Original Article

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Pathology Section

Significance of Mast Cells in Uterine Cervical Lesions: A Cross-sectional Study

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ABSTRACT

Introduction: Cervical cancer is the leading cancer in Indian women. Mast cell distribution and density support a diagnosis in addition to routine histopathological examination.

Aim: To study the variation of mast cell distribution and density in different uterine cervical lesions.

Materials and Methods: A total of 352 cases of cervical lesions were studied cross-sectionally from June 2018 to May 2020, in the Department of Pathology, Subharti Medical College, and associated Chhatrapati Shivaji Hospital, Meerut, Uttar Pradesh, India. These included both hysterectomy (296 cases) and cervical (56 cases) biopsies. Routine histopathological examination was done with Haematoxylin and Eosin stain (H&E) to categorise the lesions into non-neoplastic and Neoplastic lesions. For identification of mast cells 1% toluidine blue stain was applied to evaluate mast cell distribution and density. Data analysis was done using analysis of variance (ANOVA): one-way t-test was applied. A p-value of ≤0.05 was taken as significant. R-Software was used for analysis of data.

Results: Out of 352 cases, 302 (85.7%) were non-Neoplastic and 14% 48 (13.6%) were neoplastic. Two cases were of unremarkable pathology. Chronic cervicitis was observed as the most common lesion 293 (97%) out of 302 cases. Age of the patients ranged from 21-78 years. Most of the patients were in the reproductive age group: 21-49 years of age 274 (77.8%) cases. Mean mast cell count decreased from premalignant cases to malignant cases. In case of cervical intraepithelial lesion, mean mast cell count was found to be 26.5/10 High Per Field (HPF) and in cases of malignancy mean mast cell count of 17.5/10 HPF was observed. Mean mast cell count showed an inverse relationship with the grade of dysplasia and from dysplasia to cases of malignancy.

Conclusion: Mast cell distribution and density support a diagnosis in addition to the routine histopathology, especially in cases of small cervical biopsies which are difficult to interpret.

Keywords: Biopsy, Cervical cancer, Hysterectomy, Toluidine blue

INTRODUCTION

Mast cells are present in all mammalian and nonmammalian tissue. Von Recklinghausen in 1863 first noticed the granularity in the mast cells [1]. Then, Paul Ehrlich in 1878 in his doctoral thesis defined the mast cells based on their metachromatic staining characteristics and the large granules which reacted basophilically with aniline dyes. Mast cells are recognised easily as round or elongated cells with diameter ranging from 8-20 μm [2]. Mast cells are originated from the haematopoietic stem cells in the bone marrow, foetal liver, and the cord blood. They migrate to different organs where they mature and proliferate by the action of various microenvironmental growth factors. Mast cells normally reside in the proximity to the cell surface like the skin, mucosa of gastrointestinal, respiratory system and the genitourinary system, breast parenchyma, myocardium, lymph nodes, synovium and conjunctiva. They are usually located around the blood vessels and are not found in nonvascularised tissue such as cornea, mineralised bone, cartilage [3].

Uterus is comparatively rich in mast cells when compared to other tissues of the body. These cells normally vary from being abundance in the myometrium to only scant in the endometrium [4]. Role of mast cells in immune regulation is well known, there role has been established as modulators of tumour growth and angiogenesis [5].

As very few studies on density of mast cell in cervical lesions are available in the existing literature [6-8], the present study was undertaken to study the mast cell distribution and density in various uterine cervical lesions and to compare the mast cells in various grades of cervical intraepithelial lesions, which might prove to be beneficial in differentiating non-neoplastic and neoplastic lesions and also in predicting the prognosis.

MATERIALS AND METHODS

A cross-sectional study was conducted in the Department of Pathology, Subharti Medical College and associated Chhatrapati Shivaji Hospital, Meerut, Uttar Pradesh, India for a period of two years from June 2018 to May 2020. Approval for the study was taken from the Institutional Ethical Committee (IEC no- SMC/IEC/2018/14/2998).

Inclusion criteria: A total of 352 cases received in the Histopathology Department, for hysterectomy specimens and cervical biopsies in the defined time period of study were included.

Exclusion criteria: Inadequate specimens, poorly preserved tissue, cases with incomplete clinical details were excluded from the study.

Study Procedure

The selected 352 cases of the Department were analysed for the significance of mast cell distribution and density in various cervical lesions. Out of 352 cases, 296 (84%) were hysterectomies and 56 (15.9%) cases were cervical biopsies.

Specimens were fixed in 10% formalin. After adequate fixation, samples were processed to make paraffin blocks. Two sections were taken from anterior and posterior surface of cervix. Appropriate inking was done where ever required. Cervical biopsies were embedded in toto. Two serial sections were taken from each block. One section was stained H&E and used to observe the histopathological findings under light microscope (10X and 40X). Spectrum of cervical lesions was divided into non-neoplastic and neoplastic categories.

The other section was subjected to 1% toluidine blue staining at 4.5 pH for identification and counting of mast cells. These sections were studied first under low power (10X) to evaluate the quality of staining and then under high power (40X) to assess the mast cell count. Mast cells were counted in 10 consecutive high-power fields in each section,

including the areas where maximum mast cells were seen. The mean mast cell count was calculated and taken as density [6-8].

STATISTICAL ANALYSIS

Data analysis was done using analysis of variance (ANOVA): one-way t-test was applied. Significant p-value was taken as ≤0.05. R-software was used for analysis.

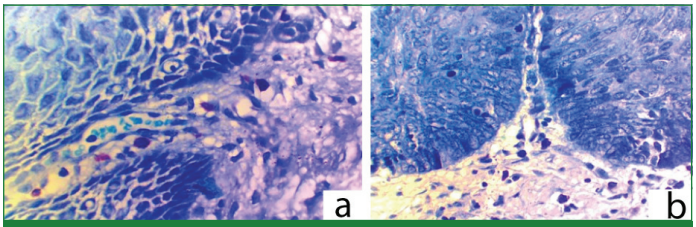
RESULTS

A total of 352 cases were included in the study which comprised of 302 (85.7%) non-neoplastic cases (chronic cervicitis, endocervicitis, microglandular hyperplasia and endometriosis) and 48 (13.6%) neoplastic cases (endocervical polyp, leiomyoma, Low-grade Squamous Intraepithelial Lesion (LSIL), High-grade Squamous Intraepithelial Lesions (HSIL), Squamous Cell Carcinoma (SCC), adenocarcinoma, small cell carcinoma) while two out of 352 cases were of unremarkable pathology. These cases were included for analysis. The age distribution of various uterine cervical lesions is shown. According to World Health Organisation (WHO)-2006, the reproductive age for the female is considered to be from 15-49 years [9]. Majority of the cases were in 21-49 years age group 274 (77.8%) [Table/Fig-1].

Age group (in years)	No. of cases	Percentage (%)
21-30	16	4.54
31-40	81	23
41- 50	177	50.28
51-60	47	13.3
61-70	30	8.52
71-80	01	0.28
Total	352	100

[Table/Fig-1]: Age wise distribution of cases.

The mast cell distribution was observed mainly around the areas of inflammation, around the blood vessels, around the dilated glands in cases of non-neoplastic lesions. In case of neoplastic lesions, mast cells were distributed predominantly around the tumour deposits [Table/Fig-2].



[Table/Fig-2]: Mast cells in case of (a) chronic cervicitis- toluidine blue at pH 4.5, 400X; (b) HSIL- toluidine blue at pH 4.5, 400X. (mast cells seen beneath the surface epithelium in both cases (a) & (b).
HSIL: High-grade squamous intraepithelial lesions

The mast cell density in various non-neoplastic and neoplastic lesions were shown in [Table/Fig-3,4]. In cases of non-neoplastic lesions highest mean mast cell count 72.5/10 HPF was observed in cases of chronic cervicitis followed by single case of endometriosis 69/10 HPF, endocervicitis 54/10 HPF and least by microglandular hyperplasia 36.5/10 HPF in a decreasing order of frequency [Table/Fig-3].

Among neoplastic lesions of cervix, maximum mean of mast cell count 58/10 HPF was observed in cases of endocervical polyp. Rest of the lesions in decreasing order of mean mast cell count were leiomyoma (52/10 HPF), benign mesenchymal lesion (48/10 HPF), LSIL (32.5/10 HPF), HSIL (25.5/10 HPF). In the malignant group, highest mean mast cell count were seen in a case of adenocarcinoma (17.5/10 HPF) and least in cases of SCC (10.5/10 HPF). A single case of small cell carcinoma showed a mean mast cell count of 16/10 HPF [Table/Fig-4].

Mast cell count	Non-neoplastic lesion	No. of cases	Range (/10HPF)	Mean	SD	SE	p-value
Mast cell count/10 HPF	Chronic cervicitis	293 (97.01%)	21-124	72.5	28.21	1.65	0.231
	Endocervictis	04 (1.32%)	26-82	54	13.98	6.99	
	Microglandular hyperplasia	04 (1.32%)	14-59	36.5	20.28	9.07	
	Endometriosis	01 (0.33%)	69	69	-	-	
	Total	302 (100%)					

[Table/Fig-3]: Variation of mast cells in non-neoplastic uterine cervical lesions.
(ANOVA- one-way t-test); HPF: High per field; SD: Standard deviation; SE: Standard error; p-value <0.05 to be considered significant

Mast cell count/10 HPF	Neoplastic lesions	No. of cases	Range (/10 HPF)	Mean (/10 HPF)	SD	SE	p-value
Benign	Endocervical polyp	11 (22.9%)	20-96	58.0	33.66	9.71	0.001
	Leiomyoma	07 (14.5%)	36-68	52	16.7	5.91	
	Benign mesenchymal tumour	01 (2.08%)	48	48	-	-	
Premalignant	LSIL	08 (16.6%)	16-49	32.5	13.11	4.6	
	HSIL	08 (16.6%)	10-41	25.5	23.53	8.32	
Malignant	Squamous cell carcinoma	10 (20.8%)	08-17	10.5	7.68	2.42	
	Adeno carcinoma	02 (4.16%)	03-32	17.5	20.5	14.5	
	Small cell carcinoma	01 (2.08%)	16	16	*	*	
	Total	48 (100%)					

[Table/Fig-4]: Variation of mast cell in neoplastic uterine cervical lesion (ANOVA-one-way t-test).
bold p-values denote significance; LSIL: Low-grade squamous intraepithelial lesions; *two cases were having unremarkable pathology but were included in the study

Mast cell counts were not found statistically significant (p-value -0.231) in non-neoplastic cases, as majority of the cases observed in the category were of chronic cervicitis only. However, in neoplastic cases statistically significant results were observed (p-value- 0.001).

A decreasing trend of mean mast cell count was observed from non-neoplastic (range–14-124/10 HPF) to neoplastic lesions (range-03-96/10 HPF). Mean mast cell count showed an inverse relationship with the grade of dysplasia. The mean mast cell count in LSIL was 32.5/10 HPF, whereas in HSIL 25.5/10 HPF was observed. Mean mast cell count decreased from premalignant cases to malignant cases. In case of cervical intraepithelial lesion mean mast cell count was found to be 26.5/10 HPF and in cases of malignancy mean mast cell count of 17.5/10 HPF were observed.

DISCUSSION

Mast cells are multifunctional cells that play a role in health and diseases. They are long lived cells with the capacity to be activated several times. The microenvironment in which the mast cells reside determines its phenotype and modulates its function. Upon activation mast cells release an array of mediators. The nature of mediators released is dependent on the stimuli. Mast role has been identified in various pathologies, including cancers and autoimmune diseases [10].

Cervical carcinoma is the most common cancer in Indian women. It is a leading cause of death in women worldwide [11]. Therefore, it is important to differentiate non-neoplastic and neoplastic lesions and also to differentiate among premalignant and malignant pathologies. At times cervical biopsies can be difficult to interpret on

which the associated features like mast cell distribution and density might prove to be beneficial for interpretation. The significance of mast cell in cervical carcinoma surveillance has been studied with conflicting results.

The aim of the present study was to analyse the significance of mast cell in uterine cervical lesions and establish a relationship of mast cell density and distribution in various non-neoplastic and neoplastic lesions. In the present study, out of 352 cases, 296 (84.04%) were hysterectomies and 56 (15.9%) were cervical biopsies. Majority of the studies have also considered both hysterectomy and cervical biopsies for the analysis of mast cells [6-8].

In the present study, 274 (77.8%) cases were in the reproductive age group. Sexually active females in the reproductive age group have a higher incidence of having a cervical pathology [12].

The age of the patients in the present study, ranged from 21-78 years with a mean age of 51 years. Majority of the patients were in the fifth decade followed by fourth decade [Table/Fig-1]. Other studies have shown a similar wide range of patients presenting with cervical lesions [13-15]. Histopathological patterns obtained in the present study were predominantly non-neoplastic 302 (85.7%) cases and only 48 (13.6%) cases were neoplastic Two cases showed unremarkable pathology. These findings were comparable with study done by Purushotham R and Nagesha KR, [15]. They observed non-neoplastic, neoplastic and unremarkable pathology in 87.7%, 11.8% and 0.56% of the cases respectively.

During the present study an attempt was made to assess the distribution of mast cell. In non-neoplastic and neoplastic category (benign and premalignant group; [Table/Fig-4]) mast cells were present in the areas of inflammation, around the dilated cervical glands, around the blood vessels and beneath the surface epithelium. In cases of malignancy [Table/Fig-4] mast cells were seen predominantly around the tumour deposits. These findings were similar to the studies done by Kalyani R and Rajeshwari G, Naik R et al., [8,16]. However, Jain PC et al., observed mast cells in neoplastic cases of cervix and documented them in close proximity to the cervical glands and young proliferating fibroblasts only [12]. Overall, mean mast cell count was maximum in cases of chronic cervicitis (mean- 72.5/10 HPF and least in cases of SCC (mean-10.5/10 HPF).

Comparison of mean mast cell count in non-neoplastic and neoplastic cervical lesions in various studies was shown in [Table/Fig-5].

Studies compared	Chronic cervicitis	Endocervicitis	Cervical dysplasia	Cervical carcinoma
Naik R et al., [16] 2004	103.8	102.57	-	48.08
Mainali N and Sinha Ak, [6] 2014	81.90	114.00	6.75	13.50
Gousuddin M et al., [7] 2015	70.6	63	42	16.5
Kalyani R and Rajeshwari G, [8] 2015	48.38	66.96	41.95	34.6
Present study-2020	72.5	54	26.5	17.5

[Table/Fig-5]: Comparison of mean mast cell counts in non-neoplastic and neoplastic lesions of the cervix.

Among neoplastic lesions of cervix, maximum mean mast cell count was observed in cases of endocervical polyp. Rest of the lesions in decreasing order of mean value were leiomyoma, benign mesenchymal lesion. In cases of cervical intraepithelial lesions, mean mast cell count of 32.5/10 HPF in LSIL and 25.5/10 HPF in HSIL while in cases of carcinoma mean mast cell count of 17.5/10 HPF was seen.

These results were in concordance with study of Gousuddin M et al., [7] who documented that the mast cell count was significantly increased in cervicitis (mean -70.6/10 HPF), followed by endocervicitis (mean-63/10 HPF), cervical dysplasia (mean-42/10 HPF) and least in carcinoma cervix (mean-16.5/10 HPF) [Table/Fig-5].

However, contrasting results were found in studies done by Mainali N et al., Kalyani R and Rajeshwari G, who showed maximum number of mean mast count in cases of endocervicitis [6,8]. Mainali N et al., found a greater number of mean mast cell count in carcinoma than the cases of dysplasia. The presence of mast cells in the tumour microenvironment is still controversial. Mast cells by virtue of its secretions can cause vasodilation and oedema with protein rich exudate. Perhaps, such an environment favours tumour invasion and spread. Heparin has been shown to suppress lymphocyte function leading to inhibition of immunity. Mast cell infiltration in neoplasia is thought to be an effect rather than the cause [7].

Naik R et al., studied mast cell profile in 50 non-neoplastic and 50 neoplastic cases of cervical lesions and observed maximum density of mean mast cell count in cases of cervical polyp (mean-250) followed by cases of chronic cervicitis (mean=103.8) [16].

Jain PC et al., compared the mast cell densities in lesions of the uterine cervix showed an increase in density in inflammatory processes whereas in cases of malignancy, reduction in density to total absence of mast cells was observed [12].

Among the malignant cases of the cervix in the present study, mean mast cell count was highest in a case of adenocarcinoma (32/10 HPF, mean- 17.5) followed by one case of small cell carcinoma (range-16/10 HPF) and least by SCC (8-17/10 HPF, mean- 10.5). These findings were in concordance with the studies done by Mainali N et al., [6] and Naik R et al., [16] which showed a mean mast cell count of 28 and 58.93 respectively in cases of adenocarcinoma which were relatively higher when compared to SCC.

No statistical significance was noted within the non-neoplastic cases (p-value 0.231). This could be attributable to the fact that majority of the cases in the non-neoplastic spectrum were that of chronic cervicitis only 293 (97.01%) while, statistically significant results were observed in the neoplastic cases (p-value=0.001).

Limitation(s)

The limitation of the study was that the follow-up of the patient could not be done to assess the prognosis especially in cases of cervical intraepithelial lesions and carcinomas.

CONCLUSION(S)

Uterus is considered to be relatively rich in mast cells when compared to other tissues. The role of mast cell in inflammation is well established but the role of mast cells in neoplastic condition is still controversial. The literature is scarce regarding the distribution and relationship of mast cells in various conditions of the cervix. Mast cell distribution and density support a diagnosis in addition to the routine histopathology, especially in cases of small cervical biopsies which are difficult to interpret. In the present study, progressively decreasing mean mast cell distribution and density were observed from non-neoplastic to neoplastic lesions. It was also observed that mean mast cell density is inversely proportional to grade of dysplasia and may act as a good prognostic indicator.

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