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Expression of Mast Cell Density and Angiogenesis in Carcinoma in Situ and Invasive Squamous Cell Carcinoma of the Cervix

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ABSTRACT

Background & Objective: Studies have shown that tumor cells produce angiogenic factors that directly trigger the endothelial cells to develop. Mast cell density (MCD) and micro-vessel density (MVD) cause more aggressiveness, metastasis, poor survival rate and higher morbidity. But the exact relationship between MVD and MCD is not yet known. The present study was undertaken to find the status of MCD and MVD in carcinoma in situ (CIS) and invasive squamous cell carcinoma (ISCC) of cervix and their relationship in the context cervical carcinoma.

Methods : The present cross-sectional analytical study was conducted in the Department of Pathology, Rajshahi Medical College (RMC), Rajshahi, and Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka over a period of two years between March 2017 to February 2019. Seventy blocks were taken from the cervical colposcopic biopsy materials and from the total abdominal hysterectomy specimens of patients, admitted in the Gynae and Obstetrics Department of RMCH with CIS or ISCC (diagnosed clinically and confirmed histopathologically). The sample comprised of ISCC (n = 42), CIS (n = 18) and healthy control (n = 10). The exposure variables of the study were MCD and MVD and outcome variables were CIS and ISCC.

Result: In the present study, ISCC formed the main bulk (60%) of the sample followed by CIS (25.7%) and healthy control (14.3%). Nearly 80% of the cases of ISCC in our study were of Grade II and Grade III. The present study demonstrated that MVD was lowest in control subjects (8/HPF) and increases progressively from CIS (27/HPF) to Grade I (41/HPF), Grade II (45/HPF) and Grade III (51/HPF) of ISCC. The mean MVD and MCD in CIS were 27/per HPF and 33/per 10 HPF respectively. The MVD and MCD were much higher in grade I ISCC (41per/HPF and 47/per 10 HPF). But in grade II and grade III tumors, the MVD increased to 45 and 51/ per HPF respectively with the corresponding decrease in MCD to 37 and 38 per/10 HPF respectively.

Conclusion: The study concluded that the MCD in ISCC is much higher compared to that in CIS. As the tumour angiogenesis progresses in ISCC with increase in the number of MVD, the MCD decreases. The MCD in cervical ISCC does not increase parallelly with the increase in MVD; rather it decreases with the advance of carcinoma from grade I to grade II & III.

Key words: Expression of Mast Cell Density, Angiogenesis, Carcinoma, in Situ, Invasive Squamous Cell Carcinoma, Cervix etc.

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INTRODUCTION:

Carcinoma of the cervix was reported to be the fourth leading cause of cancer death in women worldwide, causing an estimated 265,700 deaths a year.[1] It is the second leading malignancy in terms of incidence and mortality among Bangladeshi women.[2] About 11,956 new cervical cancer cases are diagnosed annually in Bangladesh. The human papilloma virus in particular subtype 16 & 18 are related to E6 & E7 proteins. E6 promotes the degradation of p53 while E7 inactivates retinoblastoma protein.[3] Degradation of p53 is responsible for activating angiogenesis through production of Vascular Endothelial Growth Factors (VEGF), Interleukin (IL-8), and Fibroblast Growth Factors (FGFs) from mast cells.[4]

Several studies have highlighted that the increase in mast cell density (MCD) is associated with augment angiogenesis. Angiogenesis is evaluated as micro-vessels density (MVD). A study of forty-eight cases carried out in India by Mondal and associates,[5] of which 38 cases were of invasive cervical carcinoma, 6 carcinomas in situ (CIS), 4 micro-invasive carcinomas and control sections from 10 normal cervical mucosa. The value of MCD and MVD were found higher in invasive cervical carcinomas, when compared to those in CIS & normal cervical tissue.[5] The activation of mast cell causes degranulation and releases of heparin, histamine, serine proteases, tryptase, VEGF, phospholipids which are responsible for inflammation, immunity, vascular permeability and angiogenesis.[6] Tumor associated mast cells present in the microenvironment of tumor.[7] Tumor cells produce Stem Cell Factors (SCF), which activates mast cell kit receptor. VEGF, PGE2 and histamine are chemotactic for mature mast cells and cause degranulation.[7] Mast cells are the main source of VEGF and are responsible for neo-angiogenesis. Early application of mast cell inhibitors and anti-angiogenic agents in target therapy can be able to stop neo-angiogenesis and it may cease the progression of CIS to invasive cervical cancer and prevent metastasis.

Tumor cells produce angiogenic factors that directly trigger the endothelial cells to develop. Mast cell density (MCD) and micro-vessel density (MVD) cause more aggressiveness, metastasis, poor survival and higher morbidity.[5] During the progression from CIS to invasive cervical carcinoma, MCD and MVD can be a measure of tumor angiogenesis. Detection of MCD and neo-angiogenesis through measuring MVD may predict the risk of tumor development progression and metastasis. Therefore, detection of MCD with special stain Toluidine Blue and angiogenesis by immunomarker CD-34 can be an important measure for evaluation of disease progression and survival rate of the patients. The present study was therefore, intended to see MCD and angiogenesis in carcinoma in situ and invasive squamous cell carcinoma of the cervix. The findings obtained from the study may have significant impact in the assessment of biological behavior and prognosis of cervical cancer.

METHODS:

This cross-sectional analytical study was conducted in the Department of Pathology, Rajshahi Medical College (RMC), Rajshahi on approval of the thesis protocol by the Institutional Review Board (IRB) of the RMC. Diagnosed cases of carcinoma in situ (CIS) or invasive squamous cell carcinoma (ISCC) of the cervix were included in the study. Patients diagnosed as a case of CIN-I, CIN-II and chronic cervicitis, other variant of cervical carcinoma or patients who received chemotherapy or radiotherapy before biopsy were excluded. A total of 70 blocks were collected from Pathology Department of RMC; of them 66 blocks were collected from colposcopic biopsy specimens and 4 blocks from total abdominal hysterectomy specimens; of the collected blocks, 42 were diagnosed as invasive squamous cell carcinoma, 18 were as carcinoma in situ, 10 as non-significant lesions and were taken as control. Seventy (70) paraffin blocks were sectioned in 5 micrometer thickness. After deparaffinization with xylene and rehydration with decreasing graded alcohol, three sections were made for each case-one stained with Haematoxylin and Eosin stain, one with Toluidine Blue stain and the third one was immunohistochemically analyzed with CD-34 immunomarker. Routine Haematoxylin and Eosin

stains and Toluidine Blue were done in the Department of Pathology, RMC. The Immunostaining was done at Department of Pathology, BSMMU, Dhaka. For immunohistochemistry, the section was mounted on poly-L-lysin coated slides.

RESULTS:

The findings obtained from the study showed that there was no significant difference between the subjects with CIS and those with ISCC. However, both these groups were significantly different from the control subjects ($p < 0.001$). Two-thirds (66.7%) of CIS, 54.8% of ISCC and 60% of control subjects were < 12 years old at age of menarche, with mean age of the subjects at age of menarche in the three groups being 11.1, 11.7 and 11.9 years respectively ($p=0.285$). Two-thirds (66.7%) of CIS and 70% of the control subjects were overweight & obese as compared to 40.5% of the ISCC subjects ($p=0.090$) (Table I).

4.4 Reproductive & obstetric profile:

Of the total 70 sample, 39(55.7%) were at premenopausal stage & 31(44.3%) were at menopausal stage. Data show that there was no significant difference between subjects with CIS and those with ISCC with 50% being premenopausal and 50% menopausal. However, they differ significantly from the control group, with all of the latter group being at premenopausal stage ($p = 0.010$). Grand multipara demonstrated their significant presence in the CIS group (38.9%) compared to ISCC group (23.8%). In the control group 70% were multipara, although none of them was grand multipara. The three groups were significantly heterogeneous ($p=0.002$) (Table II).

Histological grading of 42 invasive squamous cell carcinoma cases revealed that over half (52.4%) of them were of Grade-II, 26.2% grade-III and 21.4% grade-I (Fig. 1). Histological typing showed that nearly two-thirds (64%) of the invasive squamous cell carcinomas were large-cell non-keratinizing and 36% large-cell keratinizing out of 42 cases of invasive squamous cell carcinoma (Fig. 2). The mean mast cell density (MCD) has no difference between samples with CIS and those with ISCC, although they were much higher compared to that of control group ($p<0.001$). However, mean MVD of ISCC (46±14 per HPF) was staggeringly higher than that of CIS (27±10 per HPF), which was again much higher than that of control group (9±3 per HPF) ($p<0.001$) (Table III). The mean MVD and MCD in CIS were 27/per HPF and 33/per 10 HPF. The MVD and MCD were higher in grade I ISCC (41per/HPF and 47/per 10 HPF respectively). But in grade II and grade III tumour the MVD increased to 45 & 51/ per HPF respectively with the corresponding decrease in MCD to 37 and 38 per/10 HPF respectively (Fig.3).

Table I. Distribution of the study subjects by their demographic characteristics (n = 70)

Demographics	Group			p-value
	CIS (n = 18)	ISCC (n = 42)	Control (n = 10)	
Age# (years)				
≤ 40	7(38.9)	10(23.8)	8(80.0)	
41 – 50	4(22.2)	16(38.1)	2(20.0)	
51 – 60	5(27.8)	13(31.0)	0(0.0)	
> 60	2(11.1)	3(7.1)	0(0.0)	
Mean ± SD	49.8±13.3	48.2 ±10.9	32.4 ± 7.4	< 0.001
Age at menarche# (yrs.)				
< 12	12(66.7)	23(54.8)	6(60.0)	
≥12	6(33.3)	19(45.2)	4(40.0)	
Mean ± SD	11.1 ± 1.4	11.7 ± 1.7	11.9 ± 1.3	0.285
BMI* (kg/m2)				
< 18.5	0(0.0)	1(2.4)	1(10.0)	
18.5 – 25.0	6(33.3)	24(57.1)	2(20.0)	0.090
≥ 25.0	12(66.7)	17(40.5)	7(70.0)	

Figures in the parentheses denote corresponding percentage.
#Data were analyzed using **ANOVA statistics** and were presented as **mean ± SD**.
*Data were analyzed using **Chi-squared** (χ^2) Test and were presented as **n(%)**

Table II. Distribution of the study subjects by their reproductive profile (n = 70).

Reproductive & obstetric profile	Group			p-value
	CIS (n = 18)	ISCC (n = 42)	Control (n = 10)	
Reproductive state				
Premenopausal	9(50.0)	20(47.6)	10(100.0)	0.010
Menopausal	9(50.0)	22(52.4)	0(0.0)	
Parity				
Primipara	0(0.0)	1(2.4)	3(30.0)	
Multipara	11(61.1)	31(73.8)	7(70.0)	0.002
Grand multipara	7(38.9)	10(23.8)	0(0.0)	

Figures in the parentheses denote corresponding percentage.
*Data were analyzed using **Chi-squared** (χ^2)

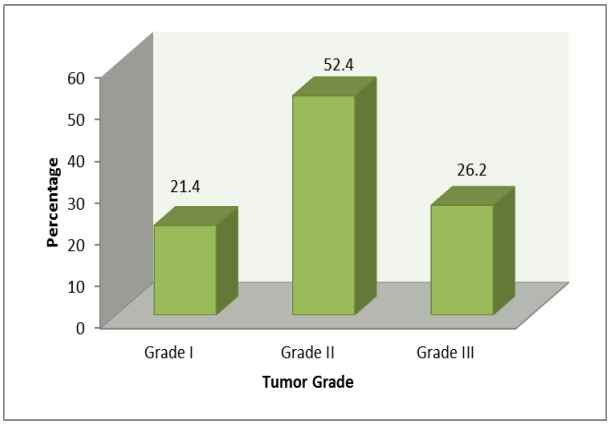

Fig. 1: Distribution of patients by histological grading of tumor (n = 42)

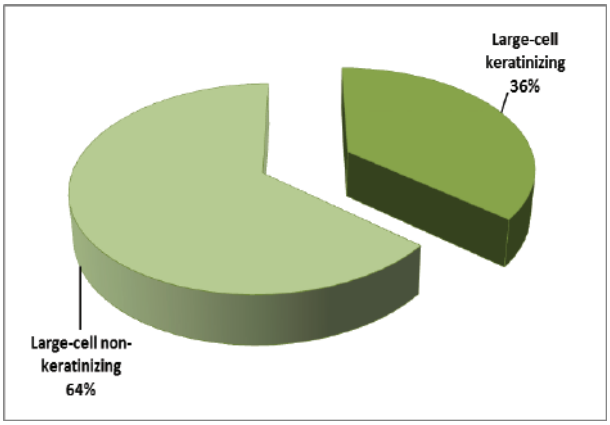

Fig. 2: Distribution of ISCC subjects by tumour type.

Table III. Comparison of MCD and MVD among the study groups (n = 70)

MCD and MVD	Group			p-value
	CIS (n = 18)	ISCC (n = 42)	Control (n = 10)	
MCD (per 10 HPF)	33 ± 16	37 ± 18	8 ± 3	< 0.001
MVD (per 1 HPF)	27 ± 10	46 ± 14	9 ± 3	< 0.001

#Data were analyzed using **ANOVA statistics** and were presented as **mean ± SD**.

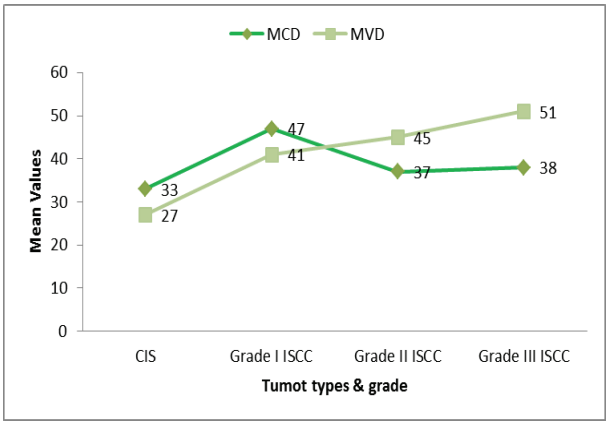

Fig. 3: Relationship between MCD and MVD

DISCUSSION:

In the present study, ISCC formed the main bulk (60%) of the sample followed by CIS (25.7%) and healthy control (14.3%). Mondal and associates[5] noted that during their study period of 2 years, they obtained a large number of cases of invasive cervical carcinoma (n = 38), compared to the number of cases of CIS (n = 6) and microinvasive carcinoma (n = 4). Nearly 80% of the cases of ISCC in our study were of Grade II and Grade III. The presence of higher number of moderate and poorly differentiated cervical carcinoma might be due to poor socioeconomic condition and lack of awareness among patients of developing countries like ours, where patients seek medical help at later stage of the disease, thus contributing to the large number of cases of invasive carcinoma that we found.

The present study demonstrated that MVD was lowest in control subjects & increased progressively from CIS to Grade I, Grade II and Grade III of ISCC. However, the MCD did not increase parallel to the increase in MVD; rather it decreased with the advance of tumour from grade I to grade II and grade III. The data on relationship between MCD and MVD in cervical cancers and pretumoral conditions are limited.[8] Angiogenesis is the process of new blood vessel formation from pre- existing ones, & it has been shown to be associated with the growth and progression of malignant tumors. Folkman and Shing[9] suggested that mast cells and macrophages could be attracted by chemotactic molecules produced by tumor cells and could be an important source of proangiogenic factors.[9] Evidence showed that micro-vessel counts might be one of the most important clinical prognostic factors. Although the importance of angiogenesis in tumor progression of many solid tumors is well-recognized, there is little information about the clinical significance of MVD in cervical SCC.[10]

The role of mast cells in tumor formation and progression is complex. Studies show both positive and negative relationships between MCD and tumor progression.[11-13] Mondal and colleagues[5] found that both MCD and MVD increased from normal cervical samples through CIS to invasive carcinoma. Mast cells were thought to play a pivotal role in

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angiogenesis due to their close association with blood vessels and lymphatic channels. These cells were also found to accumulate in substantial numbers in richly vascularized tissues, such as, hemangiomas, polyps, & tumors.[14] Cabanillas-Saez and associates[15] reported that the MCD remained constant through the Grades 1-3 of CIN, but it significantly increased in invasive cervical carcinoma. They concluded that the mast cells provide an effective mechanism to create the vascularized microenvironment necessary for tumor cells to proliferate and disseminate. Mast cells and macrophages are of vital importance in the development of tumor associated blood & lymphatic capillaries in cervical carcinoma.[16] Benítez-Bribiesca et al[16] found that the MVD in normal epithelium and in dysplasia was similar but they observed a significant increase in CIS and invasive cervical carcinoma. An increase in MVD was reported from the control samples through CIS to microinvasive cervical carcinoma.[17] A similar increase in both MCD and MVD was observed in the study Wilk et al[8]. In contrast, Naik et al[18] reported that mast cells increase in inflammatory conditions of cervix, but decrease in malignant conditions.

Previous studies have shown the presence of mast cells in some malignant epithelial tumors such as rectal carcinomas,[19] pulmonary adenocarcinoma,[20] cervical carcinoma of the uterus[21] and breast carcinomas.[22] Although the functions of these intratumoral mast cells are unclear, they have been shown to be related to patients' prognosis.[19] Two theories have been advocated about the intratumoral mast cells. One is that mast cells may play a role in tumor growth, invasion and angiogenesis.[23] The other is that mast cells have cytotoxicity to tumor cells by an immunologic mechanism.[24] An earlier study found a direct correlation between mast cell count and patient prognosis in pulmonary adenocarcinoma indicating that mast cells had a cytotoxic, rather than an angiogenic effect in tumors.[25] Mast cell cytotoxicity was reported with mast cell to tumor cell ratios greater than 20:1. Conversely, when mast cell: tumor cell ratios were between 10:1 to 1:100, tumor progression was enhanced.[26] Therefore, the effect of mast cells against cancer cells might depend on the concentration of mast cell products in the microenvironment. But in most of the tumors, as well as in our cases, the concentration of mast cells in the tumors belong to the latter situation, that is, mast cells might be implicated in the tumor angiogenesis.

The significance of MVD lies in evaluating prognosis of cervical SSC. However, previous studies evaluating the prognostic significance of MVD as measured by IHC detection of non-specific vascular markers (Factor-8, CD34, CD31) have reported conflicting results.[27,28] In the largest of these studies, Obermair et al[29] reported the worse outcome in women with high tumor MVD. The 5-years survival rate for women with high MVD (> 20 vessels/hpf) was 63% vs. 90% for women with low MVD. Several studies have supported these findings;[11,30] however high MVD has also been shown to be associated with improved survival[12,13] or not correlated with the outcome at all.[31,32]

Summarizing the findings of the study we find that mast cells accumulate in both CIS and ISCC, but the accumulation of MCD in cervical ISCC is much higher compared to that in CIS. With the progress of tumour angiogenesis, the MCD decreases in ISCC and assumes a much lower value in grade II and grade III carcinoma than that was in grade I tumour suggesting that MCD in ISCC does not increase corresponding to the increase in MVD; rather it decreases with the increase in grading of the tumour.

CONCLUSION:

From the findings of the study, it can be concluded that the mast cell density (MCD) in cervical invasive squamous cell carcinoma (ISCC) is much higher compared to that in carcinoma in situ (CIS). As the tumour angiogenesis progresses in ISCC with increase in the number of MVD, the MCD decreases and assumes a much lower value in grade II and grade III carcinoma than that in grade I tumour. Thus, the study showed that MCD in ISCC does not increase parallel to the increase in MVD, rather it decreases with the advance of tumour from grade I to grade II and grade III, because the more the mast cells degranulation takes place, the more angiogenic factors are released causing more angiogenesis.

This study will help understand the significance of mast cell in angiogenesis and tumour grading.

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