



## STUDY OF SIGNIFICANCE OF MAST CELLS IN IDENTIFYING MICROINVASION IN NON NEOPLASTIC LESIONS OF UTERUS.

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### ABSTRACT

Background: Mast cells are myeloid immune cells that take part in various physiological and pathological processes in our body. Their role in initiating angiogenetic mechanisms and in promoting tissue remodeling of neoplasms along with the prognostic significance of their presence in various tumors is still not completely understood. Mast cells have been noted in myometrium and observed in association with smooth muscle tumors of the uterus. This study attempts to study their role in Leiomyomas. **Objectives:** 1. To compare the mast cell density in leiomyomas of the uterus with the adjacent myometrium. 2. To compare the mast cell density in various degenerations in leiomyoma. 3. To compare the percentage of mast cell index in leiomyomas and adjacent myometrium. **Materials and Methods:** A prospective observational study of 100 consecutive hysterectomy specimens operated for Leiomyoma. Sections taken from the leiomyoma and the adjacent myometrium were stained with hematoxylin and eosin and toluidene blue stains. Mast cell density was calculated. **Results:** The average number of mast cells in the leiomyoma and adjacent myometrium was  $11.65 \pm 15.813$  and  $37.16 \pm 23.008$  respectively. There was a statistically significant difference noted between the two ( $p < 0.001$ ). **Conclusion:** A higher mast cell density was noted in the myometrium adjacent to the leiomyomas. This may indicate that they may be playing a role in the growth of leiomyomas by providing appropriate growth factors.

**Key words:** Leiomyoma, Mast cells, mast cell density, myometrium, smooth muscle tumor

### INTRODUCTION:

Mast cells were first described by Paul Ehrlich in 1878.<sup>1</sup> In the past two decades mast cells have gained importance for their involvement in both physiological and pathological processes.<sup>2</sup> They are involved in physiological processes like inflammation, angiogenesis, wound healing, fibrosis, tissue remodeling, and pathological conditions like asthma. In addition, they contribute to the pathogenesis of a variety of benign and malignant lesions involving various organ systems.<sup>1</sup> They are major components of the cancer microenvironment and are present in peri and intra-tumor sites. On stimulation mast cells releases enzymes like histamine, tryptase, chymase, VEGF, TNF- $\alpha$ , MMP, FGF, TGF- $\beta$ , and interleukins which have a pro-tumorigenic and anti-tumorigenic response.<sup>3</sup> Several types of tumor cells exhibit an increased production of stem cell factor, which stimulates mast cell migration,



proliferation, and degranulation.<sup>4</sup> Literature reports a close correlation between mast cells and angiogenesis in neoplasms. Mast cells were reportedly noted around the periphery of the tumor, in the adjacent connective tissue, and near lymphatics and blood vessels. It is suspected that they play a role in tumor development and progression as well as in angiogenesis.<sup>4</sup> The presence of mast cells in tumors has also been described as evidence of host immunologic antitumor response by inhibiting tumor through cytotoxic factors like TNF- $\alpha$  and granzyme B.<sup>3</sup> Accumulation of mast cells may be part of a generalized inflammatory reaction described in some tumors.<sup>5</sup> In the uterus, mast cells have been reported in the myometrium and uterine smooth muscle tumors.<sup>6</sup> Most of them were observed in close association with uterine smooth muscle cells, as well as in the vicinity of fibroblasts and collagen, and it appears they may play an important role in the reconstruction of uterine tissues during the menstrual cycle.<sup>2</sup> The significance of mast cells in non-neoplastic and neoplastic lesions of the uterus and cervix has been studied with conflicting results.<sup>4</sup> Mast cells' effect on uterine leiomyomas is still unclear. The present study was conducted in an attempt to know the role of mast cells in uterine leiomyomas.

#### OBJECTIVES:

1. To compare the mast cell density in leiomyomas of the uterus with the adjacent myometrium.
2. To compare the mast cell density in various degenerations in leiomyoma.
3. To compare the percentage of mast cell index in leiomyomas and adjacent myometrium.

#### Inclusion criteria:

1. All transabdominal hysterectomy cases performed for uterine leiomyomas.

#### Exclusion criteria:

1. Specimens consisting of autolyzed or necrosed tissue.
2. Non-appreciable myometrial tissues were excluded.
3. Specimens received in saline were excluded from the study.

#### MATERIALS AND METHODS:

A prospective observational study was conducted at Sree Balaji Medical College and Hospital in Department of histopathology for a period of 1 year from June 2022 to July 2023. The study was conducted after informed consent from the patients and The Ethics Committee of Bharath University of Medical Sciences approved this study. The institutional ethical committee at Bharath University of Medical Sciences approved all study protocols (Ref.No.008/SBMCH/IHEC/2022/2012). The 100 hysterectomy specimens received in the department of histopathology during the study were collected. From the hysterectomy specimens 2 sections were taken from the leiomyoma and its adjacent myometrium. These sections were processed routinely using formalin fixation and paraffin embedding. The blocks obtained were cut into 4 $\mu$ m sections and stained with Hematoxylin and Eosin stain for routine examination and 1% toluidine blue stain for detection of mast cells. Toluidine blue stains mast cells granules purple to red color. Regular H and E sections were studied by the pathologist and Mast cells were counted under 40X magnification for 10 consecutive fields in each slide in areas where maximum mast cells were seen and mast cell density (MCD) was calculated by the pathologist. The number of MCs/10hpf in leiomyoma was compared with the adjacent myometrium. The mast cell density in leiomyomas with degenerations were recorded and compared.

#### STATISTICAL ANALYSIS:

The data were analysed using STATISTICA 10 PL (StatSoft, USA). All results of mast cell counts were expressed as mean  $\pm$  standard deviation ( $\pm$ SD). The significance of differences between the groups was assessed with the Tukey's test at  $P \leq 0.05$ , which consider statistically significant.



## RESULTS:

Out of 100 uterine leiomyoma cases, 41% cases were with leiomyomas and 17% cases were leiomyoma with hyaline degeneration. The average number of mast cells in the leiomyoma and adjacent myometrium was  $11.65 \pm 15.813$  and  $37.16 \pm 23.008$  respectively. There was a statistically significant difference noted between the two ( $p < 0.001$ ).

Out of 100 cases of uterine leiomyomas, 36% of leiomyomas showed degeneration and 64% of leiomyomas showed no degeneration. The average number of mast cells per 10 high power fields in leiomyomas with degeneration and without degeneration was  $12.181 \pm 18.714$  and  $11.359 \pm 14.0759$  respectively. There was no statistically significant difference between the two ( $p$ -value 0.805). The average number of mast cells per 10 high power fields in adjacent myometrium of uterine leiomyomas with degeneration and without degeneration were  $36.931 \pm 19.0350$  and  $37.289 \pm 25.1102$  respectively and there was no statistically significant difference between the two ( $p$ -value 0.941).

Among the 36 cases displaying degeneration, 34 cases had hyaline degeneration and 2 cases had myxoid degeneration. The average number of mast cells in the adjacent myometrium of uterine leiomyoma per 10 high-power fields with hyaline degeneration and with myxoid degeneration was 39 and 27 respectively.

## DISCUSSION

Mast cells are a component of almost all vascularised tissues.<sup>8</sup> Mast cells are a type of leucocytes that originate from the bone marrow. Paul Ehrlich reported the presence of mast cells 130 years ago.<sup>8</sup> The role of mast cells in the pathogenesis of various lesions is still unclear. However mast cells play a role in homeostasis in the immune system. Cajal et al found that mast cells were closely associated with some epithelial tumors thereby suggesting their importance in the host defense mechanism.<sup>9</sup> Mast cells are involved in the pathogenesis of allergic reactions, pathogen immune response during infection and parasitic tissue responses. But beyond all those, they are now being recognized to play a significant role in the development of tumors.<sup>10</sup> The major point linking mast cells to cancer is their capacity to secrete and release potent angiogenic compounds.<sup>11</sup>

A study by Linling Jiang et al shows that mast cell count may be a useful index in differentiating benign leiomyomas from leiomyosarcomas.<sup>12</sup> The stroma surrounding the tumor tissue contains mast cells which secrete angiogenic cytokines and proteases.<sup>13</sup> Several tumors are found to contain a striking number of mast cells.<sup>14</sup> The human uterus, particularly the myometrium, is considered to be proportionately rich in mast cells when compared to all other tissues of the body. These mast cells are found in relation to smooth muscle cells and connective tissue.<sup>15</sup> Very few studies are found to have explored the relationship of mast cells with leiomyoma in general and leiomyomas with a component of degeneration in specific. Mast cells are developed in the bone marrow and these cells mature under local tissue microenvironment in the tissues. Mast cells are normally present around blood vessels, skin, gastrointestinal tract and lungs, sites where it is exposed to foreign /external agents. Mast cells act as a first line of defense against invasion by outside agents. Mast cells also play an important role in tumor progression, the role of which is complex and poorly understood. Mast cells promote cancer growth by modulation of cancer microenvironment which includes fibroblasts, myofibroblasts, newly formed blood vessels and inflammatory cells. Mast cells act in cancer microenvironment as both promoter and inhibitor of tumor growth (Dyduch et al., 2012).



In the present study, a statistically significant difference ( $p < 0.001$ ) in the mean mast cell count between uterine leiomyomas and adjacent myometrium was observed. Average number of mast cells per 10 HPF in uterine leiomyoma was found to be 11.65 and that in the adjacent myometrium was found to be 37.16. Several studies conducted by different authors revealed similar results, viz. Apurva V et al<sup>1</sup>, who found 40 number of mast cells in the myometrium per 10 HPF, Gousuddin M et al<sup>9</sup> found 85.5 number of mast cells in the myometrium per 10 HPF, Orii et al<sup>10</sup> found 37 mast cells in the myometrium per 10 HPF and Erol A Y G et al<sup>4</sup> found 41 number of mast cells in myometrium per 10 HPF (Table 4).<sup>1,4,9,10</sup> This observation of a greater number of mast cells noted in the myometrium adjacent to the leiomyoma further compounds the suspicion that these cells may be playing a role in tumor angiogenesis.<sup>4</sup>

Mast cells release many proangiogenic and mitogenic factors and are involved in the degradation of the extracellular matrix. Histamine released by mast cells induces tumor proliferation through H1 receptors and suppress the immune system through H2 receptors and Interleukin-10 (IL-10), and tumor necrosis  $\alpha$  (TNF- $\alpha$ ). Mast cells also release proangiogenic factors such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), transforming growth factor- $\beta$  (TGF- $\beta$ ), IL-8 and matrix metalloproteinases which are involved in extracellular matrix degradation. Mast cells release chemical mediators that cause vasodilatation, edema with protein rich exudate. Perhaps such a milieu favours tumor invasion and spread. Average number of mast cells per 10 HPF in uterine leiomyoma with degeneration and in the adjacent myometrium was 12.181 and 36.931 respectively. A decreasing trend in the mean number of mast cells in the adjacent myometrium of uterine leiomyoma with degeneration was observed.

A study done by Abeyratne N et al found a low mast cell count in leiomyomas with hyaline degeneration.<sup>10</sup> As not many studies have been conducted in this regard, further research is suggested to ascertain the probable explanation for low mast cell counts in cases of leiomyomas with degeneration.

Erol A Y G et al conducted a study between microvessel density (MVD) and MCD (mast cell density) and found that no significant correlation existed between MVD and MCD in leiomyomas. They suggested that the presence of mast cells would indicate the benign nature of the myometrial lesions and this may be important in the assessment of malignant and premalignant lesions.<sup>15</sup>(Table 4)

#### **CONCLUSION:**

It is observed that there is higher mast cell density in the myometrium adjacent to the leiomyomas. This may indicate that they may be playing a significant role in the growth of leiomyomas by providing appropriate growth factors. The presence of mast cells would indicate the benign nature of the myometrial lesions and this may be important in the assessment of malignant and premalignant lesions and for the treatment response in patients with malignancy.

#### **Limitations of the study**

Study duration is 1 year and the sample numbers are limited.

#### **Ethical issues**

The research followed the tents of the Declaration of Helsinki. The Ethics Committee of Bharath University of Medical Sciences approved this study. The institutional ethical committee at Bharath



University of Medical Sciences approved all study protocols (Ref.No.008/SBMCH/IHEC/2022/2012). Accordingly, written informed consent taken from all donor participants before any intervention.

#### **Conflicts of interest**

The authors declare that they have no competing interests.

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**Table 1: Comparison of mast cell between Leiomyoma and Adjacent Myometrium**

	Group	N	Mean	Standard deviation	P value
Mast cells	Leiomyoma	100	11.6550	15.81342	<0.001
	Adjacent Myometrium	100	37.1600	23.00800	
	Leiomyoma with degeneration	100	6.6770	8.6780	

**Table 2: Comparison of Mast cells in Leiomyoma and adjacent myometrium between Degenerative and Non-degenerative Groups**

	Degeneration	N	Mean	Standard. Deviation



Mast cells in leiomyoma	Non-degenerative	64	11.359	14.0759
	Degenerative	36	12.181	18.7148
Mast cells in adjacent myometrium	Non-degenerative	64	37.289	25.1102
	Degeneration	N	Mean	Std. Deviation
Mast cells in leiomyoma	Non-degenerative	64	11.359	14.0759
	Degenerative	36	12.181	18.7148
Mast cells in adjacent myometrium	Non-degenerative	64	37.289	25.1102

**Table 3: Number of mast cells in adjacent myometrium with leiomyoma showing degeneration**

Degeneration	Number of cases (n%)	Average number of mast cells in adjacent myometrium per 10 hpf
Hyaline Degeneration	39	34 (94.4%)
Myxoid Degeneration	27	02 (5.6%)

**Table 4: Comparison of various studies in the literature with the current study**

Sl.no	STUDY	NUMBER OF MAST CELLS IN MYOMETRIUM
1	Erol A Y G et al	41/10 H
2	Apurva V et al	40/10 HPF
3	Gousuddin M et al	33/10 HPF
4	Orii et al	85.5/10 HPF
5	Present study	37/10 HPF

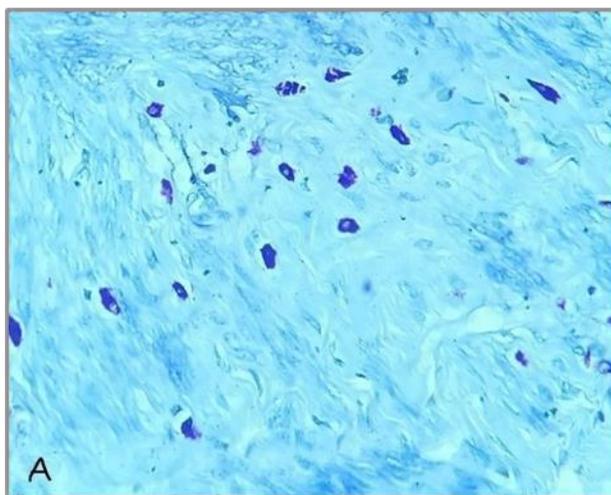
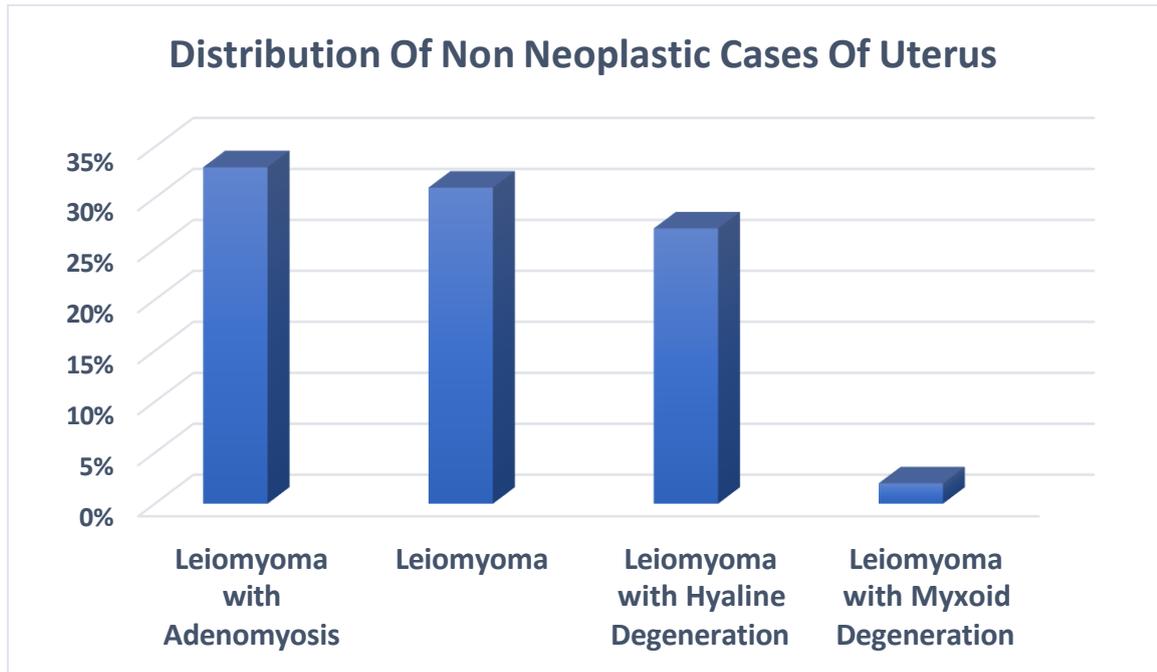


Figure 1 A, Toluidine blue, 40 x, showing mast cells stained with blue in the case of myometrium adjacent with leiomyoma with mast cell index 34%

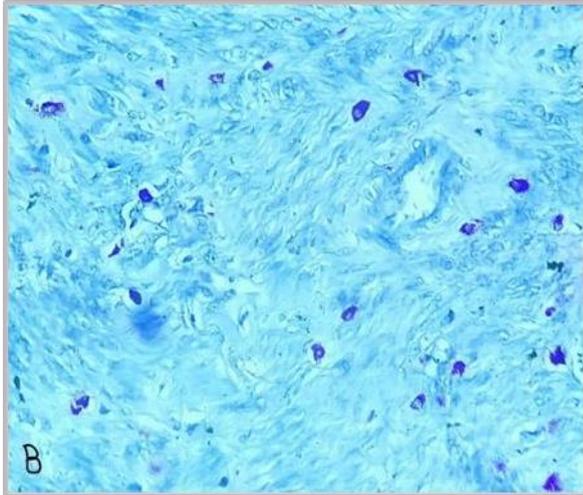


Figure 1 B Toluidine blue, 40 x, showing mast cells stained with blue in the case of Leiomyoma with degeneration with mast cell index 20%

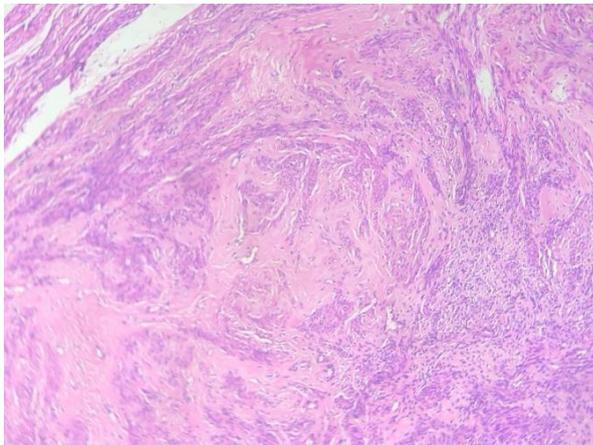


Figure 2 H&E stain of uterine Leiomyoma at low power with myxoid degeneration

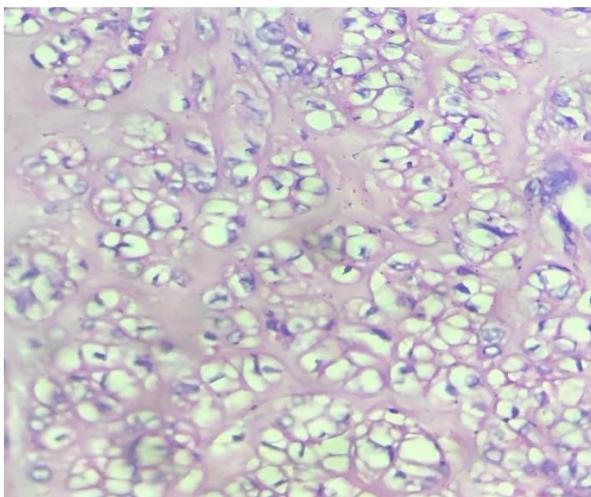
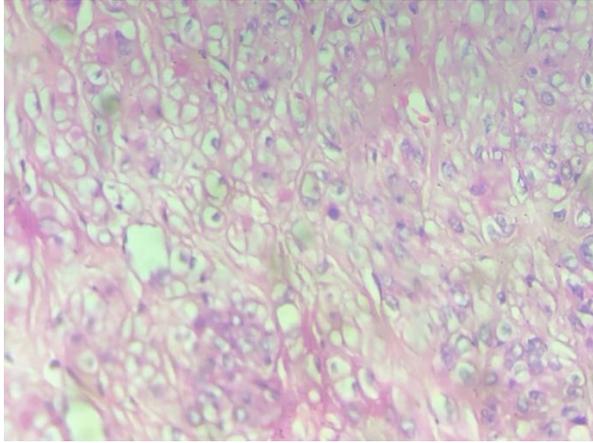


Figure 3: H&E stain of uterine leiomyoma with hyaline degeneration



**Figure 4: H&E stain of uterine leiomyoma showing mast cell at high power**