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Mast cell responses in Oral Squamous Cell Carcinoma and Leukoplakia

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ABSTRACT:
Background: Mast cells are multitalented cells and they act as key players in the progression of oral lesions. They are local residents of the connective tissue stroma, they releases pro inflammatory and mitogenic cytokines upon activation. Oral squamous cell carcinoma (OSCC) and Leukoplakia are commonly occurring oral lesions.
Aim – of our study is to estimate mast cell population in OSCC and Leukoplakia and correlate progression in mast cell count between pre malignant and malignant condition.
Materials and Methods – Ten cases each of OSCC and Leukoplakia archive tissue blocks were used from the department and 1% toluidine blue stain was used.
Result – Mast cells plays a vital role in angiogenesis and tumour progression. We found the values have increased in malignant condition but did not reach statistical significance.
Conclusion – the study needs to be done with larger number of sample size. Further detailed and better understanding might open therapeutic aspects to treat the lesions.
Key words: Mast cells, Oral Squamous Cell Carcinoma, Leukoplakia.

INTRODUCTION

Mast cells were first described by German medical scientist Paul Ehrlich in 1878. In 1950s mast cells were recognised as the primary repository of histamine and their crucial role in anaphylactic reactions was understood.(1)They are located at the interface of host and external environment, mast cell maturation, phenotype and function vary based on local microenvironment. They are strategically placed hence responds hastily to any kind of change.(2)Mast cells canspecifically recognize and respond to various stimuli through the release of an array of biologically active mediators.(3)These features enables mast cells to play a defensive role inharmful situations and respond to changes in their environment by

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communicating with a variety of other cells implicated in physiological and immunological responses. The role of mast cells in allergic diseases, anaphylaxis, and autoimmunity have been well documented. Their role in innate and adaptive immunity, including immune tolerance, has gained increased prominence. Mast cell studies in the past two decades have expressed their involvement in physiological and pathological processeshowever, their role in the pathogenesis of oral diseases is still being understood. In the year 1891 Westphal reported the presence of mast cell at the periphery of tumor areas, but their role in tumor progression and metastasis is still controversial.(4)

Most common oral lesions in our country - oral leukoplakia, oral lichen planus, oral submucous fibrosis and oral squamous cell carcinoma are characterised by chronic inflammation. Mast cells aresecretory cells known to release mediators of inflammation. In previous studies conducted in other parts of India, mast cells appeared more atypical and granularin inflamed parts of the lesionsas compared to typical mast cells.Mast cells are found in large numbers at active tumour sites. Evidently, there appears to be a clear connection between oral pathologies and mast cells.Aim of our present study is to comparemast cell progression inpre malignant conditions (oral leukoplakia) and malignant conditions (oral squamous cell carcinoma) in Eastern India.

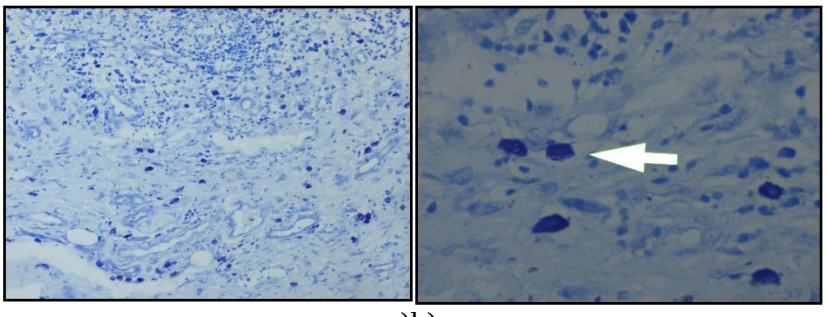
MATERIALS AND METHODS

Informed consent and Ethical Committee clearance was obtained for the study. 20 paraffin embedded tissue blocks obtained from the archives ofDepartment of Oral and Maxillofacial Pathology,Kalinga Institute of Dental Sciences, Bhubaneswar.

10 sections of both OSCC and Leukoplakiaeach.Samples were sliced into 5µm sections using the semi-automated microtome (Leica) and lifted on to the adhesive coated glass slides.Slides were placed in the slide warming table to melt the wax and then transferred to a Coplin jar containing xylene. Three changes of 5 minutes each with xylene were done followed by two changes of 10 mins each with decreasing grades of alcohol. Sections were then washed for 10 dips in distilled water.Staining done by covering the slides with 1% toluidine blue stain for 10 seconds followed by washing under a running tap.Differentiation in 100% alcohol was done for 2 min and clearing was done in xylene. Finally, the sections were mount in DPX and covered by coverslips.

Stained sections were examined under bright field light microscope to count mast cells at a magnification of 40x in Z pattern from left to right of 5 fields and the data obtained was statistically analyzed.

Mast cell granules are purplish red and the nuclei appears bluein color. Toluidine blue stains the mast cell granules metachromatically due to its reaction with sulphatedmucopolysaccharides. All the other components of the sections were seen in different shades of blue as seen in Figures 1through 8.Statistical analysis of the data was carried out using the Statistical Package Social Sciences(SPSS) 17.0 software (SPSS Inc., Chicago, IL, USA). Statistical significance was studiedusing the independent ‘t’ test.



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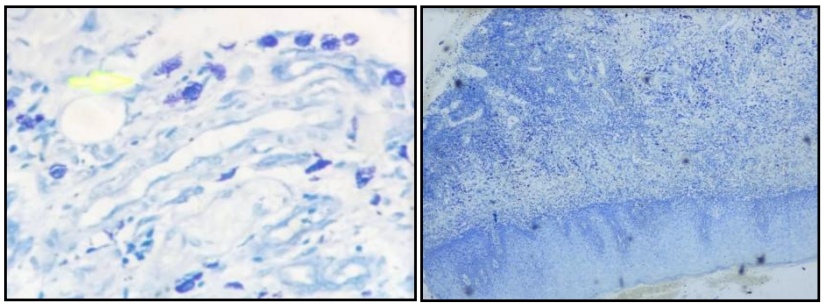

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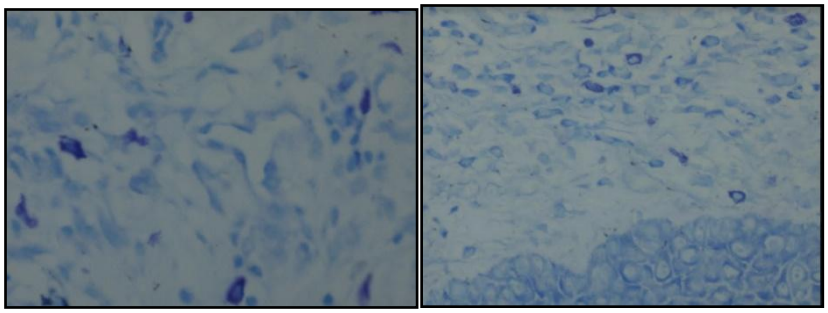

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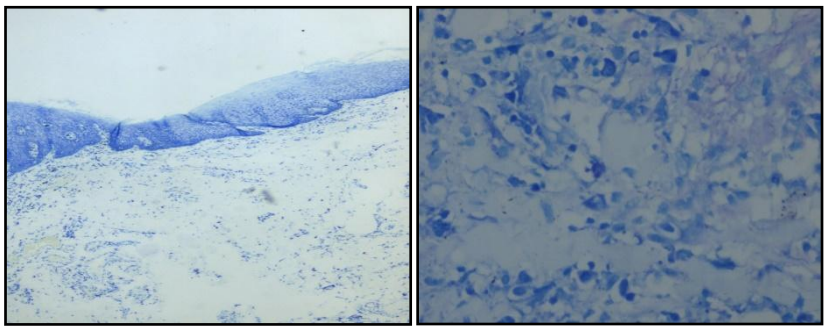

g)h)

Figure a) Mast cells in toluidine blue stained section of Oral Squamous Cell Carcinoma (10x)
Figure b) Mast cells in toluidine blue stained section of Oral Squamous Cell Carcinoma (40x)
Figure c) Mast cells in toluidine blue stained section of Oral Squamous Cell Carcinoma (40x)
Figure d) Mast cells in toluidine blue stained section of Oral Squamous Cell Carcinoma (4x)
Figure e) Mast cells in toluidine blue stained section of Leukoplakia (40x)
Figure f) Mast cells in toluidine blue stained sectionof Leukoplakia (40x)
Figure g) Mast cells in toluidine blue stained section of Leukoplakia (4x)
Figure h) Mast cells in toluidine blue stained section of Leukoplakia (40x)

RESULTS

The number of mast cells found in OSCC is explained in (Figure 9), most sections the count varied from 20 to 30 and in one section the count ranged upto 50 to 60.In comparison with Leukoplakia (Figure 10), in 4 sections the number of cells varied from 20 to 30 and in one section it was 50 to 60.t test value was conducted, p value – 0.3688 was generated. Since the sample size is small, the value is very much negligible to draw a significant estimation.

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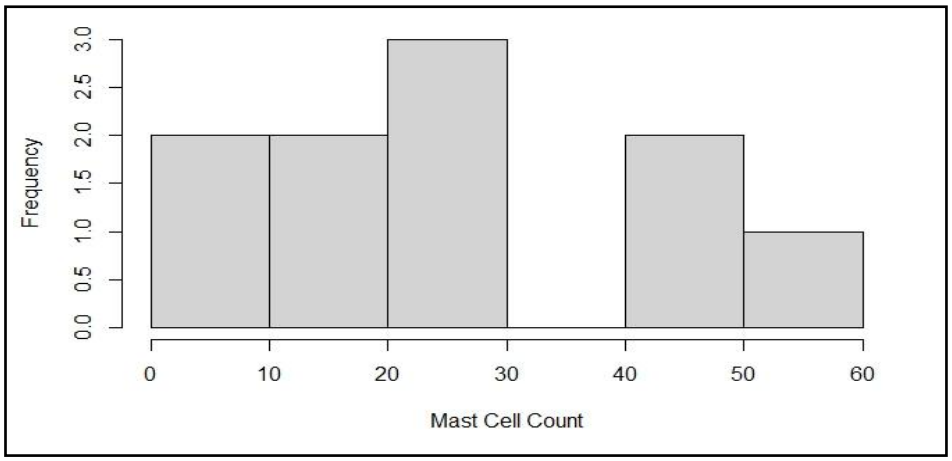

Figure 9. Histogram of mast cell count for OSCC

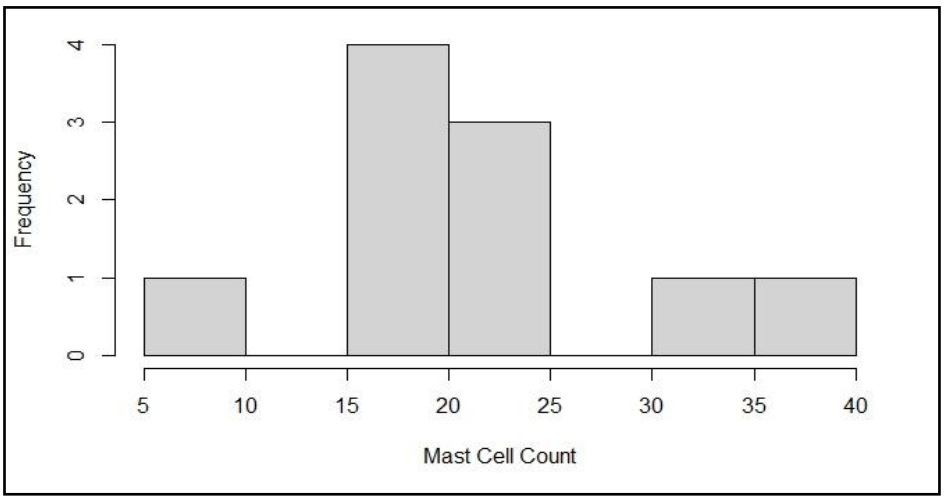

Figure 10. Histogram of mast cell count for Leukoplakia

- t- test

	MEAN	'n'
OSCC	27.3	10
LEUKOPLAKIA	21.6	10

The p value of the test is 0.3688

TABLE 1

DISCUSSION

The actuation of mast cells are followed by an array of biological steps such as mitogens activation, degradation of extracellular matrix, angiogenesis, changes in microvascularhyperpermeability and inflammatory cells are recruited. Mast cells releases proteases, they act on plasma albumin to release histamine which favours mast cell activation. Histamines, cytokines and chemokines these substances play a role in oral inflammation and carcinoma.[2] Tumor necrosis factor (TNF) alpha is the most important cytokine released because of its association with inflammation in the oral cavity.[5]

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Mast cells causes angiogenesis, induces vessel proliferation leading to epithelial proliferation and local invasion. Proteases degrades the extracellular matrix and causes angiogenesis. Matrix metalloproteinases (MMP) causes extracellular matrix degradation. In different malignant tumors the increased mast cell density is associated with poor prognosis.[6]OSCC is associated with inflammation, angiogenesis and immune reactions. Increase in mast cell is seen during transition from dysplasia to carcinoma, therefore the role of mast cells needs to be assessed. In our study, we found the number of mast cell count was more in OSCC in comparison to Leukoplakia. Mean value of mast cells in OSCC - 27.3(Table 1).In a study by A. Anuradha et al (2014), there was an increase in average number of mast cells in OSCC when compared to controls.No relevant statistical significance was found in the average number of mast cells on comparison of moderately and well differentiated cases. Average mast cell count under 400x magnification was found 3 in OSCC and 2 in controls.[7]Rooney et al suggested heparin released from mast cells causes vasoproliferation and increases the half life of basic FGF which leads to tumor angiogenesis and further invasion.Mast cells releases IL-1 which causes epithelial proliferation.[8]

On the other hand in case of leukoplakia the mean value is 21.6. (Table 1).Observations by Biviji et al 1973, showed a mean increase in the number of mast cells /unit microscopic field in oral leukoplakia compared to normal oral mucosa. It was inferred that the biologically and pharmacologically active agents in the mast cells contribute to inflammatory reaction seen in leukoplakia.[9]Proangogenic and angiogenic factor like histamine, heparin, chymase , beta fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF) causes increase in the density of microvessels. Histamine cause increased mucosal permeability, which could facilitate increased access for the antigen to the connective tissue. Increased angiogenesis is associated with neoplastic progression and metastasis. The accumulation of mast cells releases pro angiogenic and angiogenic factors which favorstumor progression. Yodavudh and Gulubova(2008) concluded mast cells can be used as a prognostic marker. It has been suggested by Chan et al that by counting mast cell density one can calculate mast cell accumulation.[4]

Costa et al (2009) stated the number of mast cell density was higher in OSCC of the lip in comparison to normal mucosa. This explains the change in tumour microenvironment during squamous carcinogenesis.[10]Michailidou et al (2008) stated there is a role of mast cells in progression from premalignant oral lesions to OSCC, the number of mast cells increases in leukoplakia compared to normal mucosa and further increases in severe dysplasia followed by OSCC.[6]PusaNelaGaje, Ra et al (2016) in a review article studying mast cells in inflammation and oral squamous cell carcinoma stated that if the potential of mast cells are studied elaborately then it can lead to new perspective and development of avenues towards better treatment and prognosis.[11]

Kalraet al (2012) showed a decrease in mast cell count in well differentiated carcinoma in comparison to low differentiated ones. An inverse relationship is seen in the number of vessels with tumor grade.[12] Similarly Sharma et al (2008) observed higher mast cell count in moderate stage than in well differentiated. This concludes mast cells plays a more pivotal role in promoting angiogenesis than its cytotoxic properties. The low and moderately differentiated carcinomas are more aggressive.[13]Contradictory results were seen in a study by Oliveira Neto et al (2007) mast cell counts were seen to be lower in OSCC and in other premalignant lesions compared to normal control group. They explained it to be a migration failure of mast cells during tumorinitation and progression.[11]The cytotoxic functions of the mast cells are suppressed by the angiogenic properties. This depends on the presence of the concentration of mediators in the certain microenvironment.

A study comparing mast cell in oral epithelial dysplasia, oral submucous fibrosis and oral squamous cell carcinoma and their association with inflammation and angiogenesis by

NeethuTelagi, Ahmed Mujib et al(2015) concluded there is a marked increase in atypical and granular mast cells in more inflamed areas compared to typical mast cells implying that malignant lesions express higher of mast cell count compared to premalignant lesions.(2) In a review article,Meera K Pynadath, Anthony George et al concluded mast cells contain a series of adhesion molecules, immune response receptors and surface molecules which gives them the ability to react with non specific and specific stimuli. Mast cells play a definite role in angiogenesis which precedes malignancy. More studies in this field will mediate improving the quality of life of these patients. In a review study Role of mast cells in inflammatory and reactive pathologies of pulp, periapical area and periodontium by HS Sheethal, KN Hema et al,(2019) they stated mast cells plays a critical role in inflammation of dental infections, so therapeutic drugs can directly influence mast cell secretion to control the lesions.(14)
Studies conducted by Theoharides TC et al(2004) and Mohtasham N et al (2010) reported high mast cell density before angiogenesis in malignancies. Angiogenic regulation in cancer is biphasic – early premalignant phase which then progresses to the cancer phase. Premalignant phase characterized by hyperplasia and dysplasia shows infiltrating mast cells which degranulate and activate fibroblasts leading to rapid angiogenesis. (8) Coussens et al (1996) and Vu TH et al (1998) studied MMP progelatinase B, activated by mast cells, which contributes to extracellular remodelling and is a key regulator of angiogenesis. In the later phase as tumor advances the cancer cells upregulateangiogenic growth factor gene and directly control neovascularisation instead of depending on inflammatory cells for the same. This could explain the increase in density of mast cells in early stages of cancer and no significant change in mast cell count between histologically well differentiated and moderately differentiated SCC.(9)
In 1863 Rudolf Virchow linked cancer and inflammation.Since then, numerous studies have tried understanding its role in treatment and prevention.The mast cell mediators vary in different diseases. Mast cells play a part in mediating link immunologic factors and outside angiogenic agents.

CONCLUSION

Present study sample size was small and results should be consolidated with other studies using larger sample sizes. A study incorporating more types of oral lesions and comparing them to a control sample of normal oral mucosacan give a broader outlook.The intact, spreading and degranulated mast cells should be studied in various lesion and their positions. The better understanding of the role of different mediators is required which might lead to new avenues in therapeutic aspects.

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