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Study of density and distribution of mast cells in endometrium

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Abstract: *Introduction:* Mast cells are heterogenous group of immune cells involved in multiple biological events they play vital role in various inflammatory and immunological reactions, linking humoral and cell mediated phases of processes. *Aim and Objective:* In this study we have tried to compare the density and distribution of mast cells in various endometrial lesions. *Material and Methods:* A prospective study with 101 cases of post hysterectomy were studied. Hysterectomy specimens were cut open from anterior wall, incorporated endometrium and myometrium, fixed in 10% formalin after routine processing, embedded in paraffin, 5 micron thickness section taken and stained with Haematoxillin-Eosin, and toludine blue to visualize mast cells. *Results:* It showed a significant p value which was < 0.001. *Conclusion:* Mast cell profile may be an additional diagnostic/prognostic tool in different endometrial lesions.

Keywords: Mast Cell, Density Distribution, Endometrial Layer and Lesions.

Introduction

Mast cell was identified and named by Paul Erlich in 1878. The mast cell origin, distribution, structure, mediator and function of which are much debated as remained a cell of interest for workers. Its origin is attributed to CD34+ pluripotent, progenitor cells of bone marrow [1]. It circulates in a immature form, only maturing either around connective tissue or mucosal tissue site [2]. Mast cells are often known as histogenous mast cells to distinguish them from the hematogenous mast cells (basophilic leucocytes of blood). Their origin is not clear and functions are conjectural [3].

Human mast cells are long life tissue resident immune cells characterized by granules contains the proteases chymase and tryptase. Their phenotype is modulated by their tissue micro-environment [4]. They are widely distributed throughout the connective tissue of the body and are particularly concentrated around blood vessels [5]. The uterus has a nutrition role in development and growth of foetus, after which is subjected to various lesions (atropy, carcinoma, etc). The study attempts to observe for density of most calls in endometrial lesions and phases of menstrual cycle and its possible significance in diagnosis and prognosis [6].

The study of its histology and distribution of different cell types especially those having immunologic roles carries outstanding importance. Mast cells are placed in close proximity to fibroblast and collagen fibers during menstrual cycle, which indicates they have important role in uterine tissue reconstruction. During secretory phase intensification of, mast cells increases leading to extra cellular tryptase. Comparison of mast cell densities in different phases of menstrual cycle and endometrial lesions showed an increase in inflammatory process, while decrease in carcinomas.

The significance of mast cells in uterine tumor surveillance has been studied with conflicting results. The presence of mast cell in tumor has been described as evidence of immunologic and tumor response with good prognosis [7]

Material and Methods

The study includes endometrial sections in post hysterectomy specimens received in

department of pathology for 2 years which numbered to be 101 cases (Table-1).

Table-1: Following cases were taken for study	
Histopathological diagnosis	**No. of cases**
Proliferative phase	43
Secretory phase	38
Atrophic endomterium	08
Cystoglandular hyperplasia	03
Endometrial polyp	06
Endometrial carcinoma	03
Total	**101**

Inclusion criteria:

- Proliferative phase
- Secretory phase
- Atropihic endometrium
- CGH
- Endomterial hyperplasia
- Adenomatous hyperplasia
- Atypical hyperplasia
- Endometrial carcinoma

Exclusion criteria:

- Lesion like adenomyosis/ neoplastic lesions of myometrium proper.
- Pregnant uterus of caesarean hysterectomy.

All the samples were fixed in 10% formalin. The hysterectomy specimens were cut open, sections from anterior wall of uterus incorporated endometrium with adjoining myometrium. After routine processing, tissues were embedded in paraffin, 5 micron thickness sections taken, were cut and stained with haematoxillin-eosin and toludine blue stain to visualize the density and distribution of mast cells.

Staining procedure: The sections were taken on albunised slides and kept at 60^{o} for ½ an hour for fixation. The slides were kept in Xylene for 15 minutes for deparaffinization. Then brought to water through different grades of alcohol and water. The slide was placed in 1% toludine blue solution for 1 minute. Then rinse in water, differentiated in 95% alcohol clear in Xyelene and mounted with DPX.

Results

Total number of Cases Studied were 101 Cases. Below table-2 shows mean mast cell count per 10hpf in normal and endometrial lesions of uterus.

Fig-1: Bar chart showing % and frequency distribution of various lesions of endometrium

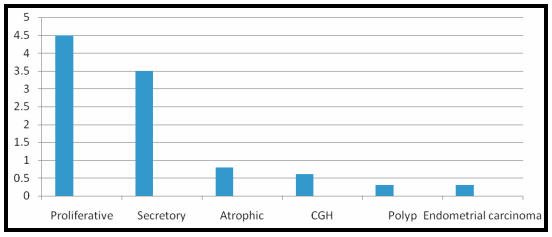


Table-2: 1: Mean mast cell count per 10 HPF in normal and endometrial lesions	
Diagnosis	**Mean mast cell count per 10 HPF**
Proliferative phase	12.43
Secretory phase	19.34
Atrophic endometrium	16.68
C.G.H.	7.63
Polyp	19.6
Endometrial Carcinoma	1.27
F-value	108.05
ANOVA p-value	P<0.0001
d.f.	5.95

Analysis of variance (ANOVA) followed by Turkey Kramma multiple comparison test-Statistical significance of difference in mast cell count (table-3).

Table-3: Turkey Kramma multiple comparison test- Statistical analysis		
Comparison	**P-value**	**Remark**
Proliferative phase vs secretory phase	P<0.001	Significant
Proliferative phase vs atrophic endometrium	P<0.001	Significant
Proliferative phase vs CGH	P<0.001	Significant
Proliferative phase vs Polyp	P<0.001	Significant
Proliferative phase vs Endometrial carcinoma	P<0.001	Significant
Secretory phase vs atrophiv endometrium	P<0.001	Significant
Secretory phase vs CGH	P<0.001	Significant
Secretory phase vs Polyp	P>0.05	Significant
Secretory phase vs Endometrial cancer	P<0.001	Significant
Atrophic endometrium vs CGH	P<0.001	Significant
Atrophic endometrium vs Polyp	P>0.05	Insignificant
Atrophic endometrium vs Endometrial cancer	P<0.001	Significant
Polyp vs Endometrial cancer	P<0.001	Significant
CGH vs Polyp	P<0.001	Significant
CGH vs Endometrialcancer	P<0.001	Significant

Discussion

Density and distribution of mast cells in endometrium as assessed in present study reveals close association of mast cells with endometrial morphology. In all the endometrial tissues studiedthe density of mast cells varied from area to area and in the same area from case to case (Table: 4). This important observation emphasizes that comparative study of mast cells density requires correct representation of various layers of endometrium of uterine wall.

Table- 4: Distribution of mast cells in various layers and lesions of endometrium						
Layer	**Proliferative phase**	**Secretory phase**	**Atrophic endrometrium**	**CGH**	**Polyp**	**Endometrial Ca.**
Subepithelial	0.02	0	0	0	0	0
Stroma	0.79	0.84	0.25	0.33	12.67	0
Peri Vascular	0.91	0.42	0.13	0	0	0
Periglandular	1.35	0.92	0.88	0.50	6.00	0
Basalis	8.47	11.16	6.63	4.50	4.67	0
Endomyometrial junction	14.4	19.76	17.13	9.67	17.67	0.67
Myometrium	36.23	63.58	58.25	23.17	57.00	5.67
Total	62.16	96.68	83.25	38.17	98.00	6.33

The present study is designed to, study more respective lesions of endometrium. Our study reveals increased number of mast cells in basal endometrium and endo-myometrial junction (Fig. 2 & 3 respectively). Similar observations when compared were seen in the study conducted by Louise Drudy et al and in addition it was also seen increased number in myometrium [8].

Fig-2: Mast cells in basalis-toludine blue stain 20X

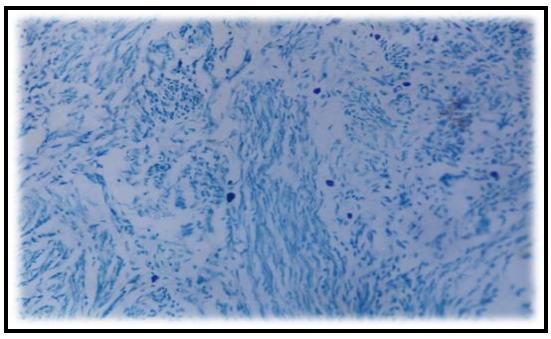
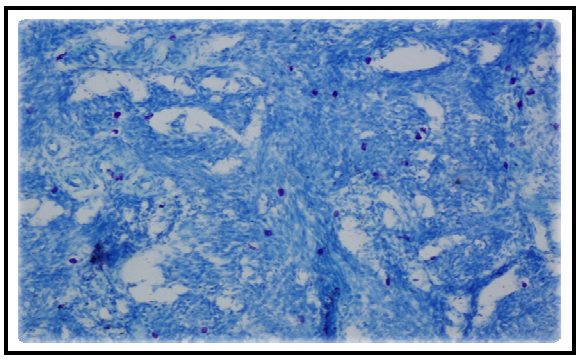


Fig-3: Mast cells in endo-myometrial junction – toluidine blue stain 20 X



The mast cells when observed in our study, in the loose endometrial stroma, they were round and ovoid, whereas in the intramuscular connective tissue of the myometrium they were elongated or spindle shaped (Fig. 4 & 5 respectively). Louise Drudy et al, who suggested that shape of mast cells is dependent on density of connective tissue [9], in the present study mast cells in the endometrium were not seen around the blood vessels except occasionally. This shows that endometrial mast cells did not show any preferential distribution [10].

Fig-4: Mast cells in myometrium –toluidine blue stain 20 X

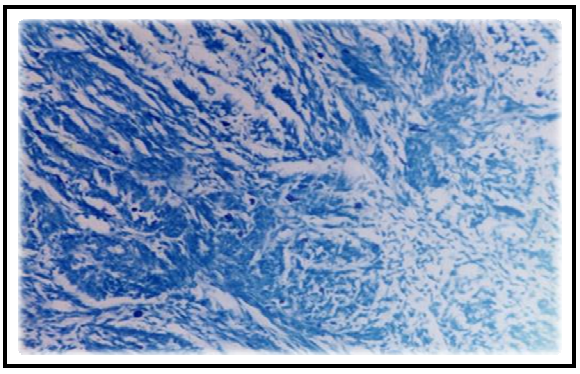
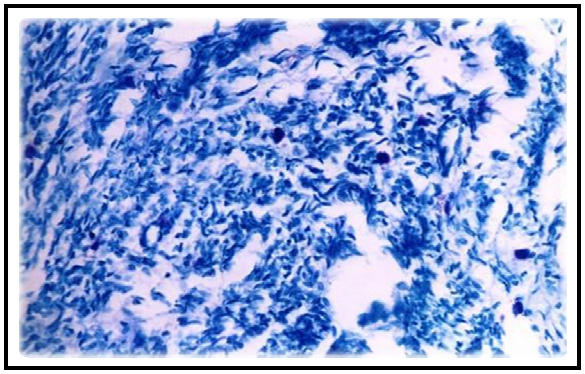


Fig-5: Mast cells in stroma –toluidine blue stain 40 X



Uterus is comparatively rich in mast cells when compared to other tissue of the body these cells are abundant in myometrium to only scant in endometrium their role has been established as modulator of tumor growth and angiogenesis [11].

Among all the layers of uterus in which mast cell distribution was seen in the sub epithelium in few of the cases, mast cells were present subepithelially in endometrial polyp and secretory phase. This proves that there is certain cyclical variation throughout the menstrual cycle [12]. Mast cells are formed in varying numbers is practically all tissue. They are positioned as sentinels at body's portal within mucosal membranes lining genital systems surrounding blood vessels [13] In this study the mean mast cell count in proliferative phase is 12.4/10 hpf and secretory phase - 19.34/10hpf and showed statistically significant P value which was < 0.001. (Table: 1).

The relationship of mast cells, nerves and fibrosis was studied, found that early stage of fibrosis, mast cells were many in numbers in mucosal layer and as fibrosis increased, the association between the mast cells and neural tissue was retained in the submucosa [14]. Dang H et al shows that stabilization of brain mast cells alleviate lipopolysacchirdes induced neuroinflammation by inhibiting microglia activation and memory impairment [15].

In the cases of Atrophic endometrium in our study the mean mast cell was 16.68/10hpf when compared to proliferative phase and secretory phase, there was statistically significant P value<0.001, but was not significant statistically with endometrial polyp as the P value >0.05. This phenomenon is explained on the hormonal basis and good number of mast cells in the myometrium can be attributed to their association with collagenous connective tissue [16].

The mean mast cell in cases of Cystoglandular hyperplasia (CGH) was 7.63/10hpf in this study which showed statistically significant P value <0.001 when compared with proliferative phase (Table 1). This suggested

that there was an inverse relation observed between mast cells and the morphology of endometrium reflecting increased levels of oestrogen [16]. An increased mean mast cell count was noticed in this study in cases of endometrial polyp and there was statistically significant of P value <0.001 (Table: 1). This states that polyps are focal hyperplasia of endometrium in response to excessive oestrogenic stimulation [17].

In cases of endometrial carcinoma in the present study, there were few mast cells in the tumour mass. These observations suggest that the presence of increased number of mast cells to indicate the benign nature of endometrial lesions and malignant neoplastic stroma is not a favorable site for mast cells. Among all lesions of endometrium in this study considering the mean mast cell count and P value, there was higher P value in secretory phase and lower P value in endometrial carcinoma (Table: 1).

Although there are various stains used to stain mast cells in this study 1% toluidine blue was used, as it was least time consuming procedure and also provided good contrast. The mast cells were stained purplish pink and background was light blue. It is obvious that significant mast cell alterations are seen in variety of uterine lesions. However further proof for the hormonal basis of these variations can only be obtained from studies which will correlate sequential mast cell counts, with simultaneous biochemical estimations of hormones.

There is a significant variation in distributional pattern of mast cell in the same section (Table: 4). This prompts need for further study on sections from various parts of the uterus. The number of mast cells increased during the fertile period of oestrous cycle in mice and reaches maximum during oestrous cycle when female is sexually receptive. They are placed in closed proximity to fibroblast and collagen fibers during menstrual cycle which indicates they have an important role in uterine tissue reconstitution [18].

Mast cells have unique capacity to neutralize / degrade toxic proteins, hypothesized as being able to adopt two alternative polarization profiles. Among the immune cells there are mast cells, neutrophils and macrophages which contribute to physiology of reproductive system. The MCT subtype is abundant in endometrium, myometrium during all stages of uterine cycle. Mast cells Tc subtype found in all layer of endometrium it was reported oestrogen increases recruitment of mast cells in uterus and increases degranulation in-vitro [19].

Hourane 2021, their study showed that there are tumor associated macrophages recruitment. Directly various components involved in tumor suppression and tumor growth [20]. Cine L study showed that significant high mast cells density and presence of mtyometrial invasion was seen in endometrial CA suggesting a role of mast cells interaction with tumor [21].

It was concluded that;

1. Density of mast cells varies with endometrial lesions.
2. In the present study, when sections were stained with toluidine blue, identification of mast cells was better in Secretory phase,
3. Highest density of mast cells was seen in secretory phase.
4. Lowest densities of mast cells wereseen in carcinoma of endometrium (Table: 2).

Conclusion

Mast cell profile (Density and distribution in endometrium) may be an additional diagnostic /prognostic tool in different endometrial lesions.

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