ABSTRACT

**Background:** Paul Ehrlich first described mast cells in 1878 based on their unique staining characteristics and large granules and are considered part of the immune system. When triggered by locally produced cytokines or bacterial products the cells can release a number of pre stored mediators. They are characterized by the presence of densely packed cytoplasm with bright red granules that stain metachromatically with Toluidine blue, Azure A, Bismarch brown and Thionin. **Aim & Objective:** The present study was conducted to determine the distribution of mast cells and correlate their presence with the state of vascularity and inflammation with the metachromatic dye- 1% Toluidine blue. **Results:** The distribution was predominantly mild in most of the cases (80.95% cases) and moderate/severe in 19.05% cases. The distribution of vascularity was found to be mild in 12.6% cases, whereas in rest of the 87.30% cases, the vascularity was found to be increased. The concentration of inflammatory cells was moderate to severe in 49 cases (77.77%), while only 14 cases (22.22%) showed mild inflammatory changes. **Conclusion:** The correlation between inflammation and mast cell distribution was thus, found to be inversely related whereas there was direct correlation between overall vascularity and inflammation in all the samples taken.
KEYWORDS: Paul Ehrlich, metachromatically, Toluidine blue, Azure A, Bismarch brown and Thionin.

INTRODUCTION
A mast cell (MC) is a resident cell of areolar connective tissue and is distributed preferentially about the microvascular bed in oral mucosa. They contain many granules rich in histamine and heparin. Paul Ehrlich first described mast cells in 1878 based on their unique staining characteristics and large granules and are considered part of the immune system.[1] In addition to IgE and antigen, anaphylatoxins, cytokines, hormones and neuropeptides can trigger mast cell activation, leading to degranulation and secretion of preformed, granule stored mediators.[2,3] MCs arise from bone marrow-derived precursors circulating in the blood and become differentiated after entering tissues. They are long-lived cells, able to survive for months or years. Despite being terminally differentiated, they can proliferate in response to appropriate signals. All mature mast cells have a common fundamental morphology with prominent electron dense granules in their cytoplasm.[4] MCs have a diameter of about 12 microns, heterogenous in shape and are packed with granules and a life span of weeks to months.[5] MCs play important role in mucosal inflammation, host defense and tissue repair. When triggered by locally produced cytokines or bacterial products for e.g., lipopolysaccharides, the cells can release a number of prestored mediators. They are characterized by oval to round nuclei and densely packed cytoplasm consisting of bright red granules.[6] These granules stain metachromatically with Toluidine blue, Azure A, Bismarch brown and Thionin.[5]

Mast cell densities or count have been studied in various lesions ranging from common inflammatory lesions to neoplasms. Some of the oral reactive lesions that are commonly encountered in the dental practice are pyogenic granuloma, inflammatory hyperplasia and granulations tissue. The present study was conducted to determine the distribution of mast cells and correlate their presence with the state of vascularity and inflammation with the metachromatic dye- 1% Toluidine blue.

MATERIAL & METHOD
A total of 63 cases were taken for the study out of which 21 cases each of inflammatory hyperplasia, granulation tissue and pyogenic granuloma. Two serial sections of 5 microns thickness of each of these cases were made and stained with H &E (Fig 1 & Fig 3) and 1% Toluidine blue (TB) solution (Fig 2, 4 & 5). The total number of mast cells was counted.
throughout the TB stained sections in 10 representative and consecutive grid fields using an oculometer grid under a magnification of X40. The observations were subjected to statistically analyzed & graphically represented. The parameters for counting were formulated as accordingly:

Mean MCs count per field in each case:
- 1-5 (Mild cases)
- 5 (Moderate / Severe cases).

Fig 1: Photomicrograph showing H & E stained section of inflammatory hyperplasia.

Fig 2: Photomicrograph showing mast cells in stained with 1% Toluidine blue in inflammatory hyperplasia.
Fig 3: Photomicrograph showing H & E stained section of pyogenic granuloma.

Fig 4: Photomicrograph showing mast cells in stained with 1% Toluidine blue in pyogenic granuloma.

Fig 5: Photomicrograph showing mast cells in stained with 1% Toluidine blue in granulation tissue.
RESULTS
The present study showed a constant mast cell distribution in all the 63 cases of inflammatory lesions and the correlation of the vascularity and inflammation was found statistically significant with that of the mast cell distribution.

In inflammatory hyperplasia, mast cells distribution was seen to be mild in 85.7% (18) cases and moderate/severe in 14.28% (3) cases. In 19.04% (4) cases vascularity was seen to be mild and in 80.95% (17) cases it was found to be moderate/severe. The inflammatory reaction was seen to be mild in 23.8% (5) cases and 76.19% (16) cases it was moderate/severe. [Graph 1].

Graph 1: Distribution of mast cells, vascularity & inflammation in inflammatory hyperplasia.

In granulation tissue, mast cells were seen to be mild in 85.7% (18) cases and moderate/severe in 14.28% (3) cases. In 9.52% (2) cases vascularity was found to be mild and in 90.47% (19) cases it was found to be moderate/severe. The inflammatory reaction was seen to be mild in 14.28% (3) cases and 85.7% (18) cases it was moderate/severe. [Graph 2]

Graph 2: Distribution of mast cells, vascularity & inflammation in granulation tissue
In pyogenic granuloma, mast cells were seen to be mild in 71.42% (15) cases and moderate/severe in 28.57% (6) cases. In 9.52% (2) cases vascularity was seen to be mild and in 90.47% (19) cases it was found to be moderate/severe. The inflammatory reaction was seen to be mild in 28.5% (6) cases and 71.4% (15) cases it was moderate/severe. [Graph 3].

![Graph 3: Distribution of mast cells, vascularity & inflammation in pyogenic granuloma](image)

The distribution was predominantly mild in most of the cases (51 out of 63; 80.95% cases) and moderate/severe in only 12 out of 63 cases (19.05%) as seen in Graph 4. The distribution of vascularity was found to be mild in only 8 out of 63 cases (12.6%), whereas in rest of the 55 cases (87.30%), the vascularity was found to be increased [Graph 5]. This is in contrast to the distribution of mast cells, which is noticed, in a milder form in most number of cases. Similarly, the concentration of inflammatory cells was moderate to severe in 49 cases (77.77%), while only 14 cases (22.22%) showed mild inflammatory changes [Graph 6]. The correlation between inflammation and mast cell distribution was thus, found to be inversely related whereas there was direct correlation between overall vascularity and inflammation in all the samples taken.

Thus, the role of mast cells is well explained, once the neo-vascularization and inflammation has already set in. The continued presence of mast cells in inflammatory hyperplasia showed marked inflammatory changes which might indicate the continuous process of inflammation.
DISCUSSION

Mast cells derive from a distinct precursor in the bone marrow and mature under the influence of stem cell factor and various cytokines. Depending on their location or stage of maturation, mast cells express different amounts of surface antigens, some of which are involved in activation and others in cell recognition. Mast cells also express numerous chemokine receptors that do not induce degranulation but could render them susceptible to
human immunodeficiency virus infection.[7] Mast cells can secrete mediators without overt degranulation, through differential or selective release this process is probably regulated by the action of distinct protein kinases on a unique phosphoprotein.[8] There are numerous literature that has proposed the role of mast cells in the pathogenesis of various pathological conditions and development of inflammation in oral mucosa and dental pulp.[3]

The discovery that mast cells produce and release variety of multifunctional cytokines, points to new mechanisms by which mast cells might significantly influence either IgE-dependent or IgE-independent responses, extending their potential functions from pro-inflammatory effector cells to regulatory components of the immune system, thus contributing to development and amplification of specific and non-specific inflammatory responses.[9]

The mast cell helps in the initiation of neo-vascularization and further in the inflammatory reactions. Degranulation of mast cell activates endothelium through TNF dependent mechanism which may be critical to the elicitation phase of inflammation.[10]

The findings in our study were in stark contrast to those reported earlier which showed a direct relation between the number of mast cells, vascularity and inflammation.[3,11] In cases of moderate/severe vascularity, the distribution of mast cells were predominantly low as shown in our study [Graphs 4 & 5]. Similarly, in cases of established inflammation, the mast cell distribution was only mild in most of the cases [Graphs 4 & 6]. This contrast in the function of mast cells could be attributed to their degranulation that would have occurred much before the stimulation of angiogenesis and subsequent inflammatory reaction. It has been reported in earlier literature that mast cells have got variable mediators within their granules. According to Kamal R et al. (2011) there was an increase in average MC count observed in pyogenic granuloma as compared to normal oral mucosa.[12] According to Spoorthi BR et al. (2013), the average no of mast cells were shown to be increased in inflammatory hyperplasia followed by pyogenic granuloma and periapical granuloma.[13] de Oliveira Rodini C et al. (2004) found in their study that an increase in average MC count was present in periapical inflammatory lesions. They proposed that mast cells may lead to inflammatory changes by release of their chemical mediators.[9] As stated by Krishnaswamy et al. (2001) degranulation of mast cells releases preformed granules containing mediators such as histamine, tumor necrosis factor, serotonin and numerous proteases responsible for most of the mast cell dependent functional responses.[14] Therefore, determining whether mast cell is intact or degranulated may be a good indicator to assess whether mast cells are
involved in a particular biological process. Thus, in this study, toluidine blue stain was selected to demonstrate intact mast cells and their granules as well as degranulation of mast cells.

Once activated, mast cells secrete numerous vasoactive and proinflammatory mediators. These include pre-formed molecules such as histamine, serotonin, TNF, kinins and proteases stored in secretory granules. Leukotrienes, prostaglandins and platelet activated factor are synthesized during mast cell activation from arachidonic acid liberated by the action of phospholipases. In addition, a number of cytokines (e.g. IL-1, 2, 5, 6, 8, 9, 13, and TNF) and vascular endothelial growth factor (VEGF) are synthesized de novo and released several hours after stimulation.\(^{[15]}\)

Once the mast cells were stimulated, they degranulate and bring about the required biological action. So it is only reasonable to presume that the predominance of mast cell under degranulation would take place in a stage much before the active stimulation of angiogenesis and subsequent inflammatory reaction. This early stage could be defined as a pre-inflammatory stage before the active vascularity and inflammatory cells are increased. Thus, there is a clear reduction in the number of mast cells in most of the well established inflammatory lesions in our study. Hence, it is evident from our study that there was an inverse relation between the distribution of mast cells, vascularity and inflammation. The present study demonstrated showed an inverse relationship existing between vascularization and inflammation and it can thus, be proposed that a pro-inflammatory stage might exist, during which the active role of mast cell begin and once the inflammation and vascularization has established, the role of mast cells gradually diminish showing an inverse relationship.

**CONCLUSION**

Mast cells serve a critical role in the development of inflammation in the oral mucosa, during the early vaso-inductive events and during transformation from acute to chronic inflammation. These cells are ideally poised to serve as "gatekeepers" of the microvasculature in the oral cavity due to the unique characteristics. An appreciation of the multiple interactions among mast cells, endothelial cells, nerves, and other cells of the immune system provide a basis for therapies for targeting mast cell responses. In the future, it may be possible to develop novel approaches that influence the release of pro-inflammatory molecules or neuropeptides during the pre-inflammatory period to ameliorate mast-cell-driven inflammation in the oral mucosa and dental pulp.
REFERENCES


