Innovations

The Csaba Stain's Power in Distinguishing Mature and Immature Mast Cells in Oral Inflammatory Lesions

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Abstract

Aim: *To evaluate and compare the staining efficacy of Csaba stain, Toluidine blue and H&E stains in the identification of MCs.* ***Objectives:*** *To assess the staining effectiveness of Csaba stain, toluidine blue, and H&E stains for identifying mast cells. To evaluate the efficacy of Csaba stain in differentiating mature from immature cells. To compare the effectiveness of Csaba stain, toluidine blue, and H&E stains in identifying mast cells within inflammatory lesions.* ***Methods:*** *A total of 30 tissue samples from retrieved tissue blocks of patients with oral inflammatory conditions were analysed, with 10 samples stained using the Csaba stain, 10 samples using toluidine blue and other 10 samples using H&E stains to examine mast cells. The staining effectiveness of the three methods were histologically analysed to categorize and compare the staining characteristics of mast cells.* ***Results:*** *The application of the Csaba stain revealed clear morphological differences between mature and immature mast cells. Mature mast cells displayed reddish pink colour granules, while immature mast cells showed blue coloured granules. Analysis indicated a distinct prevalence of mature mast cells in the samples stained with the Csaba stain, highlighting its effect in identifying mast cells.* ***Conclusion:*** *The Csaba stain proves to be an effective method for distinguishing between mature and immature mast cells in oral inflammatory conditions. This approach enhances our understanding of mast cell's role in inflammation and may guide future research.*

Keywords: *Csaba stain, H&E stain, mature mast cells, immature mast cells, metachromatic granules, Toluidine blue stain*

Introduction:

In histopathology, the standard method for examining tissue samples involves hematoxylin and eosin (H&E) staining, which helps visualize both normal structures and pathological changes.(1) Hematoxylin binds to acidic components like nuclei, coloring them blue, while eosin highlights basic elements such as cytoplasm, red blood cells, muscle fibers, nerve tissues, and bone in various shades of pink.(2)

Despite its widespread use, H&E staining sometimes fails to clearly distinguish between different tissue types, as they can appear similar, making diagnosis difficult. To address these limitations, pathologists use a variety of special stains designed to highlight specific tissue elements and assist in identifying the origin of the tissue. For example, Masson's Trichrome(3) and Van Gieson stains are useful for detecting collagen, Carbol chromotrope and Congo red stain help identify eosinophils, Von Kossa stain is used for bone tissue, and the Periodic Acid-Schiff (PAS) stain is effective for visualizing fungal organisms. Likewise, toluidine blue is a specialized stain used to detect mast cells, which are essential in immune defense.(4) These targeted staining techniques enhance diagnostic precision in complex cases.

Mast cells were first discovered in 1878 by Paul Ehrlich(5) while he was still a student of medicine. In his doctoral thesis, he described a group of connective tissue cells that stained positively with aniline dyes and contained cytoplasmic granules showing metachromasia. He named these cells "Mastzellen," a term that has since been widely adopted.

Mast cells (MCs), also referred to as mastocytes or labrocytes, are prominent connective tissue cells distributed widely across the body, especially in proximity to capillaries. They are characterized by a cytoplasm densely packed with basophilic granules, which frequently obscure the visibility of the nucleus under microscopic examination(6).Mast cells (MCs) are integral to immune system function and contribute to the regulation of hematopoietic stem cell production. They are derived from pluripotent progenitor cells in the bone marrow and undergo terminal differentiation within the specific tissue microenvironments in which they localize, a process modulated by stem cell factors. Unlike many other immune cells, mature mast cells are not typically found circulating in the bloodstream; rather, their precursors migrate from the bone marrow into peripheral tissues, where local cytokines and stem cell factors drive their maturation(7).Mast cell granules are rich in bioactive mediators such as heparin and histamine, rendering them pivotal in various physiological and pathological mechanisms. Their involvement has been implicated in conditions such as inflammation, oral lichen planus, oral squamous cell carcinoma, pyogenic granuloma, and periapical cysts(8).Mast cells are frequently elevated in neurofibromas and are considered a valuable histopathological marker for the diagnosis of this condition.

Mast Cells (MCs) as key effectors in immune system function and hematopoietic stem cell production, residing in tissues and playing roles in

inflammation and various disorders. Toluidine blue being a gold standard stain in identifying mast cells by staining their metachromatic granules(9), but the effectiveness of Csaba stain in distinguishing precursor mast cells into fully developed forms is not well understood.Distinguishing between mature and immature mast cells can offer significant diagnostic insights into previously undetected systemic disorders.

The current study is designed to assess and compare the staining effectiveness of Csaba stain, toluidine blue, and hematoxylin and eosin (H&E) in the detection of mast cells within inflammatory lesions. Additionally, it aims to evaluate the capability of Csaba stain in distinguishing between mature and immature mast cells.

Materials and Methods:

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 tumours without secondary inflammation were not included in the study.

The samples were categorized into three groups according to the staining method applied: Group 1 – Csaba stain (identifying mature and immature mast cells), Group 2 – Toluidine blue stain, and Group 3 – H&E stain, with 10 samples in each group. Three 4-µm sections were obtained from each tissue block, followed by deparaffinization, rehydration, and staining with hematoxylin and eosin (H&E), Toluidine blue, and Csaba stains. The standard protocol for H&E staining was adhered to. Fresh solutions for both Csaba and Toluidine blue stains were prepared immediately prior to staining.

Toluidine Blue Staining:

The deparaffinized sections were subjected to two changes of xylene, each lasting 10 minutes, followed by hydration with 70% and 60% alcohol for 5 minutes each. After rinsing the sections in running tap water for 5 minutes, they were immersed in freshly prepared Toluidine blue solution for a duration of 2 to 5 minutes. The slides were then washed with water and dehydrated through two changes of 95% alcohol. Subsequently, the sections were blot-dried, cleared in xylene, and mounted using DPX.

Csaba Staining:

Deparaffinized sections was brought to water via xylene and ethanol.The sections were then immersed in the freshly prepared Csaba stain solution for 10-20 minutes.At last rinsed with water, dehydrated with tertiary butanol, cleard with xylene and mounted using a resinous medium (DPX).

Csaba Stain- Preparation (100ml)	
Alcian blue	0.36g
Safranin	0.18g
Ferric ammonium sulfate	0.48g
Walpole's acetate HCl buffer	100ml

The effectiveness of Csaba stain, Toluidine blue, and H&E stains was assessed in terms of their ability to identify mast cells. Each stain was also assessed for their ability to differentiate mature and immature mast cells. The intensity of staining was evaluated using Allred scoring system. The staining intensity assessed by selecting 10 random fields with positive staining and scored as follows:

Score	Intensity
0	No stain
1	Mild stain
2	Moderate stain
3	Intense stain

Armamentarium:

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

Fig: 2 Alcian blue, Safranine, Ammonium Ferric sulphate, Sodium acetate anhydrous, Hydrochloric acid

Statistical Analysis:

The collected data were recorded in a Microsoft Excel 2019 spreadsheet for statistical analysis. The samples were then analyzed using SPSS Statistics software, version 26.0. Multiple group comparison was performed by ANOVA test to depict the difference in the variables with statistical significant difference p value kept as equal toor less than 0.05 as statistical difference in the study.

Results:

Among the thirty cases analyzed, the staining intensity was assessed and scored based on the Allred scoring system for Csaba, Toluidine blue, and H&E stains across all cases [Figure 3, Table 1], yielding a P-value of 0.003 [Table 2]. The staining intensity was found to be prominent in all cases for Csaba, Toluidine blue, and H&E stained sections, indicating that all three stains are equally effective in enhancing granule visualization.

The Csaba stain exhibited a distinct granular staining property, enabling differentiation of mature and immature mast cell granules by imparting varying colors. This was clearly observed and scored for all cases [Figure 3, Table 3], with a P-value of 0.173 [Table 4]. No such distinction was observed in the Toluidine blue or H&E stained sections.

Table 1: Represents the Descriptive Statistics among the Study Samples

Study Samples	Scoring	Frequency	Percentage
Csaba	0	1	10.0
	1	1	10.0
	2	2	20.0
	3	6	60.0
Toluidine Blue	0	-	-
	1	-	-
	2	2	20.0
	3	8	80.0
H & E	0	2	20.0
	1	2	20.0
	2	6	60.0
	3	-	-

Table 2 Represents the Intergoup Comparison between the Study Samples

Study Samples	N	Mean	S.D	95% Confidence Interval		Sig
				Lower	Upper	
Csaba	10	2.30	1.059	1.54	3.06	**.003**
Toluidine Blue	10	2.80	.422	2.50	3.10	
H & E	10	1.40	.843	.80	2.00	

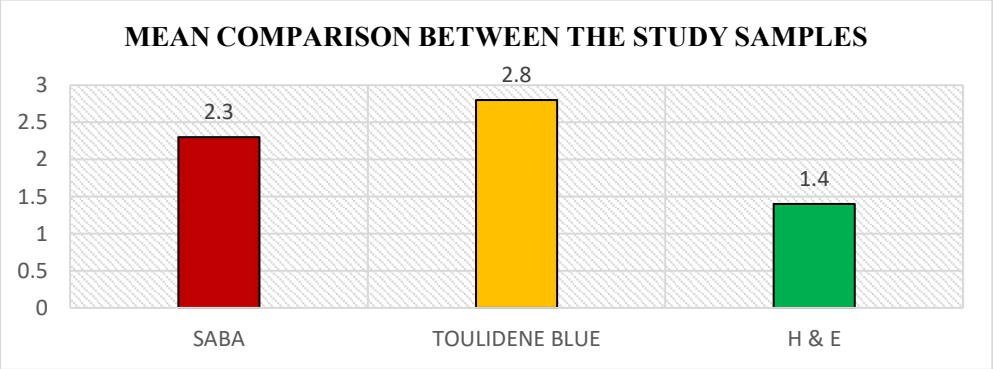
Table 3. Represents the Descriptive Statistics in Csaba Stain Study Samples

Study Samples	Scoring	Frequency	Percentage
Mature	0	4	13.3
	1	1	3.3
	2	1	3.3
	3	4	13.3
Immature	0	1	3.3
	1	1	3.3
	2	2	6.7
	3	6	20.0

Table 4. Represents the Descriptive Statistics in Csaba Stain Study Samples

Study Samples	N	Mean	S.D	95% Confidence Interval		Sig
				Lower	Upper	
Mature	10	1.50	1.434	-1.984	.384	0.173
Immature	10	2.30	1.059			

Graph: 1- Mean comparison between Csaba, Toluidine blue and H&E stains



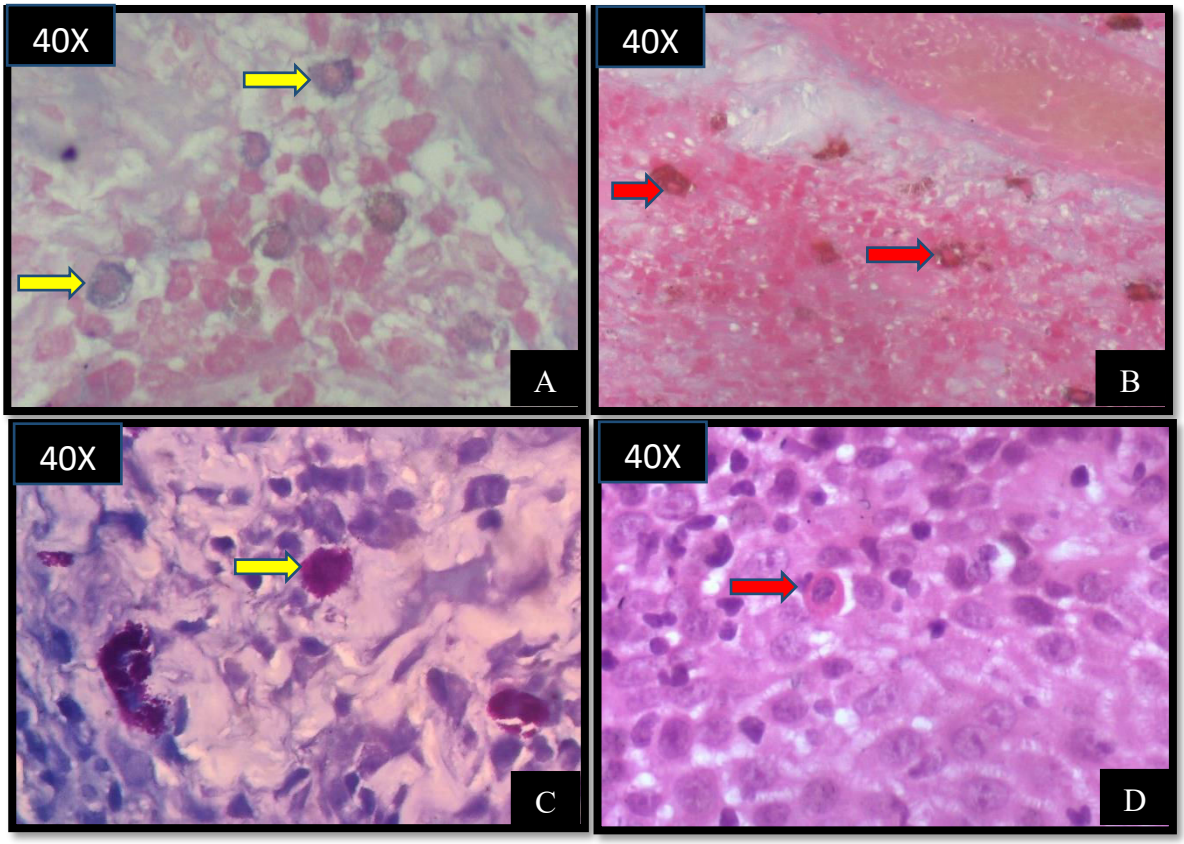


Figure: 3- Csaba staining, Toluidine blue staining and H&E staining for mast cells. (A) Csaba stain showing Immature mast cells (Yellow arrow) (Bluish granules) (40x), (B) Csaba stain showing mature mast cells (Red arrow) (Reddish brown granules) (40x), (C) Toluidine blue stain showing mast cells (Yellow arrow) (Magenta pink granules) (40x), (D) H&E stain showing mast cells (Red arrow) (Pinkish granules) (40x)

Discussion:

This research aimed to compare the staining efficiency of Csaba, H&E and Toluidine blue in detecting mast cells within common inflammatory oral lesions like peripheral reactive proliferations, inflammatory mucosal lesions, inflamed odontogenic cyst, allergic mucosal manifestations. Key features evaluated including stain intensity, granule visibility and specificity to differentiate mature from immature mast cells. While Toluidine blue is the standard(10), Csaba and H&E stains—both simple to prepare in routine histopathology labs—remain underexplored. Findings revealed that all three stains provided comparable results across the evaluated parameters, highlighting their equal effectiveness in clearly identifying mast cell granules with consistent uptake.

Mast cells function as primary effectors in the innate immunity, predominantly recognized for their function in initiating the inflammatory cascade. When IgE on the mast cell surface binds to an external antigen and upon Fc receptor crosslinking, the cell undergoes degranulation, releasing mediators such as histamine, tryptase, chymase, and TNF-α. Histamine promotes leukocyte chemotaxis, bronchoconstriction, and vascular permeability. Following

degranulation, mast cells synthesize and release lipid mediators like prostaglandins and leukotrienes, along with cytokines that amplify inflammation and recruit immune cells. Beyond defense, mast cells contribute to tissue repair and angiogenesis. Independently of IgE, they secrete procoagulant factors, inflammatory lipid mediators and platelet-stimulating factor upon injury. Heparin, tryptase, and t-PA regulate blood flow, enhancing delivery of nutrients and immune cells. Additionally, mast cells promote fibroblast and endothelial cell activity through VEGF, FGF-2, and other cytokines, supporting wound healing, angiogenesis, and even nerve regeneration(11).

In human bone marrow, mast cells can be classified into four distinct morphological types according to their stages of maturation. These include the tryptase-positive nongranulated blast cells, metachromatic blast cells, and the atypical mast cell type II, also known as promastocytes, Mature MC(12). The immature forms of Mast cells are primarily observed in mast cell leukemia, as well as in acute and chronic myeloid leukemias and other myelodysplastic conditions. Immature mast cells stain blue, while mature mast cells take on a red coloration. Variations in sulfated proteoglycan content lead to differences in mast cell staining with Csaba stain, ranging from red to blue or a combination of both colors. Cells that are Alcianophilic appear blue, indicating immaturity, whereas those that are Safraninophilic stain red, signifying maturity.

Pathologically, mast cell degranulation leads to type I hypersensitivity reactions like asthma, urticaria, and anaphylaxis. Mastocytosis, caused by KIT mutations, results in mast cell overgrowth with symptoms such as flushing and GI upset(13). Mast cells are identified by metachromatic staining due to acidic granules; dyes like toluidine blue stain them red-violet, while standard H&E stains are less specific.

Tan et al. (2004) (14)have demonstrated MC in various stages of hemangioma using Csaba stain. Surendra Lakshminarayana et al. (2022)(12)demonstrated the use of Csaba stain to distinguish mature from immature MCs. Our study seems to be one among the few studies conducted to assess the effectiveness of Csaba stain in the identification of MCs.

Conclusion:

With my study results, it can be concluded that Csaba, Toluidine blue and H&E stains effectively detect mast cells in inflammatory lesions, eventhough toluidine blue stand as a gold standard stain. The novelty of this study is highlighted by its ability to show distinct staining patterns of mature and immature mast cells using Csaba stain, which aids in differentiating the two types of mast cells.

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Conflicts of interest

No conflicts of interest exist.

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