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MAST CELL DISTRIBUTION IN SOME COMMONLY ENCOUNTERED BENIGN AND MALIGNANT SALIVARY GLAND NEOPLASMS

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ABSTRACT

Background: Mast cells with a battery of crucial chemical mediators in their typical metachromatic granules are known to play a role in health and various disease states in man. This study was undertaken to evaluate the mast cell profile in some commonly encountered benign and malignant salivary gland neoplasms.

Materials & Methods: The present study was carried out in the Department of Pathology, M.R. Medical College, Gulbarga over a period of 5 years (3 years retrospective i.e., from August 2003 to August 2006 and 2 years prospective study i.e., from September 2006 to August 2008) which included a total of 50 cases each of control and study groups. Sections were stained with H&E and 1% aqueous toluidine blue (pH=4) for mast cells. The mast cell count was performed per 10 HPF, tabulated, analyzed and statistically evaluated.

Results: A significant ($p<0.001$) increase of mast cell was observed in commonly encountered salivary gland neoplasm's when compared with that of control group. Mast cell count was significantly increased in Pleomorphic adenoma as compared to basal cell adenoma and oncocytoma. Mast cell count was significantly more ($p<0.05$) in malignant tumor as compared to benign tumors of salivary gland.

Conclusion: The present study documents striking mast cell alteration in some common salivary gland lesions. Mast cell profile may be used as an additional diagnostic or supportive parameter to differentiate between malignant versus benign lesions of salivary gland in addition to other diagnostic parameters.

Key Words: Mast cells; Salivary gland lesions; Pleomorphic adenoma.

INTRODUCTION

Mast cells with a battery of crucial chemical mediators in their typical metachromatic granules are known to play a role in health and various disease states in man. Many developments have occurred in the field of salivary gland lesions. Very few studies have shown increased number of mast cells in chronic sialadenitis, Pleomorphic adenoma, and cystadenolymphoma. Mast cells are distributed throughout the human organism and in whole occupy roughly the volume of spleen. Mast cells are ubiquitous though they are being found in varying numbers in practically all tissues their proportion is increased within mucosal membranes lining the respiratory, digestive, urogenital systems, throughout the dermis and vicinity of surrounding blood vessels[1]. In adults mast cells are located around blood vessels where they tend to aggregate in small groups[2]. The highest concentration of mast cells is present in the skin, respiratory tract, gastrointestinal tract, urinary bladder, lymphoid tissue, synovium, serosal surfaces and uterus. Thus, it could be summarized that mast cells are located in tissues particularly at the portals of entry of external substances[1]. Mast cells are relatively long lived and undergo a very slow turnover[2]. Mast cells originate from pleuripotent progenitor cells in the bone marrow, and then enter the circulation in small number from the bone marrow, which may only be recognized by their cytoplasmic expression of messenger RNA for stem cell factor, the so called C-kit or steel factor receptor. From the blood, the precursors migrate into the tissues, where, under the influence of local micro-environmental factors, they undergo their final phases of differentiation and maturation into recognizable mast cells. Several growth factor cytokines are known to affect the growth and differentiation of mast cells, which include stem cell factor, IL-3, IL-4, IL-9, IL-10 and nerve growth factor (NGF) [3].

Mast cell granules store a wide variety of mediators of inflammation. Because of the multifarious role of mast cells in edema formation, angiogenesis and fibrogenesis, it is logical to infer that mast cell alteration could be found in various inflammatory and neoplastic disorders. The term 'mastocytosis' was used to designate the entire class of morbid conditions characterized by abundant proliferation of mast cells[4].

Mast cell distribution has been shown to be altered in various fibro proliferative disorders like pterygium[5], wound healing[6, 7] and rhinoscleroma[8]. Prominent increase in mast cells was observed in lesions of breast like mammary dysplasia, fibro adenoma and scirrhous carcinoma of breast[9]. Mast cell alteration has been documented in neoplasms like squamous cell carcinoma of the cervix[10] gastric carcinoma[11, 12]. Many developments have occurred in the field of pathology of salivary gland lesions. Despite the advancement in fields of diagnosis, surprises never cease. Very few studies on salivary gland lesions have highlighted that mast cells are normally present in the stroma and capsule of the salivary gland[13]. Increased number of mast cells discharging their granules is observed in sialadenitis[14], Pleomorphic adenoma[15] and cystadenolymphoma (Warthin's tumor) [16]

A careful search of the literature revealed a marked paucity of knowledge regarding the variation of mast cells in various salivary gland lesions. With this stimulus of lacunae in the knowledge of mast cells in various salivary gland lesions, an attempt is made to evaluate the mast cell profile in some commonly encountered benign and malignant salivary gland neoplasms.

MATERIALS AND METHODS

The present study was carried out in the Department of Pathology, M.R. Medical College, Gulbarga after the institutional ethical clearance. Specimens were collected from the Basaveshwar Teaching & General Hospital, Government General Hospital and other hospitals and private laboratories in and around Gulbarga and also from Peripheral Cancer Centre.

The study included cases from 3 years retrospective study (August 2003 to August 2006) and 2 years prospective study (September 2006 to August 2008). About 50 cases commonly encountered benign and malignant tumors of salivary gland and 50 individuals with normal salivary gland were used in the study. Histopathological diagnosis of the common salivary gland lesions made from the histopathological features was included in the study. Patients with malignant neoplasm's, Skin and adnexal tumors, Soft tissue tumors, Metastatic tumors were excluded from the study.

Processing and Staining

The tissues for the histopathological study were fixed in 10% buffered formalin, processed in different strengths of alcohol, cleared in xylene and were embedded in paraffin wax. The sections were cut at 4-5 microns thickness and staining was done with H&E as routinely and also with 1% aqueous toluidine blue for mast cells. The one stained with H&E was observed for the confirmation of the lesion. Other sections stained with 1% toluidine blue were used to study mast cells. The study comprised of common salivary gland benign tumors like Pleomorphic adenoma, Basal cell adenoma, Warthin's tumor and Oncocytoma. The malignant tumors included are Mucoepidermoid carcinoma, Adenoid cystic carcinoma, and Malignant mixed tumor.

Mast cell alteration in different lesions of salivary gland was compared without emphasizing on their variants or grades of neoplasm. Mast cells in the control salivary gland specimens were counted and compared with mast cells in various groups. Control samples were chosen from normal salivary gland tissues from cadavers of anatomy department.

Processing and Staining Techniques

Mast cell staining and counting: To identify the mast cells with typical metachromatic granules, special stain 1% aqueous toluidine blue (pH= 4) was used.

Toluidine blue staining method[17]: involves the preparation of staining solution: One gm of toluidine blue powder is dissolved in 100 ml of distilled water and the pH is adjusted to 4. The solution is filtered before use. The sections were taken on albumenized slides and kept at 60º for half an hour. The slides were kept in xylene for deparaffinization for 15 minutes. Then, the slides were brought to water in descending grades of alcohol i.e., 100%, 90%, 70%, 50% alcohol and then water wash. The slides were then placed in 1% toluidine blue solution (pH=4) for 1 minute. Then, the slides were rinsed in water, dehydrated in absolute alcohol, cleared in xylene and mounted in DPX. Mast Cell Granules stains purple and Background tissue stains blue.

Mast Cell Counting and Observations:

Toluidine blue stained sections were examined under high power magnification. The number of mast cells present in ten consecutive high power fields was counted in all the sections. Findings were tabulated and were statistically evaluated. On the basis of observations, an attempt was made to study mast cell profile in the common lesions of salivary gland. A possible explanation for the significant mast cell alteration if any was attempted. Diagnostic Histomorphological Criteria was based on observations of Fletcher Christopher DM [18].

Statistical Analysis: The data's were analyzed for the statistical significance using students't' test. P< 0.05 was the level of significance.

RESULTS

In the present study, an attempt was made to study the distribution of mast cells in some commonly encountered benign and malignant salivary gland neoplasms. The study included about 50 cases of each commonly encountered salivary gland lesions and 50 individuals with normal salivary gland were used in the study.

There was a significant increase ($p<0.001$) in the distribution of mast cells in Pleomorphic adenoma, Warthin's tumor, oncocytoma, basal cell adenoma, Mucoepidermoid carcinoma, malignant mixed tumor and adenoid cystic carcinoma when compared with that of controls (Table-1). Also, we observed a statistically significant ($p<0.05$) increase in malignant group when compared with benign group.

Table-1: Distribution of mast cells in various commonly encountered benign and malignant salivary gland tumors. Values are mean± S.D. n=50 each.

Type of tumor	Number of Mast cells/10 HPF			P value
	Type	Tumor	Control	
Benign	Pleomorphic adenoma	16.9±3.02	5.12±2.82	0.001
	Basal cell adenoma	9.67±3.42	5.12±2.82	0.001
	Warthin's tumor	13.02±4.01	5.12±2.82	0.001
	Oncocytoma	10.83±3.24	5.12±2.82	0.001
Malignant	Mucoepidermoid carcinoma	17.33±3.20	5.12±2.82	0.001
	Malignant mixed tumor	15.83±3.15	5.12±2.82	0.001
	Adenoid cystic carcinoma	13.16±2.94	5.12±2.82	0.001

In Pleomorphic Adenoma**,** the H&E stained sections from Pleomorphic adenoma revealed epithelial elements resembling ductal cells and myoepithelial cells dispersed within a background of loose myxoid tissue containing islands of chondroid tissue. On toluidine blue staining, the mast cell distribution in Pleomorphic adenoma cases ranged from 14-20/ 10 HPF (Figure-1).Mast cells were chiefly distributed in the connective tissue stroma and also focal connective tissues metachromasia was noted.

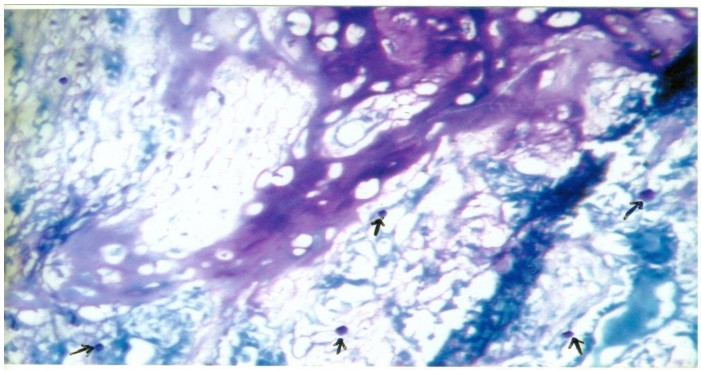

Fig-1: Photomicrograph showing increased mast cells and connective tissue metachromasia in Pleomorphic adenoma [Toluidine blue×400].

In Basal Cell Adenoma, the H&E stained section from the cases of basal cell adenoma revealed small round basaloid cells, uniform, basophilic nucleus and scant cytoplasm. Some ductal structures lined by cells with a greater amount of eosinophilic cytoplasm seen amidst the basaloid cells. On toluidine blue staining, the mast cell distribution in basal cell adenoma cases ranged between 6-12 cells/ 10 HPF (Figure-2). Mast cells were chiefly distributed in capsular connective tissue. It is to be noted that the connective tissue stroma is very scant in basal cell adenoma.

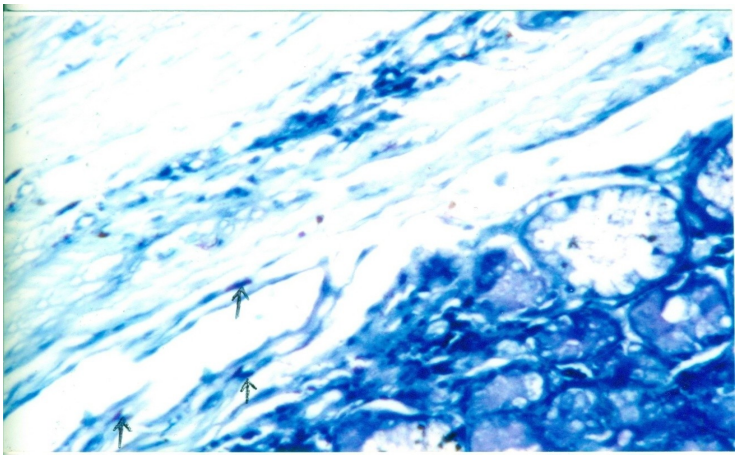

Fig-2: Photomicrograph showing increased mast cells in the capsular region of Basal cell adenoma [Toluidine blue×400].

In Warthin's Tumor, the H&E stained sections from the cases of warthin tumor revealed irregular cystic structures with the lining epithelium consisting of 2 layers of luminal layer of oncocytic columnar cells supported by a layer of Oncocytic cells. The distinct layer of basement membrane separating the cyst lining from lymphoid stroma consists of small lymphocytes and some plasma cells, histiocytes and mast cells .On toluidine blue staining, the mast cell distribution in Warthin's tumor ranged between 8-15 cells/ 10 HPF. Mast cells were seen in the lymphoid stroma and also around the periacinar region (Figure-3).

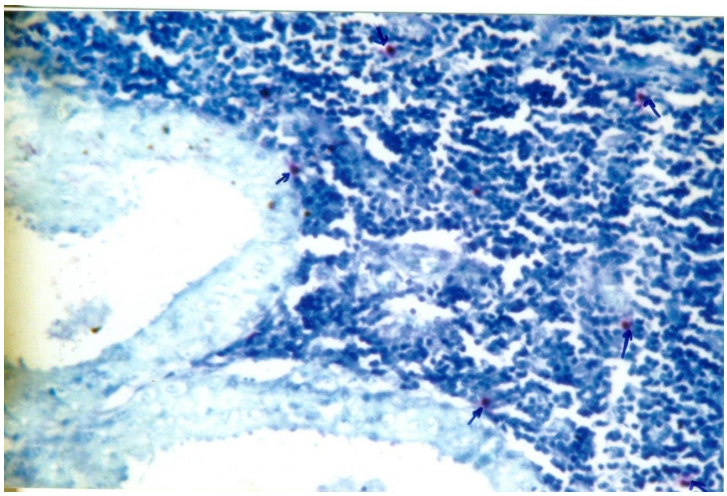

Fig-3: Photomicrograph showing increased mast cells amidst lymphoid aggregates in Warthin's tumor [Toluidine blue×400].

In Oncocytoma, the H&E stained section from oncocytoma cases revealed tumor cells arranged in diffuse sheets. Oncocytes are large polygonal to cuboidal with abundant eosinophilic granular cytoplasm, central round nuclei and with distinct nucleoli. On toluidine blue staining, mast cells in oncocytoma cases ranged between 8-14 cells/ 10 HPF. Mast cells were distributed in the stroma (Figure-4).

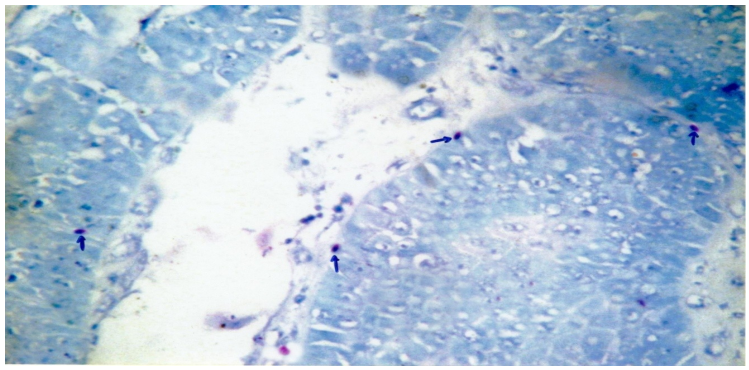

Fig-4: Photomicrograph showing increased mast cells in Oncocytoma [Toluidine blue×400].

In Mucoepidermoid Carcinoma, the H&E stained sections from Mucoepidermoid cases revealed mucin filled spaces and tumor nests composed of mucous, squamoid (epidermoid) and intermediate cells. On toluidine blue stain, mast cells in Mucoepidermoid carcinoma ranged between 14-20 cells/ 10 HPF. Mast cells were more in the intercellular stroma and around the mucin lakes (Figure-5).
In Malignant Mixed Tumor, the H&E stained sections from malignant mixed tumor cases revealed residual pleomorphic adenoma along with malignant component characterized by widespread significant cellular pleomorphism, high mitotic count, atypical mitotic figures and coagulation necrosis. On toluidine blue staining, mast cells in malignant mixed tumor ranged between 12-20 cells/ 10 HPF. Mast cells were seen in the connective tissue stroma (Figure-6).

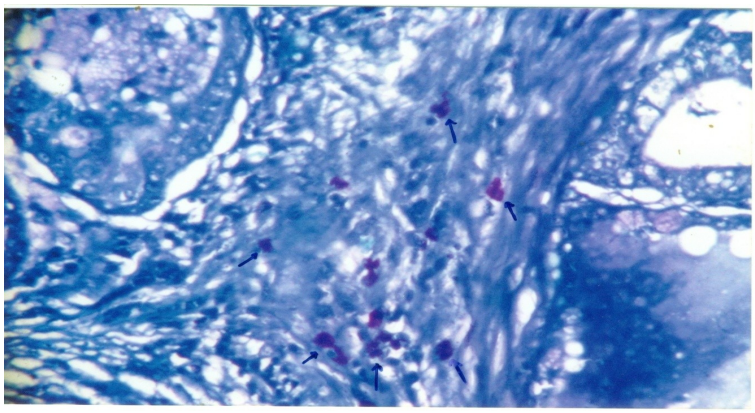

Fig-5: Photomicrograph showing increased mast cells amidst lymphoid aggregates in Mucoepidermoid carcinoma [Toluidine blue×400].

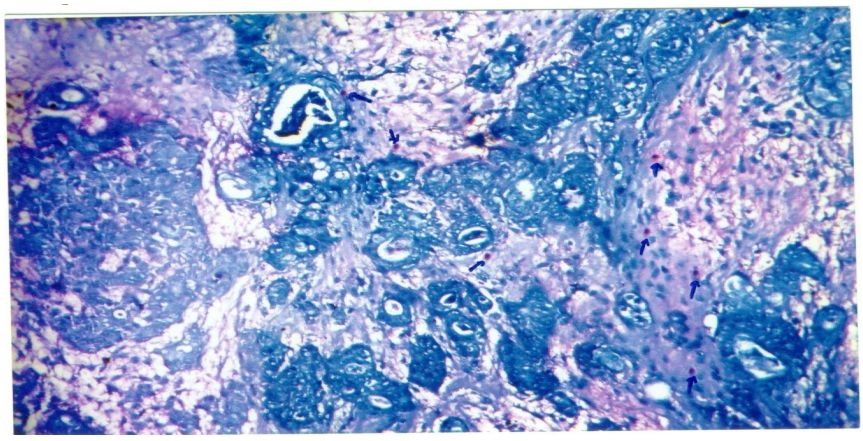

Fig-6: Photomicrograph showing increased mast cells amidst lymphoid aggregates in Malignant mixed tumor [Toluidine blue×400].

In Adenoid Cystic Carcinoma, the H&E stained sections from adenoid cystic carcinoma cases revealed cribriform structures variable sized smooth contoured, discrete and coalescent islands comprising small, uniform basaloid cells. The neoplastic basaloid cells constitute the major cell population. On toluidine blue staining, mast cells in adenoid cystic carcinoma cases ranged between 10-16 cells/ 10 HPF (Figure-7).

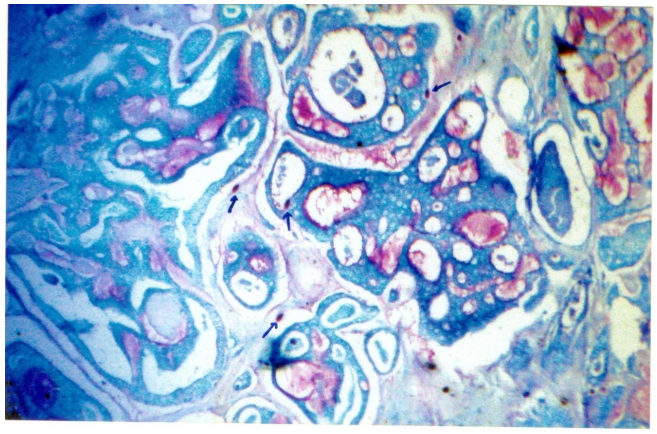

Fig-7: Photomicrograph showing increased mast cells amidst lymphoid aggregates in the stromal cells of Adenoid cystic carcinoma [Toluidine blue×400].

There was statistically significant increase ($p<0.001$) in mast cell in chronic non-specific sialadenitis, pleomorphic adenoma, Warthin's tumor, oncocytoma, mucoepidermoid carcinoma, malignant mixed tumor and adenoid cystic carcinoma versus controls. Statistically significant increase ($p<0.001$) was noted in mast cell in pleomorphic adenoma as compared to basal cell adenoma and oncocytoma. Statistically significant ($p<0.05$) increase was observed when malignant group was compared with benign group.

DISCUSSION

The association of mast cells with the tumors of man has been documented as early as 1879. Research literatures are available regarding the presence of mast cell alterations in various tumors of man and in various benign versus malignant neoplasms. Mast cell distribution has been shown to be altered in various fibro proliferative disorders like pterygium, wound healing and rhinoscleroma. Prominent increase in mast cells was observed in lesions of breast, like mammary dysplasia, fibro adenoma and scirrhous carcinoma of breast.

Katapodi and Kavantzus M (2004)[15] have shown that connective tissue stroma in pleomorphic adenomas definitely influences the actual concentration of mast cells. Pleomorphic adenomas expressed TGF-β_2, which may be associated with differentiation of inner ductal cells. Bruni and Olivi et al (1951)[19] showed diffuse connective tissue metachromasia, presumably owing to degranulation of mast cells.

Holmgren (1939)[20] studies indicated that metachromatic ground substances in connective tissue was particularly prominent at places where growth of the adjacent epithelial structures was more intense. In the stroma of tumors and regenerating epithelium there was diffuse metachromasia throughout the ground substance of connective tissue. This has been assumed to depend upon the presence of heparin, derived from mast cells. Also diffuse metachromatic staining is especially widest in various types of rapidly growing tissues. Paff et al (1952)[21] showed that the antimitotic effect of heparin is attributed to an interference by heparin with the metabolism of nucleoproteins and this process may have a prognostic significance in tumor exhibiting connective tissue metachromasia. Mast cell count in Warthin's tumor ranged between 8-15/10 HPF with a mean of 13 mast cells. The mast cell count was significantly ($p<0.001$) increased when compared to that of control group. Mast cells were seen in the lymphoid stroma and in the periacinar region. Caselitz J et al (1983)[22] showed that mast cells were associated with cystadenolymphomas. Yamamota H et al (1985)[16] showed by an immuno-histochemical study that mast cells play a special role in Warthin's tumors.

There was no statistically significant ($p>0.05$) difference on comparison of Warthin's tumor with basal cell adenoma and oncocytoma. Also, there was no statistically significant difference ($p>0.05$) on comparison of Warthin's tumor with Mucoepidermoid carcinoma, adenoid cystic carcinoma and malignant mixed tumor. It was suggested that lymphocytes may regulate mast cell production of specific lymphokines and interleukins. IL_3 and IL_4 derived from activated T cells act as growth factors for mast cells. Mast cells were predominantly more in the intercellular stroma and also around the mucin lakes.

The possible explanation regarding increase number of mast cells in malignant neoplasms may be increase release of chemical mediators like cytokines and interleukins which cause mast cell migration and proliferation. Klass Norby (2002)[23] has shown that mast cells are able to induce and enhance angiogenesis via multiple interacting pathways. Certain mast cell derived mediators like VEGF, bFGF, TGF-β, TGF-α and IL-8 act as potent angiogenic factors. Also histamine and certain lipid derived mediators induce microvascular hyperpermeability having proangiogenic effects. Also in tumors, the ECM degradation may significantly change the mast cell population in number, phenotype and function. In tumor models, mast cells have been shown to play a decisive role in inducing the angiogenic switch which precedes malignant transformation. There is strong evidence that mast cells significantly influence growth and progression in human cancers. In this context, increase mast cells in malignant salivary gland lesions may provide an additional supportive parameter in the differentiation between benign versus malignant salivary neoplasm's.

CONCLUSION

Mast cell profile may be used as an additional diagnostic or supportive parameter to differentiate between malignant versus benign lesions of salivary gland in addition to other diagnostic parameters .This study may stimulate and encourage further research in mast cell profile in commonly encountered inflammatory and neoplastic lesions of salivary gland and may offer new vistas in therapeutic approaches in salivary gland neoplasm's.

REFERENCES

1. Sorter NA and Austen KF. Mast cells in Dermatology in General Medicine. 3rd Ed., Fitzpatrick TB, Eisen AZ, Wolff K, Freedberg IM, Austen KF (eds.) McGraw Hill Book Co., New York. 426-243.
2. Galli SJ. New insights into the riddle of the mast cells, microenvironment regulation of mast cell development and phenotypicheterogenicity. Lab Invest. 1990; 50: 365-368.
3. Greggio H. les cellular granuluses (Mast Zellen) dans les tissues normaux et dans centain maladieschirurgicales. Arch Med Exp. 1911; 23: 323.
4. Edward C, Klah, Robert J Lukes et al. Benign and malignant mast cell proliferation. Cancer. 1983; 51: 119-1124.
5. Ratnakar KS, Goswamy V, Agrawal LP. Mast cell alterations in Pterygium. Acta. Pathol. 1976; 54: 563.
6. Cottennol F. Contribution a letude de la physiologie du mastocyte. Paris Librairie Arnetic The Mast Cells, Seyle H. Washington DC. Butterworths. USA: 1965.
7. Sanjay A, Pratima S, Pattankar Vl. Mast cell profile in wound healing. Dissertation submitted to Rajiv Gandhi University of Health Sciences, Bangalore.
8. Reddy BN, pattankar V. Mast cell profile in rhinoscleroma. Dissertation submitted to Gulbarga University, Gulbarga.
9. Kobal RG, Pattankar VL, Ratnakar KS. Mast cell profile in neoplastic and non-neoplastic disease of breast. In: Proceedings of 36th National Conference, IAPM at Hyderabad.
10. Weill P. Mast zellen studies on sarkomentastasen. Folia Haemat. 1919; 23: 185. 11. Quensel. Some investigations concerning mast cells. Acta Path Microbiol Scand. 1928; 5: 34.

11. Bruni C and Centonze M. Le sostanze metachromatiche mesenchimalli and cancrodello stomaco. Law 1st Amat. Univ. Perugia. 9, 129.

12. Staemmler M; Untersuchung uber vorkommen und bedeuturg der histiogenen mastzeller im menschliden korper unter normalen und pathologischen verhaltnisen Frankf. Ztschr. Pathol. 25; 391.

13. Diao Gx. Quantitative analysis for mast cells in obsructive sialadenitis. Zhonghua Kou. Quang Yi, Xau Za Zhi. 1993 March; 28(2): 93-4.

14. Katopodi AI. Kavantzas AI, Paulopoulos AI et al. The frequency and distribution of mast cells in pleomorphic adenomas of salivary glands. J Pathology. 2004 Jun; 36(3): 256-61.

15. Yamamoto H, Caselitz T, Seifert G. Cystadenolmphoma: An immunohistochemical study with special rference to IgE and mast cells. Pathol Resp Pract. 1985; 180(4): 364-8.

16. Porter KR and Bonneville MA. The structure of cells and tissues. 4th Edn., Lea & Fabiger. Philadelphia. 135-136.

17. Fletcher Christopher DM. Diagnostic histopathology of humans. 3rd Edn., Vol. 1, Churchill Livingstone, Harcourt Publishers: 2000.

18. Bruni and Olivi M. Per 1' anatomia patologica die tumor delta regione paratidea IX. Ricarche sistematiche sella esistenza di mast zellen nella parotide nelle neoplasic di questa gliandola lav. Ist Unit Perugia. 1951; 10: 73.

19. Homgren H. Uber vorkommen and Bedeutung der chromotropen (meta chromatischen) substanz in menschlichen Fern. Anat Anzg. 1939; 88: 246.

20. Paff, Sugiura HT, Boucher CA and Roth JS. The probable mechanism of heparin inhibition of mitosis. Anat Rec. 1952; 114: 499.

21. Caselitz J, Salfelder A and Seifert G. Mast cells in cystadeno-lymphomas. J of Molecular Medicine. 1984; 62(6)" 284-286.

22. Klas Norrby. Mast cells and angiogenesis. APMIS. 110(5): 355-371.