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Original Article

Pathology Section

Distribution of Mast Cells in Uterine Leiomyoma and Adjacent Myometrium: A Cross-sectional Study

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ABSTRACT

Introduction: Mast cells are involved in various physiological and pathological processes. Their role in neoplasm angiogenesis remains an enigma. Mast cells have been observed in association with smooth muscle tumours of the uterus. The role of mast cells in leiomyomas is conflicting, with some studies favoring tumour development and others showing the opposite.

Aim: To assess the distribution of mast cells in uterine leiomyomas and the adjacent myometrium.

Materials and Methods: This cross-sectional study included 100 consecutive hysterectomy specimens from patients who underwent surgery for leiomyoma over a period of one year and five months at PES Institute of Medical Sciences and Research, Kuppam, Andhra Pradesh, India. Sections from the leiomyoma and adjacent myometrium were stained with Hematoxylin and Eosin (H&E) and toluidine blue stains. Mast cell distribution was calculated. The data was collected and analysed using the Statistical Package for the Social Sciences (SPSS) (Version 23.0; SPSS Inc., Chicago, IL, USA). Mast cell counts were expressed as mean and standard deviation. The comparison between the means of different groups was performed using the "Independent sample t-test." A p-value<0.05 was considered statistically significant.

Results: A total of 100 leiomyoma cases were included in the study. The mean age of the study participants who underwent hysterectomy was 46.3±6.73 years, and the majority (32%) of them were in the age group of 46-50 years. The average number of mast cells in the leiomyoma and adjacent myometrium was 11.65±15.813 and 37.16±23.008, respectively. A statistically significant difference was observed between leiomyoma and adjacent myometrium (p-value<0.0001).

Conclusion: A higher distribution of mast cells was observed in the myometrium adjacent to the leiomyoma. This finding may indicate that mast cells play a role in the growth of leiomyoma by providing appropriate growth factors.

Keywords: Benign tumours, Degenerations, Mast cell density, Smooth muscle tumours, Uterus

INTRODUCTION

Mast cells were first described by Paul Ehrlich in 1878 [1]. Over the past two decades, mast cells have gained recognition for their involvement in physiological and pathological processes [2]. They play a role in physiological processes such as inflammation, angiogenesis, wound healing, fibrosis, tissue remodeling, as well as pathological conditions like asthma. Additionally, they contribute to the pathogenesis of various benign and malignant lesions affecting different organ systems [1].

Mast cells are components of the cancer microenvironment and are present in peri- and intratumour sites. Upon stimulation, mast cells release enzymes such as histamine, tryptase, chymase, Vascular Endothelial Growth Factors (VEGFs), Tumour Necrosis Factor α (TNF-α), Matrix Metalloproteinases (MMPs), Fibroblast Growth Factors (FGFs), Transforming Growth Factor-β (TGF-β), and interleukins, which have protumourigenic and antitumourigenic responses [3]. Several types of tumour cells exhibit increased production of stem cell factor, which stimulates mast cell migration, proliferation, and degranulation [4]. Literature reports a close correlation between mast cells and angiogenesis in neoplasms. Mast cells have been observed around the periphery of the tumour, in the adjacent connective tissue, and near lymphatics and blood vessels. It is suspected that they play a role in tumour development, progression, and angiogenesis [4]. The presence of mast cells in tumours has also been described as evidence of host immunologic antitumour response by inhibiting tumours through cytotoxic factors like TNF-α and granzyme B [3]. The accumulation of mast cells may be part of a generalised inflammatory reaction described in some tumours [5].

In the uterus, mast cells have been reported in the myometrium and uterine smooth muscle tumours [6]. Most of them were observed in close association with uterine smooth muscle cells, as well as in the vicinity of fibroblasts and collagen, suggesting they may play an important role in the reconstruction of uterine tissues during the menstrual cycle [2].

The significance of mast cells in non-neoplastic and neoplastic lesions of the uterus and cervix has been studied with conflicting results. Mast cells have been hypothesized to enhance tumour development based on their effects on angiogenesis. However, there is also mounting evidence suggesting that mast cells inhibit tumours [4]. In lesions of the cervix, Kalyani R and Rajeshwari G found a decreased number of mast cells in neoplastic lesions compared to non-neoplastic lesions [2]. In lesions of the uterus, although evidence exists that mast cells promote tumourigenesis, there are some clinical settings and experimental tumour models that suggest mast cell functions favoring the host [7]. Thus, the effect of mast cells on uterine leiomyomas is still unclear. Therefore, the present study was conducted in an attempt to determine the role of mast cells in uterine leiomyomas. A study by Abeyratne NV et al. found a low mast cell count in leiomyomas with hyaline degeneration. Since there are very few studies in the literature [8] exploring the distribution of mast cells in leiomyomas with degeneration, the present study aimed to compare the mast cell distribution in uterine leiomyoma with the adjacent myometrium and also compare the mast cell distribution in various degenerations in leiomyoma.

MATERIALS AND METHODS

This was a cross-sectional study of 100 consecutive hysterectomy specimens operated for leiomyoma and received in the Department of Pathology, PES Institute of Medical Sciences and Research, Kuppam, Andhra Pradesh, India. The study was conducted over a period of 17 months, from February 2019 to July 2020. This

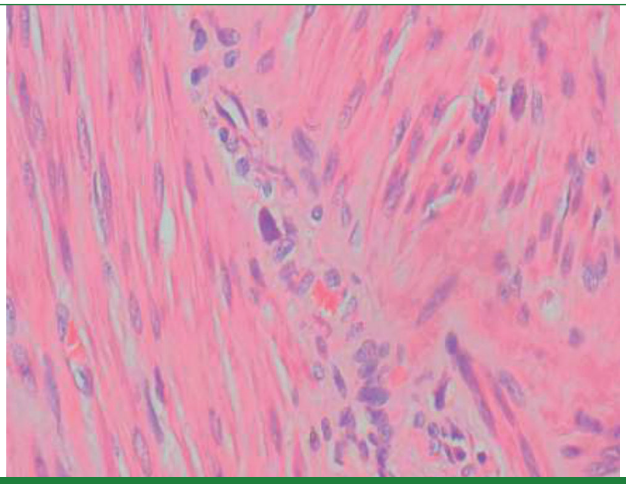
study was approved by the Institutional Human Ethics Committee (PESIMSR/IHEC/C-12/2022). Histopathological examination was conducted in the Department of Pathology.

Inclusion criteria: All hysterectomy cases with uterine leiomyomas were included in the study.

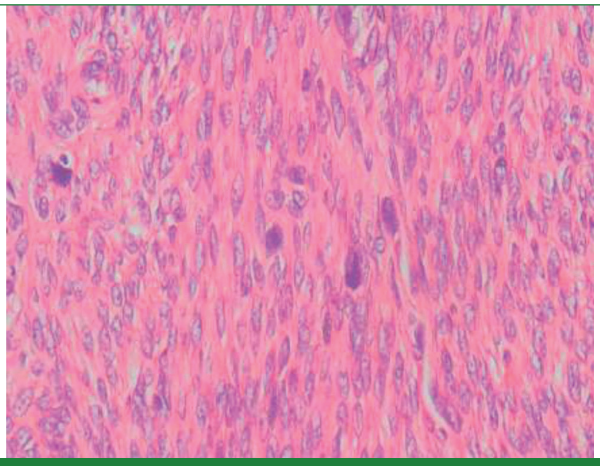
Exclusion criteria: Specimens consisting of autolyzed or necrosed tissue and non-appreciable myometrial tissues were excluded from the study.

Procedure

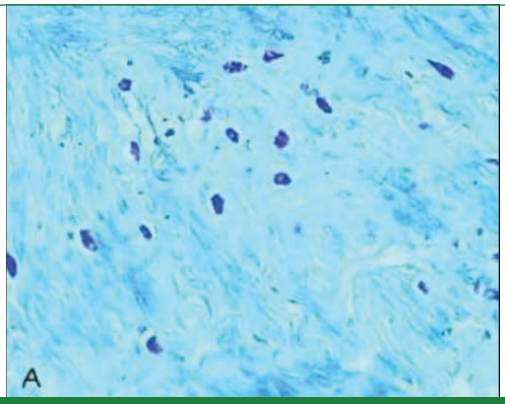
Two sections each were taken from the leiomyoma and its adjacent myometrium. These sections were routinely processed using formalin fixation and paraffin embedding. The obtained blocks were cut into 4 µm sections and stained with H&E stain for routine examination (shown in [Table/Fig-1,2]) and 1% toluidine blue stain as per routine protocol for the detection of mast cells [9]. Toluidine blue stains mast cell granules in a purple to red color (shown in [Table/Fig-3,4]).



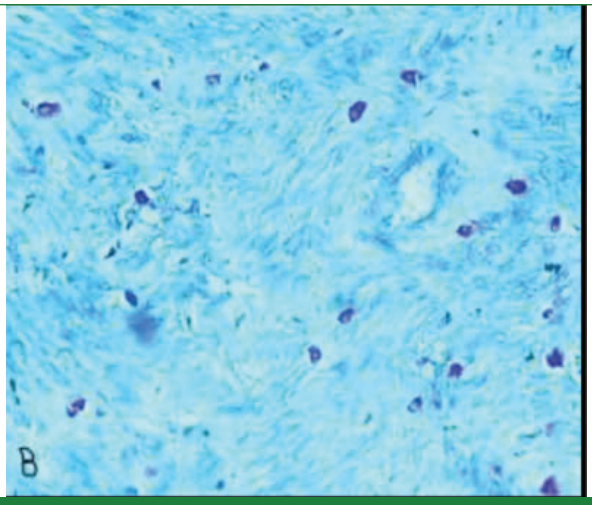
[Table/Fig-1]: Photomicrograph of mast cells in adjacent myometrium, H&E stain, x400.



[Table/Fig-2]: Photomicrograph of mast cells in leiomyoma, H&E stain, x400.



[Table/Fig-3]: Photomicrograph of mast cells in adjacent myometrium, Toluidine blue, x100.



[Table/Fig-4]: Photomicrograph of mast cells in adjacent leiomyoma, Toluidine blue, x100.

Mast cells were counted under 40X magnification for 10 consecutive fields in each slide in areas where the maximum number of mast cells were observed. The number of mast cells per 10 high-power fields (hpf) in the leiomyoma was compared with the adjacent myometrium. The mast cell distribution in leiomyomas with degenerations was recorded and compared. The distribution of different degenerations among the leiomyoma specimens, the mean and standard deviation of mast cells among the leiomyomas and their adjacent areas, and the mean and standard deviation of mast cells among degenerative and non-degenerative leiomyomas were determined in this study.

STATISTICAL ANALYSIS

The study details were collected, and the data was entered into MS Excel 2010. Further analysis was performed using Statistical Package for the Social Sciences (SPSS) (Version 23.0; SPSS Inc., Chicago, IL, USA). The mast cell counts were expressed as mean and standard deviation. The comparison between the groups was conducted using the "independent sample t-test". A p-value < 0.05 was considered statistically significant.

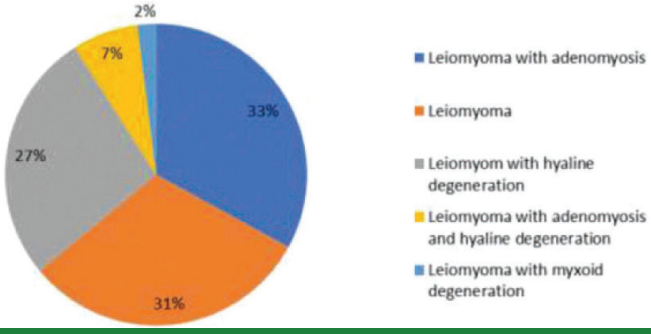
RESULTS

There were a total of 100 leiomyoma cases. The mean age of the study participants who underwent hysterectomy was 46.3±6.73 years, and the majority (51%) of them were in the age group of 41-50 years (shown in [Table/Fig-5]).

Age group (years)	Number (%)
31-40	29 (29)
41-50	51 (51)
51-60	20 (20)
Total	100 (100)

[Table/Fig-5]: Distribution of study samples based on age.

Distribution of leiomyoma cases is depicted in [Table/Fig-6].



[Table/Fig-6]: Distribution of cases.

The average number of mast cells in the leiomyoma and adjacent myometrium was 11.65±15.813 and 37.16±23.008, respectively. A statistically significant difference was noted between the two groups (p-value: <0.0001) (shown in [Table/Fig-7]).

	Group	N	Mean±SD	p-value
Mast cells	Leiomyoma	100	11.6550±15.81342	<0.0001
	Adjacent Myometrium	100	37.1600±23.00800	

[Table/Fig-7]: Comparison of mast cell between leiomyoma and adjacent myometrium.

The average number of mast cells in the leiomyoma and adjacent myometrium of leiomyoma with degeneration is shown in [Table/Fig-8].

	Degeneration	N	Mean±SD	p-value
Mast cells in leiomyoma	Non degenerative	64	11.359±14.0759	0.805
	Degenerative	36	12.181±18.7148	
Mast cells in adjacent myometrium of leiomyoma	Non degenerative	64	37.289±25.1102	0.941
	Degenerative	36	36.931±19.0350	

[Table/Fig-8]: Comparison of mast cells in leiomyoma and adjacent myometrium between degenerative and non-degenerative groups.

Among the 36 cases displaying degeneration, 34 cases had hyaline degeneration (including leiomyomas with hyaline degeneration-27 and leiomyoma with adenomyosis with hyaline degeneration-7), and 2 cases had myxoid degeneration. The average number of mast cells in the adjacent myometrium of uterine leiomyoma with hyaline degeneration and myxoid degeneration was 39 and 27 per 10 hpf, respectively (shown in [Table/Fig-9]).

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

[Table/Fig-9]: Number of mast cells in adjacent myometrium with leiomyoma showing degeneration.

DISCUSSION

Mast cells are present in virtually all vascularised tissues [10]. Derived from the bone marrow, mast cells are tissue-homing leukocytes that were initially described by Paul Ehrlich over 130 years ago [10]. The role of mast cells has been a puzzle for researchers. Cajal noted their close association with some epithelial tumours, suggesting their importance in the host defense mechanism [11]. They are known to be involved in the pathogenesis of allergic reactions and responses to parasitic tissue [11]. However,

recent studies have recognised their significant role in tumour development [12]. The major link between mast cells and cancer is their ability to secrete potent angiogenic compounds [13]. A study by Jiang L et al., suggests that mast cell count may be a useful indicator in differentiating between leiomyomas and leiomyosarcomas [7]. The stroma surrounding tumour tissue is believed to contain mast cells that secrete angiogenic cytokines and proteases [14]. Many tumours have been found to have a high number of mast cells [15]. The human uterus, especially the myometrium, is considered relatively rich in mast cells compared to other tissues in the body. These mast cells are located in proximity to smooth muscle cells and connective tissue [1]. Few studies have explored the relationship between mast cells and leiomyoma in general, and specifically in leiomyomas with degeneration [1,4,7]. Therefore, one of the objectives of this study was to compare the distribution of mast cells in various degenerations in leiomyoma, making it a unique study.

In the present study, a statistically significant difference (p-value <0.001) was observed in the mean mast cell count between uterine leiomyomas and adjacent myometrium. The average number of mast cells per 10 hpf in uterine leiomyoma was 11.65, while in the adjacent myometrium, it was 37.16. These results are consistent with similar studies that have reported the proportional distribution of mast cell numbers. For example, Apurva V et al., found 40 mast cells in the myometrium per 10 hpf [1], Gousuddin M et al., found 85.5 mast cells in the myometrium per 10 HPF, Orii A et al., found 37 mast cells in the myometrium per 10 HPF, and Erol AY et al., found 41 mast cells in the myometrium per 10 HPF [Table/Fig-10] [1,4,11,12]. However, direct comparison of mast cell numbers may not be possible due to the limited number of leiomyoma cases available for analysis and the limited number of tissue sections examined, which may not capture the full spectrum of mast cell distribution. The observation of a greater number of mast cells in the myometrium adjacent to the leiomyoma further supports the suspicion that these cells may play a role in tumour angiogenesis [4].

Study	Place and publication year	Number of mast cells/10 HPF
Apurva V et al., [1]	Muzaffarnagar, India 2016	Leiomyoma: 40.02±25.34 Adjacent myometrium: 65.70±30.96 (p-value<0.0001)
Erol AY et al., [4]	Turkey, 2011	23.2±7.8 in the stroma (p-value=0.987) 41.4±17.1 in the myometrium (p-value=0.810)
Gousuddin M et al., [11]	Kalaburagi, India 2015	Leiomyoma: 45-126 (Mean-85.5) Adjacent myometrium: Not mentioned
Orii A et al., [12]	Kyoto, Japan. 1998	Leiomyoma 33/10 HPF Adjacent myometrium: Not mentioned
Present study	Kuppam, Andhra Pradesh, India, 2023	Leiomyoma: 11.65±15.81 Adjacent myometrium: 37.16±23.00

[Table/Fig-10]: Comparison of various studies in the literature with the current study [1,4,11,12].

The 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 by Abeyratne NV et al. found a low mast cell count (<50/10 hpfs) in leiomyomas with hyaline degeneration [8]. As there have been limited studies 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 AY and Ozdemir O conducted a study on the correlation between Microvessel Density (MVD) and Mast Cell Density (MCD) and found no significant correlation between MVD and MCD in leiomyomas. They suggested that the presence of mast cells would indicate the benign nature of myometrial lesions, which may be important in the assessment of malignant and premalignant lesions [16].

Limitation(s)

The study was limited by the number of leiomyoma cases available for analysis. Histomorphological assessment of mast cells involves subjective interpretation by pathologists, which may have led to interobserver variability.

CONCLUSION(S)

It was observed that there was a higher distribution of mast cells in the myometrium adjacent to the leiomyoma. This may indicate that they play a role in the growth of leiomyoma by providing appropriate growth factors. The presence of mast cells may indicate the benign

nature of myometrial lesions, which could be important in assessing malignant and premalignant lesions.

Further studies on mast cell distribution may shed additional light on the role of mast cells in tumour biology.

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