COMPARISON OF MAST CELL COUNT AND MAST CELL DENSITY IN NORMAL MUCOSA, ORAL LEUKOPLAKIA, ORAL LICHEN PLANUS, ORAL SUBMUCOUS FIBROSIS AND ORAL SQUAMOUS CELL CARCINOMA – A STUDY ON 50 CASES

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ABSTRACT

The present study was carried out for quantitative analysis of the mean MCC/optical field and also MCD/sq. mm in oral submucous fibrosis (OSMF), oral lichen planus and oral leukoplakia (OL). The study was carried out in the Department of Oral Pathology and Microbiology, Jaipur Dental College, Jaipur. Histologically confirmed 10 cases each of OSMF, OL, OLP and OSCC were selected. The sections were obtained and stained with toluidine blue. The number of mast cells/optical field and mast cell density/sq. mm were assessed.

An assessment of the role of mast cells in the etiopathogenesis of oral submucous fibrosis, oral lichen planus and oral leukoplakia and oral squamous cell carcinoma was reviewed. The following conclusions were drawn from our study. An increase in both mean MCC/optical field and MCD/sq. mm in OSMF, OL, OLP and OSCC was selected. This increase in the values was statistically significant when compared with that of normal buccal mucosa which constituted the control group. An increase in both mean MCC/optical field and MCD/sq. mm was found in precancerous condition & lesion and also in potentially malignant and malignant oral lesions. This increase in the values was statistically significant when compared with that of normal buccal mucosa which constituted the controls.

KEYWORDS: Mast cells, Mast cell count, Mast cell density, Oral submucous fibrosis, Oral leucoplakia, Oral lichen planus, oral squamous cell carcinoma

INTRODUCTION

It has been more than a century since the discovery of the mast cell by the genius and tenacity of Paul Ehrlich 1, who described this cell when he was a medical student. Mast cells were first described by Paul Ehrlich in his 1878 doctoral thesis on the basis of their unique staining characteristics and large granules. These granules also led him to the mistaken belief that they existed to nourish the surrounding tissue and were first described by Paul Ehrlich in his 1878 doctoral thesis on the basis of their unique staining characteristics and large granules. These granules also led him to the mistaken belief that they existed to nourish the surrounding tissue and he named them "Mastzellan" (from the ancient Greek masto, "I feed"). They are now considered to be part of the immune system.

Mast cells are very close to basophil granulocytes (a class of white blood cells) in blood; the similarities between mast cells and basophils have led many to speculate that mast cells are basophils that have "homed in" on tissues. However, current evidence suggests that they are generated by different precursor cells in the bone marrow. The basophils leave the bone marrow already mature, whereas the mast cell inulates in an immature form, only maturing once in a tissue site. In a tissue site an immature mast cell chooses to settle in probably determines its precise characteristics. Two types of mast cells are recognized comprising a set from connective tissue and a distinct set of mucosal mast cells. The activities of the latter are dependent on T cell. Mast cells are present in most tissues characteristically surrounding blood vessels and nerves, and are especially prominent near the boundaries between the outside world and the internal milieu, such as the skin, mucosa of the lungs and digestive tract, as well as in the mouth, conjunctiva and nose.

Mast cells have a diameter of about 12 microns, heterogeneous in shape and are packed with granules. They have a life span of weeks to months. 2-4 Mast cells release preformed secretory mediators like histamine, heparin, tryptase, lipid derived mediators like leukotrienes B4 (LTB4), LTC4, LTD4, LTE4, pro inflammatory cytokines like TNF alpha, IL-1, mutagenic cytokines – IL-3, IL-5 and immuno-modulatory cytokines like IL-4, IL-10 5. Therefore mast cells have been studied in various conditions like wound healing, chronic inflammation, keloid, pulmonary fibrosis and angiogenesis.

As far as oral diseases are concerned, mast cells have been implicated in variety of diseases like periapical lesions 7, oral submucous fibrosis 8, odontogenic cysts 9, gingivitis 10 and pyogenic granuloma. 11 Mast cells have also been reported to be present in lichen planus, a common dermatological disorder to manifest itself in the oral cavity. 12 In the early 1970s, it was proposed that ‘tumor growth is angiogenesis dependent’. 13 Small tumors could be nurtured with oxygen and nutrients by diffusion, but tumor growth would require newly formed blood vessels. It is also an important event in variety of physiological processes such as embryonic development, chronic inflammation and wound repair.

The commonly occurring oral diseases like oral leucoplakia, sub mucous fibrosis, lichen planus, squamous cell carcinoma...
are the commonly occurring oral diseases, with characteristic clinical and histological features. In addition, auto-immunity which is strongly associated with oral lichen planus and angiogenesis is associated with proliferation of the carcinoma. These diseases at some stage are associated with chronic inflammation in adjacent connective tissue. Our study was done to histologically evaluate the number of mast cells in tissue sections of these diseases.

AIMS

Estimation of mast cells per optical field in normal mucosa, oral leukoplakia (OL), oral submucous fibrosis (OSMF), oral lichen planus (OLP) and oral squamous cell carcinoma (OSCC)

- To determine the mast cell density (MCD) per sq.mm unit area in normal mucosa, OL, OSMF, OLP and OSCC.
- Comparison of the mast cell count in OL, OSMF, OLP and OSCC to that of normal mucosa.
- To evaluate statistically the above parameters between normal mucosa, OL, OSMF, OLP and OSCC.

MATERIALS AND METHODS

The present study was carried out in the Department of Oral Pathology & Microbiology, Jaipur Dental College, Rajasthan. 10 cases each of OL, OSMF, OLP & OSCC were retrieved from the department archives. Biopsies of normal oral mucosa were obtained from 10 adult patients undergoing extraction of third molar with their consent, which made up the control group. From each block, two sections were made using Leica Semi-automatic microtome. The sections were standardized by maintaining the thickness at 3-4 microns. One set of sections were stained by Harris hematoxylin and eosin for histopathological diagnosis. The other section was stained with 1% toluidine blue for mast cells using Toluidine blue staining characteristics as the fibroblasts. Selective stain of 1% toluidine blue is used to remove the intraobserver bias. Mast cells are spindle to oval shaped and have the same staining characteristics as the fibroblast with hematoxylin and eosin staining. Therefore, they are difficult to differentiate from fibroblasts. Selective stain of 1% toluidine blue is used for mast cells. Mast cell granules are purplish red and the nuclei of mast cell appear sky blue in color.

Mast cells were counted in 10 random high power fields (40 X) having larger number of mast cells, with one field depth from basement membrane of the epithelium and the average per high field was determined. Further the mast cell count was expressed per sq mm using the following formula. The radius of 1 field under the high power objective (40X) was 0.235mm, measured with the occulo-micrometer. Hence the area of the field was \(22/7r^2\) that is \(22/7 \times (0.235)^2\) approximately 0.2 sq mm. Therefore the number of mast cells per one high power field (ie) 0.2sq mm was multiplied by 5 to get the number of mast cells per sq.mm. The results thus obtained by the above method were subjected to statistical analysis for obtaining significance value using ‘t’ & ‘Z’ test.

RESULTS

The present study compared 50 cases (n=50), which comprised 10 cases each of OL, OSMF, OLP and OSCC (n=40) and 10 cases of normal mucosa as controls (n=10).

In the present study, the patients with OSMF were in the age group of 18 – 55 years with mean age of 27.5 years and there was male predominance with male to female ratio of 4:1. The patients with OLP in our study were in the age group of the 18 – 60 years with the mean age of 44.5 years and there was equal occurrence among both sexes with the male to female ratio of 1:1.

The patients with OL in our study were in the age group of the 25 – 62 years with the mean age of 37.3 years and there was male predominance with the male to female ratio of 4:1. The patients with OSCC in our study were in the age group of the 32 – 85 years with the mean age of 52.4 years and there was slight male predominance with the male to female ratio of 3:2 (Graph 1). The controls of our study were in the age group of 25-62 years with the mean age of 37.3 years with 5 males and 5 females.

Staining of mast cells was carried out using Toluidine blue and enumeration of mast cells was done using the protocol. Mast cells visible in each optical field (40X) were individually counted and the total number of mast cells per optical field was determined. Further the mast cell count was expressed per sq. mm. The mean and standard deviation of mast cell count (MCC) per optical field and mast cell density (MCD) per sq. mm was calculated for normal oral mucosa, OSMF, OL, OLP and OSCC cases. All the values were expressed in terms of mean ± Sd. The results obtained were tabulated and subjected to statistical analysis.

A high positive correlation was observed between MCC/optical field of normal oral mucosa, OSMF, OL, OLP and OSCC. The mean values MCC/optical field was increased in OSMF (3.85±0.82), OLP (5.26±1.51), OL (6.10±1.81) and OSCC (8.65±3.38). The increase in the mean values of MCC/optical field when compared to the control group (2.51±0.84) was statistically significant (P <.001). (Graph 2)

Similarly the increased mean value of MCC/optical field of OL, OLP & OSCC when compared with OSMF was statistically significant (P <.001). The increase in mean values of MCC/optical field of OLP, OL & OSCC was not significant when compared between them (P >.05).

A significant difference was observed by using ‘t’ test between mean values of MCC/optical field of pre-cancerous condition (4.55±1.41) and pre-cancerous lesion (6.10±1.81) (P <.05) and also when compared with the control group (P < .001). (Graph 3)

Comparison of the mean values of MCC/optical field of normal mucosa (2.51±0.84) with potentially malignant lesions (5.07±1.71) and malignant lesions (8.65±3.38) showed a very highly significant increase of mean values (P <.001). (Graph 4)

A high positive correlation was observed between MCD/sq.mm of normal oral mucosa, OSMF, OLP, OL & OSCC. The mean values MCD/sq.mm was increased in OSMF (19.25±4.11), OLP (26.30±7.56), OL (30.50±9.07) and OSCC (42.75±17.48). The increase in the mean values of MCD/sq.mm when compared to the control group (12.56±4.18) was statistically significant (P <.001). (Graph 5)
Mast cells are sensitized with IgE to particular foreign antigens. From its derivation, ‘mast’ relates to feeding, and was applied itself of infecting organisms. Hence mast cells are to be regarded as an important component of the body’s defense mechanism for the elimination of foreign antigens.

The disease is multifactorial in etiology. The various factors have major physiological and pharmacological significance. Mast cells have been incriminated in diverse conditions such as delayed type of hypersensitivity reaction and fibronectin leading to increased fibrosis and decreased edema seen in early stages of the disease were as interleukin-8, which are round or oval in shape, having a diameter of about 12-15 μ. Their numerous cytoplasmic granules frequently obscure the small, round nucleus. In some sections, these cells seem to have undergone degranulation, so that many of the granules are located outside the cell. Mast cells are present in mucosal and connective tissue environment. In oral mucosa and skin, they are distributed preferentially about the microvascular bed, being in close proximity to the basement membranes of blood vascular endothelial cells and nerves.

Mast cells are known as "unincellular endocrine" glands, since on discharge of mast cell granules, a number of mediators are released which include heparin, histamine and serotonin, which have major physiological and pharmacological significance.

Mast cells are connective tissue cells with basophilic metachromatic granules in their cytoplasm, which contain a wide array of mediators with diverse functions. Mast cells have been incriminated in diverse conditions such as delayed hypersensitivity, allergy, asthma, chronic host versus graft reaction, neoplasms etc. It has been substantiated that mast cells and T-lymphocytes behave in a bi-directional manner thus influencing each other in various aspects. Mast cells are not prominent in routine H&E sections and hence their number in routine sections of connective tissue is often underestimated. Mast cell granules stain metachromatically with Toluidine blue, Azure A, Bismarch Brown and Thionin. They are also visualized with a number of Alcian blue methods.

Mast cells are sensitized with IgE to particular foreign antigens that have already entered the body. Thus they serve as "sentinels on look out" as it were, for the reappearance of the same antigens. When these again enter the body, they cause mast cells primed with IgE specific for these antigens to liberate content of chemical mediators and there by invoke the many facets of the inflammatory reaction. In general, this reaction serves to render the foreign antigen harmless to the body and eliminate the source of antigen if it is within the body. Hence mast cells are to be regarded as an important component of the body’s defense mechanism for ridding itself of infecting organisms.

Many investigators have tried to highlight the role of mast cells in oral health and disease conditions. Among normal oral tissues, mast cells have been proved to be present in gingival connective tissue, tongue and lining mucosa. Mast cells have also been reported in normal periodontal ligament and pulp, although in very low densities. It has been a general belief that mast cells of different phenotypes may have different roles in human health & disease. There is considerable controversy regarding the number and distribution of the mast cells in clinically normal oral tissues as compared to the inflamed or diseased tissues. Much interest has been shown by many authors in recent years regarding the density and distribution of mast cells in oral mucosa.

In the present study, the control group consisted of normal mucosa and the mean values of mast cell count/optical field was 2.51±0.84 and mast cell density/sq. mm was 12.56±4.18 respectively. This finding of mast cells/optical field is an agreement with various investigators like Shapiro et al (2.7), Bhat AP & Dholakia HM (1.02) and Madhuri Ankle R et al (1.02)⁴. The mast cell density/sq. mm was in agreement with Hansson & Nygren (12-15 cells/sq. mm)⁵.

OSMF is a chronic oral mucosal disease characterized by epithelial atrophy and progressive accumulation of collagen fibers in the lamina propria and submucosa of the oral mucous membrane. The abnormal fibrosis causes blanching and stiffness of the mucous membrane, with eventual immobility of lips, cheeks, tongue, soft palate and uvula. The disease is multifactorial in etiology. The various factors implicated in the etiology are the chillies, nutritional deficiency, genetic susceptibility, autoimmune mechanisms, increased lysyl oxidase enzyme, arecanut and cytokines. Mast cells have also been implicated in the pathogenesis of OSMF. Some authors state that majority of OSMF patients gave an important observation that they had a feeling of itching in oral mucosa, especially in early stages of the disease. This is probably due to release of histamine from mast cells. In the present study, there was a significant rise in both the mean values of MCC/optical field (3.85±0.82) and MCD/sq. mm (19.25±4.11) in OSMF cases. This findings were in agreement with the studies of various investigators like Bhat AP & Dholakia HM (4.5 cells/optical field)⁵ and Madhuri Ankle R et al (48.25 cells/sq. mm)⁶ which is slightly higher than our findings, which could be because of selection of the case as it is well documented that mast cell count decreases with increasing grades of OSMF. Various investigators have suggested that the release of mast cell granules in the connective tissue in oral sub mucous fibrosis may initiate a change in the connective tissue ground substance by changing the intra-cellular fluid or free tissue water into a mucinous fluid leading to fibrosis⁷. The effect of chemical mediators can explain the histopathological changes seen in OSMF. Histamine could probably attribute to submucosal edema seen in early stages of the disease were as interleukin-1 and tryptase causes increased production of type-I collagen and fibronectin leading to increased fibrosis and decreased mouth opening⁸.

Lichen planus is a fairly distinct mucocutaneous disease of uncertain etiology but with unique histopathological features. Many factors like genetic, psychogenetic, traumatic and immunological factors have been implicated in the etiology of lichen planus. Current opinion suggests that the pathogenesis of lichen planus is a cell mediated process and bears similarity to delayed type of hypersensitivity reaction where mast cells are known to play an important role. 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 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. In the present study, a quantitative assessment of mast cells in OLP was done and an attempt was made to enable the assessment of role of these cells in the etiopathogenesis of lichen planus. In our present study, there was a significant rise in both the mean values of MCC/optical field $(5.26\pm1.51)$ and MCD/sq.mm $(26.30\pm7.56)$. These findings were in an agreement with the findings of Hansson & Nygren $(30-32cells/sq.mm)$ \(^{23}\). However the values were less compared to Xiji JZ et al $(151cells/sq.mm)$ \(^{28}\) and Madhuri Ankle R et al $(2.39cells/optical field & 59.75 cells/sq. mm)$ \(^{24}\).

Most of the previous studies indicate an increase in mast cell count in OLP, when compared to normal mucosa. Mast cells have also been reported along the basement membrane in cases of OLP. This lining of mast cells along the basement membrane has been thought to be a response to external agents or antigenic stimuli, to release histamine. The increased mast cell count in oral lichen planus has been attributed to the fact that mast cells possibly helped in recruiting the lymphocytes to the subepithelial zone. Some workers have reported that the increase in mast cell numbers in OLP suggested a controlling role of mast cells over the lymphocytes and also a role secondary to the immune response. The pathogenesis of OLP, as such, appears to be influenced by mast cells and their products like histamine which causes trafficking of lymphocytes and submucosal edema which in turn leads to vesicles and bullae. The TNF-α factor leads to necrosis and liquefactive degeneration of basal changes\(^{29}\).

OL is more commonly occurring oral precancerous lesion has received the most attention since its close relationship to oral cancer was well established as early as 1970 through the careful observation of Butlin & others. It is innocuous in its onset and potentially hazardous in later stages with the malignant transformation rate ranging from 4.4% to 7.5%. Tobacco, alcohol, radiation, syphilis, galvanism, viruses & candidiasis are some of causes that have been implicated in etiology of oral leukoplakia\(^{10}\). More recently efforts have been made to study the presence of mast cells in premalignancies including OL and to establish their possible role in pathogenesis of this disease\(^{3}\). In the present study there was a significant rise in both the mean values of MCC/optical field $(6.10\pm1.81)$ and MCD/sq. mm $(30.50\pm9.07)$ in OL cases. These findings were in an agreement with the studies of various investigators like Biviji et al \(^{31}\), Madhuri Ankle R et al \(^{28}\) and Michailidou EZ et al \(^{32}\). Various investigators have concluded that the biologically and pharmacologically active agents in the mast cells contribute to inflammatory reaction seen in leukoplakia. These stimulated mast cells may release interleukin-1 which causes increased epithelial proliferation that is seen in leukoplakia and the lesion clinically appears as white patch. Histamine may cause increased mucosal permeability, which could facilitate increased assess for antigen to connective tissue\(^{22}\).

OSCC is an aggressive epithelial neoplasm. Despite the early detection, intervention and treatment the overall survival rate has improved only slightly. The role of angiogenesis in neoplasia has been receiving increasing attention in recent times, since it can be used as independent prognostic indicator for tumor progression and metastasis, and also as novel second target for anticancer therapy instead of direct tumor cell inhibition\(^{33}\). Angiogenesis is the process of formation of new microvessels from the pre-existing vasculature. It is the compelling force for tumor growth and metastasis by providing nutrients and oxygen for metabolism and removal of resultant waste products. Although in the beginning angiogenesis develops by incorporating existing host blood vessels, no solid tumors can probably grow more than 1-2 mm\(^3\) unless they synthesize their own network of new microvessels. Their formations require a direct or indirect role of angiogenic factors. It is thought to be initiated by an increase in the level of angiogenic stimuli and a concomitant decrease in the level of angiogenic inhibitors. These factors are produced by tumor cells, stromal cells and inflammatory cells such as mast cells and macrophages\(^{14}\).

In the present study there was a significant rise in both the mean values of MCC/optical field $(8.65\pm3.38)$ and MCD/sq. mm $(42.75\pm17.48)$ in OSCC cases. These findings were in an agreement with the studies of various investigators like Madhuri Ankle R et al $(56.75cells/sq. mm)$ \(^{24}\) and Michailidou EZ et al \(^{32}\). Rooney et al \(^{33}\) suggested that heparin from the mast cells causes 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. It was found that microvessel density increased between normal oral mucosa, dysplasia and OSCC\(^{32}\). Micro vessel density was found to depend on mast cell density. So it was concluded from their studies that mast cells get attracted at the lesion site and may turn on an angiogenic switch during tumorigenesis\(^{34}\).

In the quantitative analysis of the mean MCC/optical field showed an increase and was found to be $3.85\pm0.82$, $5.26\pm1.51$, $6.10\pm1.81$ and $8.65\pm3.38$ in OSMF, OLP, OL and OSCC. The increase in the values was statistically significant when compared with that of normal buccal mucosa $(2.51\pm0.84)$ which constituted the control group. The mean MCD/sq.mm also showed an increase and was found to be $19.25\pm4.11$, $26.30\pm7.56$, $30.50\pm9.07$ and $42.75\pm17.48$ in OSMF, OLP, OL and OSCC respectively. The increase in the values was statistically significant when compared with that of normal buccal mucosa $(12.56\pm4.18)$ which constituted the control group. This increased in the values was in agreement with the values obtained in the study by Madhuri Ankle R\(^{24}\) except that mean mast cell count of OL was more than OLP in our study.

The World Health Organization classifies oral precancerous / potentially malignant disorder into 2 general groups as follows: A precancerous lesion is "a morphologically altered tissue in which oral cancer is more likely to occur than its apparently normal counter-part". These precancerous lesions include leukoplakia, erythroplakia and the palatal lesions of reverse smokers. A precancerous condition is a "generalized state associated with significantly risk of cancer". The precancerous condition includes oral submucous fibrosis, lichen planus, epidermolysis bullosa and discoid lupus.
Conclusions
Various studies have implicated mast cells in pathogenesis of many oral lesions. However 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.

Thus, the mast cells, which still remains as a mystery in many aspects is equally important to an immunologist, pharmacologist, physiologist and last but not the least, to an oral pathologist. It is now up to the oral pathologist to solve the still existing puzzles about this “sentinel on the outlook”.

References
Karthikeyan Ramalingam et al: Study on Comparison of Mast Cell Count and Mast Cell Density


Graph 1: Showing mean age of patients in Oral Submucous Fibrosis, Lichen Planus, Leukoplakia and Squamous Cell Carcinoma

Graph 2: Showing Mean Values of MCC/Optical Field of Normal Mucosa, Oral Submucous Fibrosis, Lichen Planus, Leukoplakia & Squamous Cell Carcinoma
Graph 3: Mean Values of MCC/Optical field of Normal Mucosa, Potentially Malignant & Malignant Lesions

Graph 4: Showing Mean Values of MCD/sq. mm of Normal Mucosa, Oral Submucous Fibrosis, Lichen Planus, Leukoplakia & Squamous Cell Carcinoma
Graph 5: Showing Mean Values of MCD/sq. mm of Normal Mucosa, Potentially Malignant & Malignant Lesions

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