JOURNEY OF MAST CELL FROM PRENEOPLASTIC LESION TO NEOPLASTIC LESION USING MAY GRUNWALD GIEMSA STAIN AND TOLUIDINE BLUE STAIN

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ABSTRACT

Introduction: Mast cells are regarded as complex and multifunctional cells, playing a significant role in immunopathology and substantial role in tumor angiogenesis. They are local resident of connective tissue and considered as proinflammatory and produce mitogenic cytokines. These functions may play a significant role in pathogenesis of preneoplastic lesion like Oral lichen planus (OLP) and neoplastic lesion like Oral squamous cell carcinoma (OSCC).**Aim:** The aim of this study to the number of mast cell in Oral lichen planus and Oral squamous cell carcinoma using toluidine blue and May Grunwald Giemsa stain (MGG). **Material and Method:** 20 cases of OSCC, 20 cases of OLP and 10 cases of normal mucosa were studied for mast cell number using toludine blue and May Grunwald Giemsa stain.**Result**: Showed increased number of mast cell seen in OSCC and OLP in comparison to normal mucosa. Overall not much significant result was seen between toludine blue and MGG stain.

KEYWORDS:- Mast Cells, Mast cell Count, May Grunwald Giemsa (MGG), Normal mucosa, Oral Lichen Planus(OLP), Oral Squamous Cell carcinoma, Toluidine Blue,

INTRODUCTION

Mast cells are cells of hematopoietic origin which was discovered by Paul Ehrlich in 1977. It is granular cell of loose connective tissue and named it as "MASTZELLAN"- a well fed cell. **[1]** They are large mononuclear cells containing in them cytoplasmic granules that exhibit chemical and histochemical characteristic of acid mucopolysaccharides. Depending on their density of surrounding tissue they may assume various shapes. They may be flat, spherical and spindle shaped, stellate or almost filiform. Plump cell have diameter of 8 to 15um. In extracellular matrix a distinct cell membrane is 50-60A˚thick is noted; in addition; finger like protrusion of length of 0.3to 0.7µ. The cytoplasmic granules (10.6µ) shows fine granular and a lamellar structure. There are mitochondria, golgi zone and endoplasmic reticulum. [**2]**

They exert their influence locally and systematically by releasing a variety of potent mediators like histamine, leukotrienes and cytokines through degranulation and causes neovascularisation by producing angiogenic mediators such as FGF, TGF-beta, TNF and VEGF. They occur in various pathological state and also in benign and malignant tumor**. [3], [4], [5]** The commonly occurring oral

disease like oral leukoplakia, OSMF, OLP, OSCC have chronic inflammation in common. In addition, autoimmunity is strongly associated with OLP and angiogenesis is associated with proliferation of carcinoma.[**6**]So abnormal mast cell count is of clinical relevance and also the staining method used for evaluation of mast cell may also contribute to certain type of variation.

Therefore our present study was carried to compare the number of mast cell obtained by these two stains in case of OLP and OSCC. These stain were selected because toluidine blue is reported to be the stain of choice for mast cell identification and MGG stain is also widely used, although less in practice and often used by pathologist to stain mast cell tumors and takes almost equal time for staining and can be used as alternative method for identification of mast cell.

MATERIAL AND METHOD

20 paraffin embedded specimens of OSCC& OLP were retrived from the archives of the department of oral pathology. 10 normal oral mucosal biopsies were obtained from adult patients undergoing extraction for orthodontic treatment. Two section of 5 micron thickness each were cut ; one was stained with 1% toludine blue and the other section was stained with MGG stain for mast cells.

Mast cells were counted under light microscope at a magnification of 400x in a *Z*-pattern from left to right and the data obtained was statistically analyzed using t test. (Figure 1and 2)

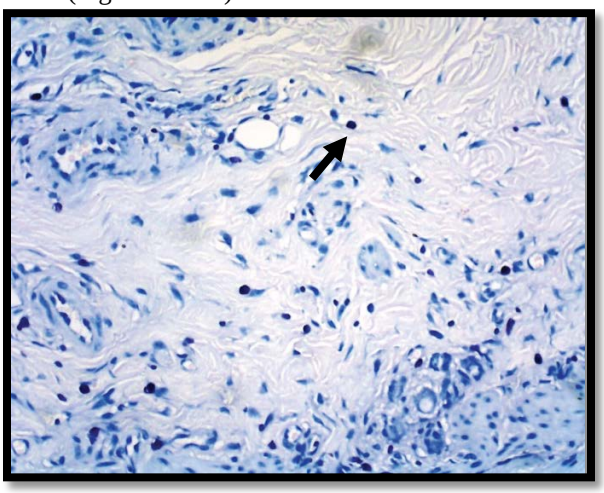

Figure 1: Microphotography showing mast cell stained with May Grunwald Giemsa Stain

CRITERIA TO IDENTIFY THE MAST CELLS

Mast cells are spindle to oval shaped. Mast cell granules are purplish red and nuclei appears sky blue in color when stained with 1% toludine blue and mast cell granules are dark purplish with sky blue nuclei when stained with May Grunwald Giemsa stains

May Grunwald Giemsa stains &1% toludine blue mast cell granules metachromatically due to its reaction with sulphated mucopolysaccharides.

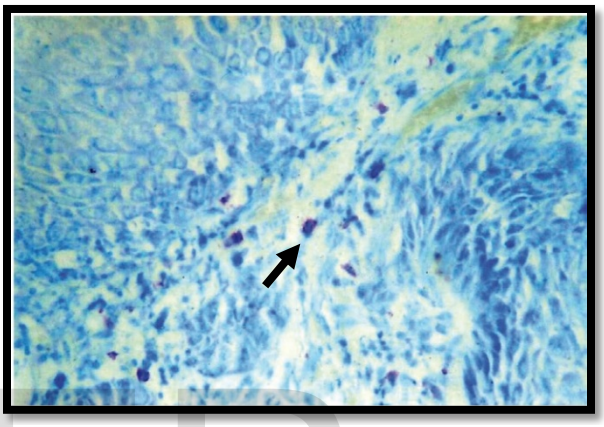

Figure 2: Microphotography showing mast cell stained with 1% Toluidine blue Stain

RESULTS

Overall No statistical difference (P = 0.144) was found in OSCC , OLP and Normal mucosa when toludine blue and MGG stains were compared. (Table 1, Figure 3)

A Significant increase in number of mast cell was observed with toludine blue when compared with MGG stain in case of Oral lichen planus . P value >0.05. (Table 2)

A slightly higher number of mast cell were observed with toludine blue when compared with MGG stain in case of Oral Squamous Cell Carcinoma and normal mucosa . P value statistically not significant (table 3, 4)

TABLE 1- Comparison between toludine blue and May Grunwald Giemsa stain in oral squamous cell carcinoma, oral lichen planus and normal mucosa.

Group 2	N	Mean	Std. Deviation	t-value	Mann whitey	P value
Toludine blue	20	40.6667	45.02515	0.98	548	0.053
MGG	20	29.7667	40.8392			

TABLE 2- Comparison between Toludine blue and May Grunwald Giemsa stain in oral lichen planus

Group 2	N	Mean	Std. Deviation	t-value	Mann whitney	p-value
TOLUDINE BLUE	20	61.80	36.18	2.52	548	0.016
MGG	20	42.63	31.73			

TABLE 3- Comparison between Toludine blue and May Grunwald Giemsa stain in oral squamous cell carcinoma .

Group 2	N	Mean	Std. Deviation	t-value	Mann whitey	p-value
Toludine Blue	10	0.80	1.13	1.47	376.5	0.147
MGG	10	0.43	0.77			

Group 2	N	Mean	Std. Deviation	t-value	Mann witney	p-value
Toludine blue	50	37.16	41.95	2.03	418	0.144
MGG	50	26.11	34.63			

TABLE 4- Comparison between Toludine blue and May Grunwald Giemsa stain in normal oral mucosa

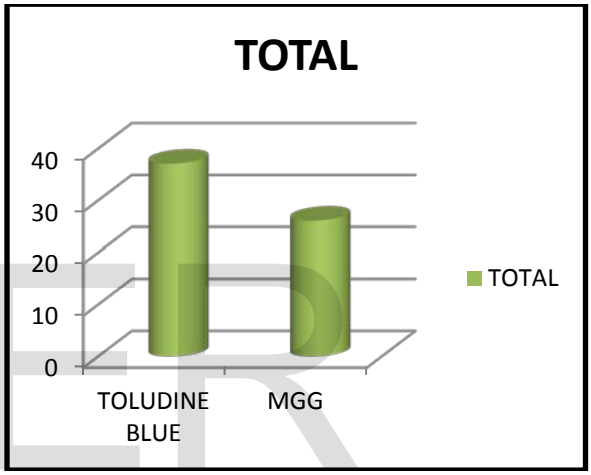

Figure 3- Graphically overall result between toludine blue and May Grunwald Giemsa stain in oral squamous cell carcinoma, oral lichen planus and normal mucosa

DISCUSSION

Paul Ehrlich in 1877 discovered a granular cell of loose connective tissue and named it as "Mastzellen"—a well fed cell. Studies on mast cells in normal and various pathologic conditions have shown them to be complex, well-engineered, and multifunctional cells playing an essential role in acquired and innate immunity**. [7]**

They take origin from multipotent CD 34+ precursor in the bone marrow, later circulate in the peripheral blood as agranular monocytic cell, and then migrate into tissues, assuming their typical granular morphology from their immature state. They are normally distributed throughout the connective tissue, adjacent to blood or lymphatic vessels, and near or within peripheral nerves. They are numerous

especially beneath the epithelial surfaces of the skin, in the respiratory system, gastrointestinal and genitourinary tracts. [**8], [9]**

Mast cell are known as unicellular endocrine glands, since on discharge of mast cell granules, a number of mediators are released which include heparin, histamines and serotonin, which have major physiological and pharmacological significance.[**10]**

Mast cells are connective tissue cells with basophilic metachromatic granules in its cytoplasm, which contain a wide array of mediators with diverse functions. **[11]** They have been studied in normal gingiva, chronic inflammatory gingivitis, desquamative gingivitis, lichen planus, oral submucous fibrosis (OSMF), and OSCC. They exhibit phenotypic plasticity and variation in the mast cell mediators with the change in the microenvironment. Volumes of literature speak about their role in the inflammatory reactions and neovascularization. In some malignancies, large numbers of mast cells were detected before the occurrence of neovascularization.[**12]**

Mast cells were first recognized by virtue of Metachromasia in 1877. The compounds responsible for metachromasia are histamines and heparins, heteroglycan rich in half – sulfate esters. Metachromasia reaction can be defined as the capacity for a stain to give a different color to selected tissue components. This phenomenon is attributable to change in absorption peak with different stain concentration.**[13]** Mast cell granules are stain metachromatically with Toluidine blue, May Grunewald Giemsa, Azure A, Bismark Brown, Alcian Blue and thionin.[**14]**

The result obtained are similarly to the studies carried out by leclere et al**[15]** as they concluded that mast cell granules are metachromatically stain with toluidine blue , May Grunwald Giemsa stain rather than Fast Romanosky stain .

Increased numbers of mast cell are seen in OLP and OSCC in comparsion to normal mucosa.
A predominance of connective tissue mast cells has been found in oral lichen planus by various investigators who suggested that mast cells could be involved in the pathogenesis of lichen planus. Mast cell products like histamine have been suggested to bring about structural changes in the epithelium and connective tissue in lesions of lichen planus and the close association of these cells with the T-lymphocytes has added impetus to the concept that these cells could be responsible for the chronicity of this lesion. **[16]**

In case of OSCC many investigators have suggested that heparin from the mast cells cause vasoproliferation and increases the half-life of basic fibroblastic growth factor (bFGF) which is a potent angiogenic substance, thereby promoting tumour angiogenesis and facilitating local tumour invasion. Interleukin-1 leads to epithelial proliferation leading to exophytic growth of lesion. Heparin causes increased vascularity of the stroma leading to tumor angiogenesis. Various investigators have studied the contribution of mast cells to neo-angiogenesis during tumorigenesis in OSCC**. [17]**
Thus through the present study one fact is proven that mast cell population is increased in case of oral lesion than in normal condition. The reason behind is that, the chemical mediators released by the mast cell granules which are known to vary with variation in microenvironment in various disease. Secondly, Toluidine blue is the stain of choice for mast cell identification but significant result was not appreciated between the two stains. Henceforth May-Gru¨nwald Giemsa stain can be a alternative for identification of mast cells.

CONCLUSION

More specific and newer staining techniques for the demonstration of mast cells and their mediators like immunohistochemical staining and enzyme histochemical procedures may provide a better insight into the role of these immunocompetent cells in the pathogenesis of potentially malignant and malignant oral lesions.

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