) Importantly, mast cells have also been identified in both keratinized and non-keratinized odontogenic cysts, emphasizing their role in oral lesions. (7) Clinically, mast cells are now well-recognized as key players in the pathophysiology of chronic inflammation, allergic reactions, and anaphylaxis. Their ability to regulate both acute hypersensitivity responses and long-standing inflammatory events underscores their dual significance in health and disease. (8)

Historically, Alcian - Blue has been widely employed to identify acidic mucins. By incorporating pre-treatments such as sialidase digestion or pH modification, researchers were able to further differentiate sialidase-resistant sialomucins and sulphated mucins (9). In addition, Alcian Blue and Alcian Blue–Safranin O staining techniques have been successfully applied to polyacrylamide films containing various glycosaminoglycans. These studies revealed that the polyacrylamide framework acts as a protective "barrier" around substrate compounds, closely resembling the physiological microenvironment where complex protein molecules also form protective barriers (10).

Beyond connective tissues, Alcian Blue has demonstrated broader histological utility, staining not only mast cells, but also goblet cells in the intestine and Clara (Club) cells in the respiratory epithelium (1). This highlights its versatility in detecting mucin-rich granule-containing cells. In histopathology, Toluidine Blue and Alcian Blue remain the most frequently employed special stains for mast cells in fixed tissue sections and cell preparations. However, occasional reports have noted performance variability between these dyes, possibly linked to batch-to-batch differences in dye composition (11). Despite such limitations, Alcian Blue continues to serve as a reliable and valuable tool in mast cell histochemistry, particularly when combined with Safranin O for improved differentiation of cell maturity.

Safranin O is a cationic (positively charged) dye, soluble in water, and originally known for its wide applications in the textile industry (12). In histology, however, it has gained significant importance as a special stain for cartilage and connective tissue studies. When used in combination with Fast Green as a counterstain, Safranin O effectively demonstrates cartilage proteoglycans. Its staining mechanism is based on the principle that the negatively charged glycosaminoglycan (GAG) components of proteoglycans form stoichiometric ionic bonds with the positively charged dye molecules of Safranin O (13). This strong interaction provides a vivid contrast, enabling clear visualization of proteoglycan-rich structures in tissue sections.

Toluidine Blue (tolonium chloride) is a basic metachromatic dye with a strong affinity for acidic tissue components, particularly sulphates, carboxylates, and phosphate groups. This property allows it to selectively stain structures such as mast cell granules and mucopolysaccharides (14). Beyond routine histochemistry,

toluidine blue vital staining has played a landmark role in oral oncology. Since the 1960s, it has been employed as a chairside diagnostic adjunct for the detection of oral premalignant and malignant lesions. A pivotal meta-analysis (1989) assessing its diagnostic accuracy in detecting oral squamous cell carcinoma (SCC) reported a high sensitivity (93.5%–97.8%) and a moderate-to-high specificity (73.3%–92.9%) (15).

The combined use of Alcian Blue and Safranin O often simply referred to as the Safranin technique is a well-established method for the demonstration of mast cells. The strength of this dual-staining approach lies in its ability to simultaneously differentiate serosal (connective tissue) and mucosal mast cells within the same tissue section.

In this method, Safranin O selectively stains serosal mast cells (connective tissue type) in red to pink, reflecting their high content of highly sulphated glycosaminoglycans, primarily heparin. In contrast, Alcian Blue imparts a blue coloration to mucosal mast cells, which contain poorly sulphatedglycosaminoglycan's, particularly heparin precursors and chondroitin sulphate E. Thus, the Alcian Blue–Safranin O stain provides not only improved visualization but also functional insight into mast cell heterogeneity, allowing the pathologist to distinguish between mature connective tissue mast cells and immature mucosal mast cells with diagnostic precision (1).

The combined Alcian Blue–Safranin O staining technique is particularly valuable for distinguishing mature and immature mastocytes on the basis of their heparin distribution. In this system, mature connective tissue mast cells stain pink with Safranin O, reflecting their abundance of highly sulphated glycosaminoglycans (mainly heparin). Conversely, immature mast cells whether connective tissue or mucosal type stain blue with Alcian Blue, owing to the predominance of poorly sulphated glycosaminoglycans such as heparin precursors and chondroitin sulphate E.

As connective tissue mast cells undergo progressive maturation, their staining pattern gradually shifts. In the intermediate stage, some cytoplasmic granules stain pink with Safranin, while others retain the blue Alcian positivity, often giving the cytoplasm a violet hue or leaving a blue peripheral rim. This transitional staining highlights the dynamic biochemical changes occurring during mast cell maturation.In contrast, mucosal mast cells consistently remain Alcian Blue–positive at both immature and mature stages, indicating a more stable glycosaminoglycan profile. (1)

The presence of mast cells in inflammatory lesions suggests that they may play a role in the inflammatory process. Additionally, mast cells have been identified in certain precancerous and malignant tumors of the oral cavity.Mast cells in the latter lesions accelerate the disease progression (Ankle et al. 2007; Kheur et al. 2013). According to Mathiesen (1973), Mast cells are typically identified by staining their sulphated mucopolysaccharides and detecting the activity of trypsin-like enzymes in their granules. Due to their spindle to oval shape and staining characteristics similar

to haematoxylin and eosin (H&E), mast cells can be challenging to distinguish from fibroblasts (Ankle et al., 2007). Because mast cells resemble fibroblasts, they are difficult to distinguish using standard H&E staining (Singh et al).
Mast cells can be identified using particular stains like Alcian blue safranin O and toluidine blue. The most common application for toluidine blue, a straightforward metachromatic thiazine dye, is mast cell identification.The Alcian Blue-Safranin O stain can be used to identify mast cells containing both sulphated mucopolysaccharides (heparin) and carboxylated mucopolysaccharides (histamine), as well as sialomucin, within their cytoplasm. (16)
Mast cell granules will show blue from Alcian blue and red/orange from Safranin O when both stains are applied to a tissue sample, making mast cells easily visible,in the evaluation of the staining effectiveness of the two metachromatic dyes as reported by Ashish Shrestha et al. (2021). They also found thatthe mean mast cell count stained with Alcian Blue-Safranin O was higher across all groups compared to Toluidine Blue staining. (16)
Our study also showed almost similar staining intensity for both Alcian blue-safranin O and toluidine blue stain.

Conclusion:
Mast cells are pivotal players in both physiological regulation and pathological processes, acting as immune system gatekeepers that respond to diverse signalling pathways. Their activity is closely linked to inflammation, carcinogenesis, and tumor metastasis (16). The Alcian Blue–Safranin O staining technique has proven to be an effective histochemical method for the identification and differentiation of mast cells across various tissue types. This dual stain method takes advantage of the glycosaminoglycan composition of mast cell granules. Alcian Blue binds to carboxylated and sulphated acidic mucopolysaccharides, marking mucosal mast cells blue, whereas Safranin O selectively stains heparin-rich granules of connective tissue mast cells in red. Such contrasting coloration not only enhances mast cell visibility within dense tissue matrices but also provides valuable insight into their maturation status and functional heterogeneity.
Importantly, this method is simple, cost-effective, and equipment-friendly, making it highly suitable for routine laboratory use. Its diagnostic utility is especially relevant in studies of allergic reactions, chronic inflammation, and the tumor microenvironment. From my study results, it can be concluded that Alcian blue safranin O can be used as an alternative metachromatic stain for toluidine blue in identifying mast cells.

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