Original Research Article

# Differential mast cell density in spectrum of benign neoplasms of breast: potential for patient stratification and personalised treatment approach

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## Abstract

**Background:** Mast cells have a clear anti-tumorigenic role in invasive breast carcinoma. However, their role in the stepwise progression of benign fibroepithelial neoplasms of breast and mesenchymal tumours of the breast is less understood. Increased C-KIT (a receptor tyrosine kinase) expression in those tumours have been found to be due to presence of mast cells and a potential for patient stratification and individualized targeted therapy especially in phyllodes tumours. The present analysis was undertaken to assess the differential mast cell density within the spectrum of benign fibroepithelial neoplasms of the breast. **Methods:** Tissue from fifty three cases of fibroadenoma and it variants including cellular fibroadenoma, fibroadenoma with increased stromal cellularityand benign phyllodes tumour were included in the study. Mast cells were clearly demonstrated in breast 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). Differential mast cell density in spectrum of benign breast neoplasms was analysed and statistical analysis was done. **Results:** Mast cell density was statistically significantly higher in tissue from fibroadenoma compared to normal breast tissue. MCD was increased in cellular fibroadenoma and fibrodenoma with increased stromal cellularity compared to fibroadenoma. MCD was increased in benign phyllodes tumour compared to fibroadenoma with increased stromal cellularity, cellular fibroadenoma and fibroadenoma. **Conclusions:** Our results indicate a relative and progressive increase in mast cells in fibroadenomas compared to normal breast tissue and comparatively increased with increased cellularity and increased stromal cellularity among the spectrum of fibroepithelial neoplasms. The study could be extended with larger sample size especially of phyllodes tumours and combined with immunohistochemical (IHC) methods (antibodies for CD 117) could be used for patient stratification and selection for personalized treatment including anti-C-KIT therapy.

**Key words**: Mast cell, Fibroadenoma, fibroepithelial lesion, Benign breast neoplasm, Phyllodes tumour

## Introduction

The incidence of cancers is on the rise worldwide resulting in significant morbidity and mortality [1]. Breast cancer is one of the widely studied cancers for developing biomarkers for early diagnosis, prognostic and predictive markers and advanced therapeutic options for increased patient survival rates as well as to improve the quality of life of patients. Individual tumour biology and the interactions within the tumour microenvironment play a major role in the progression of the tumours from benign to pre-malignant stage to initial stages of tumour development and progression of cancers [2]. Mast cells are one of the potent immune cells in the tumour micro environment which release mediators that have both pro-tumorigenic and anti-tumorigenic role [3-7]. The role of mast cells in invasive breast cancer has been predominantly found to have a beneficial role for the patient [8, 9].

Benign breast neoplasms fall in a spectrum of fibroepithelial lesions with proliferation of epithelial and stromal component of varying proportions with overlapping morphological findings. The spectrum of benign breast lesions comprise of fibroadenoma,

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cellular fibroadenoma, fibroadenoma with increased stromal cellularity, benign phyllodes tumour the latter having the highest stromal component. A step wise progression of these fibroepithelial lesions can lead to borderline phyllodes tumour and malignant phyllodes tumour. Many studies have been done to understand the underlying mechanisms in the formation of these biphasic benign tumours and the progression to each lesion towards the right of this spectrum [10,11].

Mast cells have been found to be increased in fibroadenomas, cellular fibroadenomas and phyllodes tumours. Studies have also shown step wise progression of these lesions resulting in epithelial and stromal proliferation, the increased stromal proliferation associated with presence of increased mast cells [12-15].

A subset of mast cells expresses SCF (Stem cell Factor) which can bind to its receptor C-KIT (also known as CD 117; a type of receptor tyrosine kinase). It has been shown that increased C-KIT expression noted in these spectrum of benign breast neoplasms in varying proportion is a mast cell phenomenon and mast cell derived mediators may play a role in progression to borderline and malignant phyllodes tumours [16-19].

Anti- C-KIT therapy is one of the potential treatment modalities currently used in the management of phyllodes tumours.

In this study, we have evaluated mast cell density in the spectrum of benign breast neoplasms and correlated to clinicopathological data to understand the significance with varying amount epithelial and stromal components.

## Materials and Methods

**Place and Type of Study-** The study was conducted at the Department of Pathology, SRM Medical College Hospital & Research Centre, Kattankulathur, Tamil Nadu, India during the time period between July 2011 and July 2015.

This cross sectional study was carried out after obtaining approval from the Institutional Ethics Committee.

**Inclusion criteria:** Cases with histopathological specimens of benign breast neoplasms between July 2011 and July 2015 with adequate clinical data

**Exclusion criteria:** Cases with inadequate material and those cases in which the slides/ blocks were not available were also excluded.

**Sample Collection and sampling methods-** A random sampling of histopathology specimens representing benign breast neoplasms in the above time period were included in this cross sectional study. A total of fifty three cases were included in the study. Clinical parameters like age and other relevant information were obtained from the referring departments and from hospital records. Paraffin-embedded tissue blocks and histopathological examination of H & E stained section from benign breast neoplasms were studied and diagnosis was recorded.

**Demonstration of mast cells in lymph node tissue using Toluidine blue stain:** Mast cells were demonstrated histochemically on tissue sections on all cases by staining with 1% acidified toluidine blue solution at pH2.3 [20, 21].

**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 and 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 Methods:** Data Analysis was performed using SPSS (Statistical Package for the Social Sciences, v 17.0) software.

A p-value of less than 0.05 was considered significant.

## Results

A total of fifty-three cases of benign breast neoplasms were studied. One case of giant fibroadenoma (Figure 1) was part of the study.

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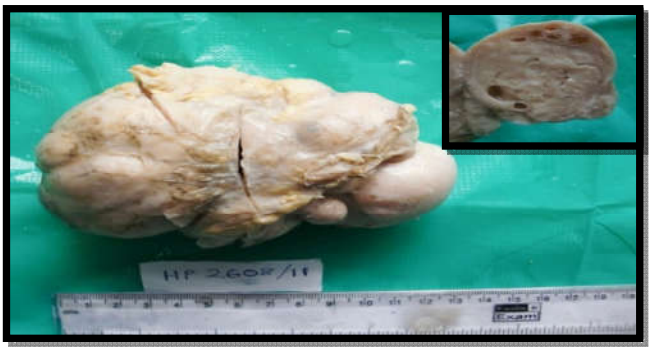

**Fig1: Giant fibroadenoma (gross appearance)**

**A well circumscribed, encapsulated breast neoplasm >12 cm in greatest dimension (Inset: Cutsurface shows solid homogenous grey white lesion with slit-like spaces and myxoid change)**

**a) Age distribution:** The age of patients in benign neoplasms ranged from 16 to 35 years with a mean age of 23.5 years

**b) Histopathology diagnosis:** Out of a total of fifty three cases of benign breast neoplasms, forty four cases were conventional fibroadenomas, five cases of cellular fibroadenoma, three cases of fibroadenoma with increased stromal cellularity and one case of benign phyllodes tumour were diagnosed (Figure 2)

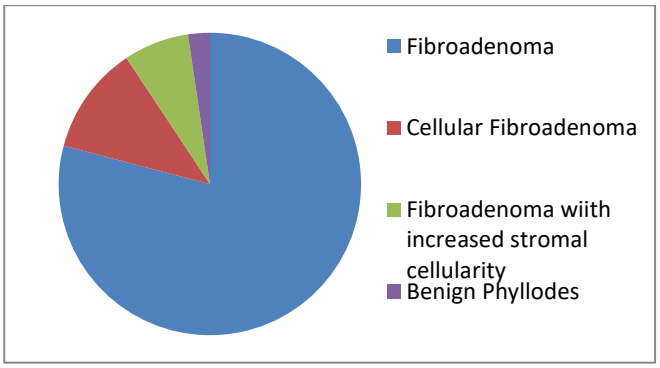


**Fig2: Histological types of benign breast neoplasms (n= 53)**

**c) Mast cell counting and Mast cell density in normal and neoplastic breast tissue:** The mast cell density in benign neoplasms of the breast was compared with the mast cell density in normal breast tissue.

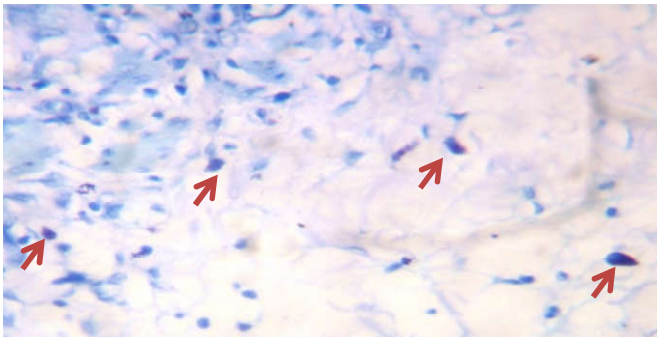

**Fig3: Mast cells were demonstrated by toluidine blue staining**

**Mast cells having violet-purple granules (arrows) (Metachromatic staining; Toluidine Blue Stain; x 400)**

The mean mast cell density across all cases of fibroadenoma was higher compared to normal breast tissue (Table 1). The difference was statistically significant (p- value <0.0001).

**TABLE 1: Mast Cell Density in Normal and Benign Neoplastic Breast Tissue.**

| Tissue examined | No. of cases | Mean MCD +/- SD |
|---|---|---|
| Control breast tissue | 10 | 0 |
| Fibroadenoma | 44 | 2.17 +/- 0.75 |
| | | **p < 0.0001**** |

The mast cell density in the case of giant fibroadenoma was not statistically significantly different from other cases of fibroadenoma

**d) Mast Cell Density in Spectrum of Benign Breast Neoplasms-** The mean mast cell density in cellular fibroadenoma was higher than conventional fibroadenoma (Figure 3, 4), but the difference was not very statistically significant (Table 2).

**TABLE 2: Mast Cell Density in Spectrum of Benign Breast Neoplasms.**

| Tissue examined | No. of cases | Mean MCD +/- SD |
|---|---|---|
| Fibroadenoma | 44 | 2.17 +/- 0.75 |
| Cellular fibroadenoma | 5 | 3.15 +/- 0.35 |
| Fibroadenoma with increased stromal cellularity | 3 | 4.75 +/- 0.45 |
| Benign Phyllodes | 1 | 5.82 +/- 0.25 |
| | | p=0.08 |

The mean mast cell density (MCD) in cellular fibroadenoma (Figure 3, 4) was higher than conventional fibroadenoma. MCD in fibroadenoma with increased stromal cellularity was higher than in cellular fibroadenoma. MCD in Benign phyllodes was slightly higher than fibroadenoma with increased stromal cellularity. However, the difference in MCD was not statistically significant (Table 2)

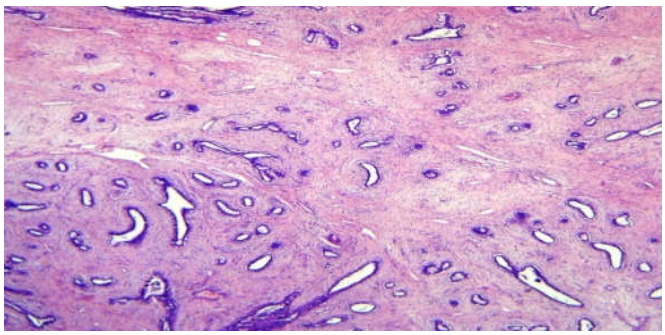

**Fig4: Cellular fibroadenoma- histopathology**

**Fibroadenoma with increased cellularity, few foci of epithelial hyperplasia (H & E x 40)**

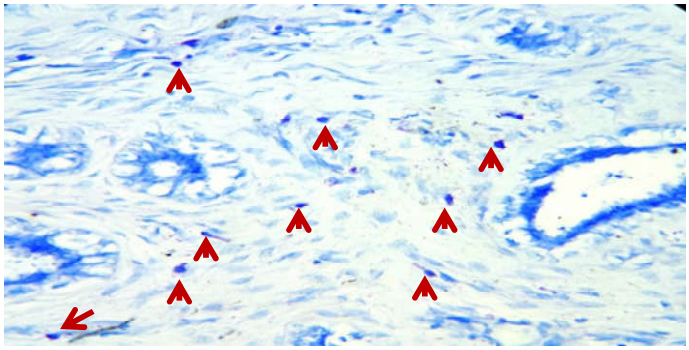

**Fig5: mast cell density in cellular fibroadenoma**

**Increased mast cells (arrow) in cellular fibrodenoma (Toluidine Blue stain; x 400)**

## Discussion

Mast cells have been shown to have both pro-tumorigenic and anti-tumorigenic role in the tumour microenvironment [3-7]. Their anti-tumorigenic and beneficial role in breast carcinoma has now been established [8, 9]. Their role in benign breast neoplasms and subsequent transformation into benign and malignant mesenchymal tumours of the breast is relatively less studied. Hussain *et al* [10] studied the stromal cellular response in breast tumours and found increased mast cell infiltration in fibroadenomas, but not in giant fibroadenomas. However, in the current study, we observed no statistically significant difference between MCD in fibroadenoma and giant fibro-adenoma. However, we have only one case of giant fibroadenoma in the present study (Figure 1).

Loke *et al* [11] have shown that there is a step wise distinctive progression by subsequent mutations in the origin of fibroadenoma and progression to other fibroepithelial lesions of the breast. Yang et al [13] reported that the spectrum of fibroepithelial lesions have overlapping morphological, immunohistochemical and molecular pathways with a stepwise progression from one stage to the other with increased stromal cellularity. Our results with gradual increase in mast cell density with increasing stromal cellularity conform to results from those reports.

Kashiwase*et al* [14] performed a quantitative analysis of mast cells in benign and malignant lesions and reported that the number of mast cells in intra-canalicular fibroadenoma was significantly lower than that in peri-canalicular fibroadenoma. We did not observe such a differential expression pattern in the present study. Kashiwase*et al* also showed that the MCD determined by Toluidine Blue staining and by immuno-histochemical methods were comparable.

Hence, Toluidine Blue staining which was also a simple and economical methodology was employed in this study to demonstrate mast cells. MCD was statistically significantly increased in neoplastic breast tissue compared to normal breast tissue [Table 1].

Djordjevic *et al* [15] reported that increased C-KIT (a receptor tyrosine kinase) expression in fibroepithelial lesions of the breast was a mast cell phenomenon, i.e. attributed to presence of increased mast cells, a subset of which expresses Stem Cell factor (SCF) which is a receptor for C-KIT (also known as CD 117). The resultsfrom the present study with increasing MCD in benign breast neoplasms as stromal cellularity increases (Table 2) indirectly conforms to those observations.

Vilela*et al* [16] have reported the use of mast cell density in distinguishing cellular fibroadenomas and benign phyllodes tumour for pre-operative diagnosis for patient stratification and personalised treatment approach. They have observed higher number of mast cells in benign phyllodes tumour compared to cellular fibroadenoma. Our results also conform to those observations but the difference was not statistically significant. However, our study has only one case of benign phyllodes tumour and this could affect statistical significance

Kondi-Pafiti et al [17] and Bose et al [19] have demonstrated the importance of C-KIT expression and indirectly the presence of differential mast cell density in the spectrum of benign breast neoplasms ending in benign phyllodes which could have step wise transformation to malignant phyllodes tumours. Hence, anti- C-KIT therapy for patients expressing the protein is one of the treatment modalities offered in the management of these tumours.

Our results show a differential mast cell density in a spectrum of benign breast neoplasms with increasing MCD correlating to increased stromal cellularity could be the reflection of a stepwise progression of these fibroepithelial neoplasms. Combined with immuno-histochemical methods (IHC) for more accurate detection of mast cell subsets, this could be helpful for potential patient stratification and targeted therapy and personalised treatment approach.

A limitation of the study is the lower number of cases of benign phyllodes and fibroadenomas with increased stromal cellularity and the study could be further extended with higher number of samples representing those neoplasms.

## Conclusion

Our study has utilized a simple and economical method to evaluate mast cell density in the spectrum of benign breast neoplasms. The progressive increase in mast cell density with increasing cellularity conforms to the previous studies. Since increasing C-KIT expressionin phyllodes tumours have been found to correlate with increased mast cells, there is potential to extend this study with larger number of samples and use for patient stratification and personalized treatment approach including possible targeted anti-C-KIT therapy. Immunohistochemical studies using antibodies to CD117 could help in identifying mast cell subsets more accurately.

**Contribution by different authors**

Dr. C.D. Anand contributed to conception of the study, study designing, literature search and review, experiment optimizing, performing the experiments, data acquisition and analysis, statistical analysis, manuscript preparation and editing; Dr.G. Shivashekar contributed to study designing, data analysis, manuscript preparation and editing; Dr. S. Muthu contributed to data analysis, statistical analysis, manuscript preparation and editing; Dr. Kalaivani Amitkumar contributed to histological data generation and data analysis; Dr. A. Sundaram contributed to enabling mast cell counting methodology, data analysis, manuscript preparation and editing;

**Additional knowledge gained from the study-** The present study is the first study in India on this topic as per standard literature review andadds new insights to existing knowledge in this area.

This data set indicates that mast cells play a modulatory role in the tumour microenvironment and in the stepwise progression withinthe spectrum of fibro-epithelial neoplasms of breast.

This is unlike a clear anti-tumorigenic and beneficial role of mast cells seen in invasive carcinoma of breast.

**Findings**: Nil; **Conflict of Interest**: None initiated
**Permission from IRB**: Yes

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