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Increased Mast Cell Density in Axillary Lymph Nodes without Metastasis in Breast Carcinoma: A Possible Protective Role

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Abstract

Introduction: One of the most important prognostic factors in breast cancer is metastasis to regional lymph nodes resulting in a reduced overall survival rate. Mast cells in tissue release both pro-tumourigenic and anti-tumourigenic substances and can determine progress of the tumour including invasion and metastasis. The present study endeavored to assess mast cell density (MCD) in regional axillary lymph nodes in cases of carcinoma breast with clinicopathological correlation to infer their biological role and pathological significance. *Methods:* Tissue from forty seven cases of invasive carcinoma of breast (mastectomy with regional axillary lymph node clearance) was analyzed and routine histological findings were recorded. Mast cells were clearly demonstrated in tissue using Toluidine Blue stain at pH 2.3. Mast cells were counted using an eyepiece grid and expressed as no. of cells / per sq. mm, i.e., mast cell density (MCD). The distribution of mast cells within all the sampled lymph nodes (with and without metastasis) was recorded. *Results:* Mast cell density was statistically significantly ($p<0.0001$) increased in regional axillary lymph nodes without microscopic metastatic deposits (n= 255; Mean MCD +/- SD: 6.0 +/- 3.4) compared to lymph nodes showing metastatic deposits (n=140; Mean MCD +/- SD: 0.57 +/- 0.59). *Conclusions:* Our results indicate a clear anti-tumourigenic role of mast cells within the regional lymph node tissue in carcinoma breast, possibly limiting the mechanisms for invasion of lymphoid tissue and limiting the metastasis. Mast cell density could thereby be a significant prognostic indicator in the management of patients with breast carcinoma.

Keywords: Breast Carcinoma; Lymph Node; Mast Cell; Metastasis; Toluidine Blue.

Introduction

Cancer is the leading cause of death worldwide accounting for 13% of all deaths [1]. Cancers of the lung, stomach, colon, and breast have been found to cause the maximum number of deaths worldwide. Mortality and morbidity rates in cancers have not reduced significantly even in this modern era of advancements in early diagnosis, therapeutics and the expanding repertoire of prognostic and predictive markers available. Newer prognostic indicators particularly reflecting on the intrinsic biological potential of the tumours are desirable for further stratification of patients and appropriate clinical management.

Mast cells are potent effector cells of the immune system that infiltrate the tumoural stroma and periphery of the tumours along with other inflammatory cells like cytotoxic T cell subsets, macrophages and fibroblasts. The diverse mediators derived from mast cells are reported to have both pro-tumourigenic and anti-tumourigenic effects. The balance between these opposing mechanisms determines their net effect on the progression or regression of the tumour at any given stage [2].

Accumulation of mast cells has been shown to have varied prognostic significance in many solid organ and haematolymphoid malignancies [3,4]. In fact, presence of mast cells is now considered an

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independent prognostic marker in certain cancers. Mast cells are also being considered a predictive marker in certain malignancies where therapeutic approaches targeting mast cells and/or mast cell mediators could be effective. In such cancers, mast cell density and distribution could enable identifying patients likely to respond to targeted anti-mast cell therapy. The detrimental or beneficial effects of mast cells in malignancies are still an ongoing debate in the scientific community.

Breast carcinoma is the leading cause of death in women with 1 million deaths reported annually. Metastasis to axillary lymph nodes is one of the most important prognostic factors in breast cancer associated with a reduced overall survival rate. The role of mast cells in the outcome of breast carcinoma is being actively studied [5-7]. This study was undertaken to infer the pathological significance of mast cells within axillary lymph nodes in carcinoma breast.

Materials and Methods

Study Design

The study was conducted at the Department of Pathology after obtaining approval from the Institutional Ethics Committee. Histopathology specimens representing carcinoma breast received in the laboratory between January 2011 and July 2013 were included in this cross sectional study.

Material

Paraffin-embedded tissue blocks and stained sections were retrieved for cases received prior to July 2011. For cases received during and after July 2011, fresh tissue and formalin fixed tissue from surgical resection specimens (mastectomy) was used. A total of 47 cases of breast carcinoma were included in this study.

Methods

Clinical parameters like age, gender, history of chemotherapy etc. were obtained from the referring departments and retrieved from the Medical Records Department. Results of previous biopsy / FNAC (Fine Needle Aspiration Cytology) reports were also recorded. Mastectomy specimens with axillary lymph node clearance were routinely sampled. The number and size of lymph nodes dissected along with gross evidence of tumour deposits (if any) was recorded.

(i) Histopathological evaluation (H &E): H & E stained tissue sections were evaluated microscopically (OLYMPUS- CX-21; Field area of 0.196 mm^2). Routine microscopic parameters in carcinoma breast including the following were recorded: (1) Histological type, (2) Histological grade, (3) Involvement of surgical margins, (4) Axillary lymph node status – number of lymph nodes with metastatic deposits (if any)

(ii) Demonstration of mast cells in lymph node tissue using Toluidine blue stain: Mast cells were demonstrated histochemically on tissue sections of all axillary lymph nodes (with and without metastatic deposits from breast) by staining with 1% acidified toluidine blue solution [8,9].

a. Material: "Microscopy- grade Toluidine Blue" (Loba Chemie; CI no: 52040; Lot no: S26701111; Dye content- 80%; Solubility- 0.1%) was used for preparing a water clear solution. An electronic pH meter (Eutech Instruments: Catalog No: 35624-02) was used to control the pH.

b. Mast cell counting: Toluidine blue stained sections were microscopically examined immediately along with the corresponding H &E stained slides. Mast cells were identified on sections due to the violet-purple metachromatic staining of their granules against the blue orthochromatic background.

Mast cells were counted on sections using an eyepiece grid (model WF-18). Each side of the large square represented one millimeter (mm) on the tissue section. The power fields were used for counting mast cells and the average density was expressed as:

Mast Cell Density (MCD) = No. of Mast Cells/ sq. mm area of the Tissue Section.

Statistical Analysis: Data Analysis was performed using SPSS (Statistical Package for the Social Sciences, v 17.0) software. Mast cell density (MCD) in lymph node sections was compared between the group of lymph nodes showing tumour deposits and the group without tumour deposits. A p-value of less than 0.05 was considered significant.

Results

A total number of 395 lymph nodes were studied from 47 cases of carcinoma breast (mastectomy specimens) with regional lymph node clearance. Numerous mast cells were observed in lymph node tissue without metastatic deposits from invasive carcinoma of breast (Figure 1, 2). A marked reduction or near absence of mast cells in lymph nodes with metastatic tumour deposits was observed even if majority of lymph node parenchyma was uninvolved

by the tumour (Figure 3,4). Mast cell density (MCD) was statistically significantly increased (p<0.0001) in the group of lymph nodes without metastatic deposits from breast carcinoma (n= 255; Mean MCD +/- SD: 6.0 +/- 3.4) compared to lymph nodes showing metastatic deposits (n=140; Mean MCD +/- SD: 0.57 +/- 0.59) of varying degrees (Table 1).

Table 1: Comparison of mast cell density in lymph node tissues with and without metastasis from invasive carcinoma of breast

Lymph node status	No. of lymph nodes	Mean Mast Cell Density in lymph node (MCD) +/- SD
Metastasis present	140	0.57 +/- 0.59
Metastasis absent	255	6.0 +/- 3.4
		p<0.0001

Fig. 1: Axillary lymph node without evidence of tumour deposits in invasive carcinoma breast (H &E; x100)

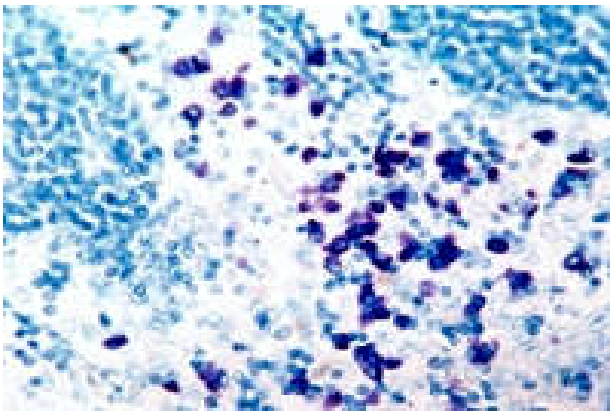

Fig. 2: Numerous mast cells (with violet- purple granules) seen in the above lymph node not involved by tumour (Toluidine Blue stain; x400)

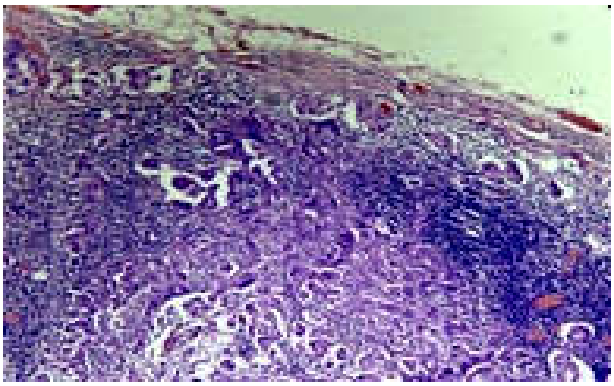

Fig. 3: Axillary lymph node with metastatic tumour deposit from invasive carcinoma breast (H &E; x100)

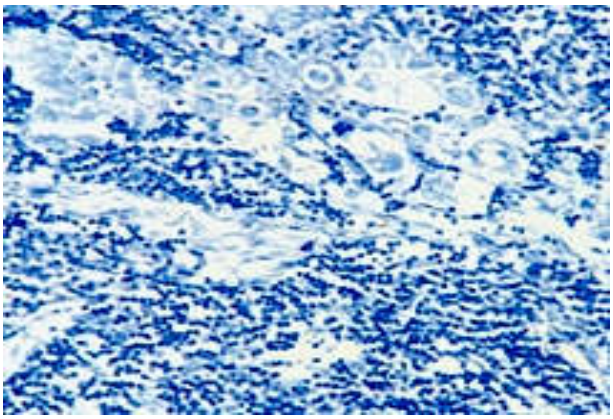

Fig. 4: Mast cells were markedly reduced/ absent in axillary lymph node with metastatic tumour deposits (of varying degrees) from invasive carcinoma breast (Toluidine Blue stain; x400)

Discussion

Mast cells were clearly demonstrated in tissue sections using toluidine blue staining method. The present study has found a statistically significant (p <0.0001) increased mast cell density in axillary lymph nodes without tumour deposits compared to those lymph nodes with metastatic tumour deposits. Metastasis from primary breast tumour through lymphatic/ and or vascular emboli involves many stages including breakdown of extracellular matrix (ECM) and migration through the ECM to reach distant sites and then enter the tumour microcirculation. The biological functions of mast cells include release of both pro-tumorigenic and anti-tumorigenic roles in the tumour microenvironment. This also includes their role in releasing or inhibiting enzymes like chymase and tryptase that degrade the ECM.

Though it would be difficult to directly infer the in-vivo function of mast cells without a functional study, these results clearly indicate a possible protective role of mast cells in the progression of breast carcinoma. Earlier, two similar studies (Mahopatra et al, Bowers et al) were done in Western population and one (Naik et al) in a North Indian population have

analyzed the significance of mast cells in axillary lymph nodes in carcinoma breast and mast cells were manually counted. The difference in the number of mast cells per sq. mm between the two groups of lymph nodes varied and was not consistent. The present study is the first of its kind in South Indian population and has also employed an objective methodology to accurately count mast cells using an eyepiece grid. Our results also showed a near absence of mast cells in lymph nodes with metastatic tumour deposits irrespective of the extent of deposits and even with partially retained lymph node parenchyma, whereas previous studies showed presence of a minimal baseline level number of mast cells in lymph nodes and variation with extent of tumour deposits.

Conclusion

Axillary lymph node status is an important prognostic indicator in the overall survival rates of breast cancer, poorer outcomes associated with axillary lymph node metastasis. Our study indicates that presence of increased mast cells in lymph node tissue could serve as a favourable prognostic factor and aid in risk stratification and better patient management. Mast cell density (MCD) data should be further correlated with prognostic information on the patients like yearly survival rates and disease progression to accurately state the utility of MCD as an independent prognostic factor. More functional studies (*in-vitro)* are warranted to infer the biological role of mast cells in breast cancer and consider targeted therapy using mast-cell directed agents.

Immuunohistochemistry (IHC) based demonstration and counting of mast cells including mast cell subtypes could help in accurately assessing their pathological significance in the micro-environment of breast carcinoma, its progression and clinical outcome.

References

1. Ferlay J, Shin HR, Bray F, Forman D, Mathers C and Parkin DM. GLOBOCAN 2012 v2.0, Cancer Incidence and Mortality Worldwide: IARC Cancer Base No. 10 [Internet]. Lyon, France: International Agency for Research on Cancer; 2012. Available from: http://globocan.iarc.fr.
2. Theoharides TC, Conti P. Mast cells: the Jekyll and Hyde of tumor growth. Trends Immunol 2004; 25(5):235–41.
3. Johansson A, Rudolfsson S, Hammarsten P, Halin S, Pietras K, Jones J, et al. Mast cells are novel independent prognostic markers in prostate cancer and represent a target for therapy. Am J Pathol 2010; 177(2):1031–41.
4. Kirchhoff D, Bischoff SC, Maurer M, Zollner TM. Mast cells in health and disease: from basic science to clinical application 4-5 July 2008, Stuttgart, Germany. Expert Opin Ther Targets 2008; 12(12):1591–4.
5. Rajput AB, Turbin DA, Cheang MC, Voduc DK, Leung S, Gelmon KA, et al. Stromal mast cells in invasive breast cancer are a marker of favourable prognosis: a study of 4,444 cases. Breast Cancer Res Treat 2008;107(2): 249–57.
6. Dabiri S, Huntsman D, Makretsov N, Cheang M, Gilks B, Bajdik C, et al. The presence of stromal mast cells identifies a subset of invasive breast cancers with a favorable prognosis. Mod Pathol Off J United States Can Acad Pathol Inc 2004;17(6):690–5.
7. Kankkunen JP, Harvima IT, Naukkarinen A. Quantitative analysis of tryptase and chymase containing mast cells in benign and malignant breast lesions. Int J Cancer J Int Cancer 1997;72(3):385–8.
8. Bancroft JD, Floyd AD, Suvarna SK. Bancroft's theory and practice of histological techniques: Churchill Livingstone Elsevier; 2013 [cited 2013 Oct 6].
9. Sridharan G, Shankar AA. Toluidine blue: A review of its chemistry and clinical utility. J Oral Maxillofac Pathol JOMFP 2012;16(2):251–5.
10. Mahapatro RC, Bowers HM., Jr Distribution of mast cells in the axillary lymph nodes of breast cancer patients. Cancer. 1979 Aug; 44(2):592–597
11. Bowers HM Jr, Mahapatro RC, Kennedy JW. Number of mast cells in the axillary lymph nodes of breast cancer patients. Cancer 1979;43(2):568–73.
12. Naik R, Baliga P, Bansal R, Pai M. Distribution of mast cells in the axillary lymph nodes of breast cancer patients. J Indian Med Assoc 1997; 95 (12):606–7.