Evaluation of Histopathological Diagnosis and Determination of Grades and Stages in Breast Cancer

ABSTRACT

Background: Breast cancer is the most frequent neoplasm in women. According to American Cancer Society in 2019, an estimated 268,600 new cases of invasive breast cancer was diagnosed among women in USA. In Bangladesh, breast cancer has rapidly become the number one cancer in female pushing cervical cancer to second position.

Objective: To evaluate histopathological diagnosis and determination of grades and stages in breast cancer.

Methods: This cross-sectional descriptive type of study was conducted in the Department of Pathology, Rajshahi Medical College. Forty-five untreated cases of breast cancer were included in this study between the period of July 2019 to June 2021 and paraffin embedded sections were obtained from representative mastectomy specimen. The sections were stained with hematoxylin and eosin stain followed by evaluation of angiogenesis by using CD34 antibody.

Results: According to histopathological grade 51.1% belonged to grade III followed by 28.9% grade II and 20% grade I. Histological stage showed most of the cases was stage II (48.9 %) followed by stage I (26.7 %) then stage III (24.4 %). Upon statistical analysis, a significant relation was obtained between MVD with increasing histologic grades and stages.

Conclusion: In conclusion we can say that histological stage showed most of the cases was stage II followed by stage I then stage III.

Key words: Angiogenesis, microvessel density, tumor, adjuvant therapies

INTRODUCTION

The mortality rate (16.9%) of breast cancer is also higher in comparison to other cancer related deaths in Bangladeshi women. In Bangladesh, breast cancer has rapidly become the number one cancer in female pushing cervical cancer to second position. High expression of CD34 indicates a high MVD [1]. Normal glandular breast tissue shows lower MVD than DCIS whereas invasive ductal breast cancer shows higher MVD than DCIS [2]. According to certain studies, poorly

differentiated and undifferentiated tumors have higher microvessel density than well differentiated tumors. There is difference between MVD in Grade I, II and III tumours. Higher mean microvessel count is found in patients of carcinoma breast with increase in TNM stage of tumour[2].

The prognosis of breast cancer is based on the age, tumour size, histological types and grades, as well as vascular invasion. MVD correlate significantly with other established prognostic features. Higher microvessel count is associated with higher tumour grades and stages and indirectly predict poor prognosis[3].

The patients with the presence of lymph node metastasis exhibit higher numbers of stained microcapillaries than the patients without lymph node metastasis[4]. Number of stained microcapillaries as evaluated by using the CD34 immunoreactivity level seems to be useful predictor for the development of local lymphnode metastases in female with invasive ductal breast cancer[4].

Ch'ng *et al* (2012) analyzed CD34 expression and identified that CD34 was highly expressed in the young group (aged ≤55 years). High vascularity was more frequent among infiltrating ductal carcinomas compared with lobular and other histologic types[5,6]. Highest mean vascular density was observed in ER-/ PR- followed by ER-/ PR+ and ER+ / PR-[7]. Ludovini *et al.* (2003) reported in their study that patients with high HER2 expression (>50% positive tumor cells) had a high MVD count and patients with low HER2 expression (≤50% positive tumor cells) had a low MVD count[7,8]. There was a significant positive correlation between presence of lymphovascular invasion and high MVD[9].

In the last few decades, targeted therapies gained the importance to treat the cancer, prevent their progression and metastasis. Angiogenesis is an important component of cancer growth, invasion and metastasis. Therefore, inhibition of angiogenesis is an attractive strategy for treatment of cancer[10,11]. Using anti-angiogenic drugs with chemotherapeutic agent are more effective in treating breast cancer[12]. Expression of MVD can predict biological behavior, rate of growth and metastasis of breast cancer. This marker will help in understanding the metastatic process [13,14]. At a time, it will act as prognostic marker (Weidner *et al.*, 1992) and predictive marker for targeted therapy (Hlatky *et al.*, 2002)[13,15].

This study was conducted to determine MVD and to find out its relation with grading and staging of breast cancer. The CD34 immunomarker can be used to find out relevant risk groups for the development of metastasis, facilitate the selection of candidates for adjuvant systemic therapy. Anti-angiogenic therapy in early stage of breast cancer can prevent further worsening and relapse. Thus, determination of MVD could provide a novel potential tumour marker for patients with breast cancer.

OBJECTIVE

Evaluation of histopathological diagnosis and determination of grades and stages in breast cancer.

METHODOLOGY

Chart 1: Study description

Type of study	This was cross-sectional type of descriptive study
Place of study	Department of Pathology, Rajshahi Medical College
Study period	July 2019 to June 2021
Study population	Females of different age groups having histopathologically confirmed breast cancer admitted in Department of Surgery in Rajshahi Medical College Hospital and Private Hospitals of Rajshahi city were selected for study population.
Sampling technique	Was purposive.
Sample Size	45 Patients

Inclusion criteria

Total mastectomy specimen diagnosed histopathologically as invasive breast carcinoma with or without lymphnode metastasis.

Exclusion criteria:

- Previously diagnosed cases of breast cancer and having chemo or radiotherapy.
- Inadequate sample, poorly preserved tissue.

Sample collection and processing

During collection of specimens, all relevant information was recorded systematically in a preformed data sheet. A questionnaire was used for socio-demographic data and clinical history.

Statistical analysis

The data were analyzed with the help of Statistical Package for Social Sciences (SPSS), version 20 for Windows. Descriptive techniques involving frequency distribution, computation of percentage, mean, standard deviation etc. were applied. Association between variables were conducted by applying chi-square test. The level of significance was set at 95% and p value < 0.05 was considered significant.

RESULT

Table I. Showed the age distribution of the patients, ranged from 25 to 77 years. The cases were grouped on the basis of decades. It was observed that 26.7% of patients belonged to the age group of $\leq$ 40 years, 44.4% of the patients were 41-50 years, 15.5% were of 51-60 years and 13.3% were more than 60 years. The mean $\pm$ SD was found 47.5 $\pm$ 12.4 years.

Table 1: Age distribution of the study sample (n=45):

Age in year	Number of patients	Percentage
≤ 40	12	26.7%
41-50	20	44.4%
51-60	7	15.5%
>60	6	13.3%

Mean $\pm$ SD = 47.5 $\pm$ 12.4 years.

Table II. Represented tumour size distribution. Tumour size of the study sample ranged from 1-8 cm. It was observed that 14 patients (31%) had the tumor size $\leq$ 2.0 cm. 25 patients (56%) had the tumor size within 2.1-5.0 cm, while the tumor sizes of 6 patients (13%) were more than 5 cm. The mean $\pm$ SD of tumor size was found 3.53$\pm$ 1.7 cm.

Table II: Tumour size distribution (n=45):

Tumour size (cm)	Number of patients	Percentage
≤ 2.0	14	31%
>2.1-5	25	56%
>5	6	13%

Mean ± SD = 3.53± 1.7 cm.

Figure 1: Histological grade of tumours in study cases:

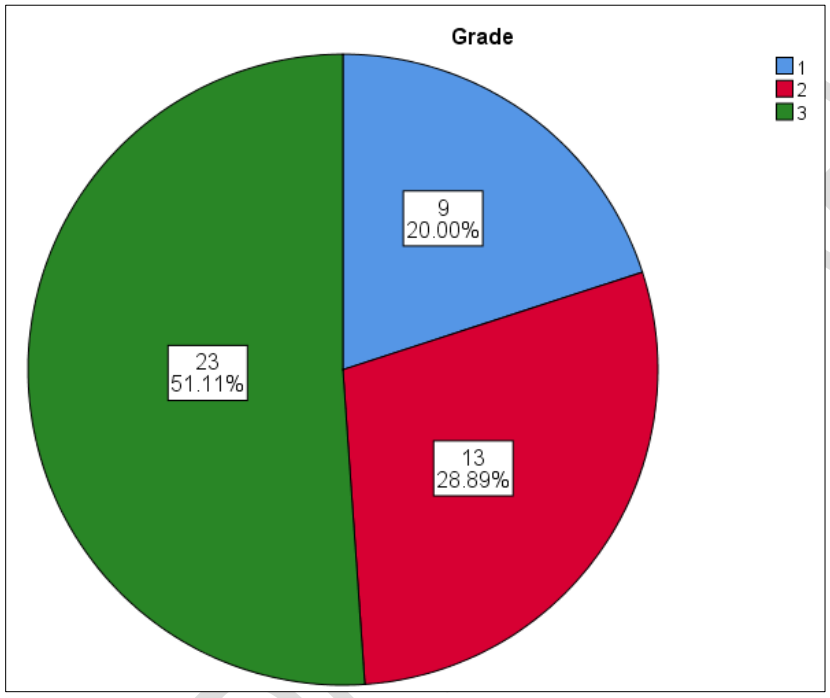

Figure 1. Pie distribution of the study sample reflected that higher number of patients 23 (51.1%) were found to have grade III disease followed by 13 patients (28.9%) with grade II and 9 patients (20%) were of grade I. The Mean ± SD was 2.31±0.79.

Figure 2: Staging of tumours in study cases:

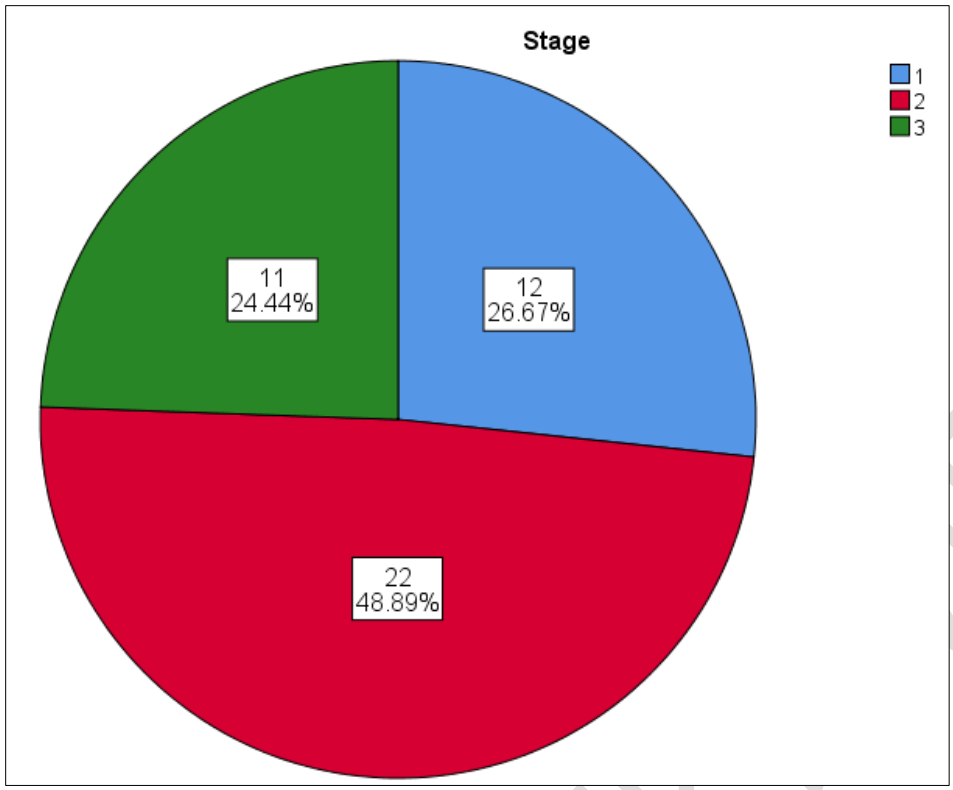

Figure 2. Distribution of staging showed by pie chart. American Joint Committee on Cancer (AJCC) staging was done in all the cases, both infiltrating ductal carcinoma and infiltrating lobular carcinoma. Where 12 (26.7 %) cases were stage I, 22 (48.9%) cases were stage II and 11 cases (24.4%) were stage III. The Mean ± SD was 1.98±0.72.

Table III. Showed that in 45 cases, there were increased MVD along with tumour sizes but not showed statistically significant relation between them. The P value was 0.115. Where Low microvessel density <18 /HPF and High microvessel density ≥18 /HPF. Out of 14 cases 10 (71.4%) showed high MVD and 4 (28.6%) showed low MVD in ≤ 2.0 cm tumour size. In >2.1-5 cm 36% showed low and 64% showed high MVD. Finally in >5 cm tumour size showed 6 out 6 (100%) had high MVD.

Table III: Relation of Tumour size with Microvessel density (MVD):

Tumour size	Low MVD (%)	High MVD (%)	Total
≤ 2.0	10 (71.4%)	4(28.6%)	14
>2.1-5	9(36%)	16(64%)	25
>5	00(00%)	6(100%)	6

Total	19	26	45

X^2 = 4.96, df = 2, P value 0.115

Table IV. Out of 45 patients there were 30 cases had lymphnode metastasis and 15 cases had no lymphnode metastasis. Positive lymphnode metastasis showed 80% high MVD and 20% low MVD. Negative lymphnode metastasis showed 86.7% low and 0nly 13.3% high MVD. The MVD and lymph nodes metastasis were found to be related in this study with a significant P value 0.001. Positive lymph node showed higher MVD than the negative, where Low microvessel density <18 /HPF and High microvessel density ≥18 /HPF.

Table IV: Relation between Lymph Node Status and MVD (n=45):

Nodal Status	Low MVD (%)	High MVD (%)	Total
Positive	6 (20%)	24 (80%)	30
Negative	13 (86.7%)	2 (13.3%)	15
Total	19	26	45

X^2 = 18, df = 1, P value 0.001

Table V represented histologic grades of our study. According to Bloom-Richardson grading system we divided our grades in Grade I, Grade II and Grade III. In Grade I all the 9 (100%) cases had low MVD. In grade II, there were 8 (61.5%) cases with low MVD and 5 (38.5%) cases with high MVD. In grade III, 2 cases (8.7%) had low MVD and 21 (91.3%) cases with high MVD. There was a significant positive correlation (P value: 0.001) between high histologic grade and high MVD.

Table V: Relation between Histological Grade of tumour with MVD:

Histological grade	Low MVD (%)	High MVD (%)	Total
I	9 (100%)	0 (0.0%)	9
II	8 (61.5%)	5(38.5%)	13

III	2 (8.7%)	21 (91.3%)	23
Total	19	26	45

X^2 = 25, df = 2, P value 0.001

Table VI. AJCC cancer staging system was done our study. Here Stage 0 and stage VI was absent. Stage I showed in the study, 10 (83.3%) cases had low MVD and 2(16.7%) had high MVD. In stage II, there were 9 (41.0%) cases with low MVD and 13 (59.0%) cases with high MVD. In stage III all 11 cases (100%) had high MVD. There was a significant positive correlation with p value 0.001 between high histologic stage and high MVD.

Table VI: Relation between Staging of tumour with MVD:

Stage	Low MVD (%)	High MVD (%)	Total
0			**Absent**
I	10(83.3%)	2(16.7%)	12
II	9(41.0%)	13(59.0%)	22
III	0(0.0%)	11(100%)	11
IV			**Absent**
Total	19	26	45

X^2 = 16, df = 2, P value 0.001

DISCUSSION

This cross-sectional study was carried out to estimate the microvessel density expression by CD34 immunomarker in invasive breast cancer with or without lymph node metastasis. It also estimated the relation between the percentage of area covered by MVD with different histologic grades and stages.

Degree of vascularization in different cancers and angiogenesis was heterogeneous within same tumor, so the areas of greatest micro vessel density were counted as “Hot spots”.

We followed the method of counting vessels as described by Weidner *et al.* (1991) by finding the vascular hotspots at low power (10x10) and counting at higher magnification (10x40). In contrast to the study done by Martin *et al.* (1997) we had taken monoclonal antibody to CD34 and counted three fields[3,16]. Earlier studies only assessed one x 200 microscopic fields for micro vessel density and this was used for statistical analysis. In this study we tried to find any association of microvessel density with prognostic factors as tumor size, axillary lymph node status, histologic grade, and stage.

Regarding age distribution, it was observed that about 44.4% of patients belonged to the age group of 41-50 years. The mean $\pm$ SEM age was found 47.5 ± 1.8 years ranging from 25 to 77 years. Similar age distribution was found by Fattahi *et al.* (2014) and Ozdemir *et al.* (2014) with the mean age 51.5 years and 50.0 years, respectively[17,18]. Almost similar mean age and age range were also observed by Reda & Hendawy (2008), whereas Younis *et al.*, (2007) found the median age of the patients 46 years ranging from 29-75 years[19,20]. On the other hand, mean age of 52.67±8.19 years ranging from 39 to 71 years was reported by ElMoneim and Zaghloul (2011)[21]. Higher mean age was observed by Suciu *et al.* (2008), where they found the average age of patients was 54 years ranging from 29-85 years[22]. The mean age and age range variation may be due to geographical variations, racial, ethnic differences, genetic causes and different life style may have significant influence on breast cancer.

In present study, it was observed that 55.6 % cases had the tumor size within 2.1-5.0 cm. The mean $\pm$ SEM tumor size was found 3.53$\pm$ 0.257 cm ranging from 1 to 8 cm. Fattahi *et al.* (2014) observed mean tumor size was 2.65 ± 1.1cm. In another study, Wang *et al.* (2012) found tumor size of $\leq$ 2 cm was 50.9% and of > 2cm was 43.6%[17,23]. Reda and Hendawy (2008) found tumor size varied from 1.5 cm to 4 cm with values between 1 and 8 cm. The above findings are in correspondence with current study[19].

In histological grading 23 (51.1 %) cases were found to have grade III disease followed by 13 (28.9%) with grade II and 9 cases (20%) were grade I. The Mean ± SEM was 2.31±0.118.

In our study, a significant positive correlation was found between the MVD and histologic grade with a P value of 0.001. Our findings are in accordance with the findings of studies done by Pyakurel *et al.* (2014), Safwat *et al.* (2009), Agnani *et al.* (2020) and Kwon *et al.* (2005)[9,24–26].

Miliaras *et al.* (1995) found no relationship to vessel count with histologic grades even though grade I tumours had lower values (45.94 $\pm$ 16.54) than grade II (53.13 $\pm$ 23.22) and grade III tumours (51.71 $\pm$ 20.64)[27]. The modest difference in tumor type between ductal and lobular carcinomas was not significant. Miliaras *et al.* (1995) also found small difference in ductal (48.7 $\pm$ 18.4) and lobular (43.5 $\pm$ 19.8) carcinoma that was not significant. In our study, we found 38 cases (84.4%) of invasive ductal carcinoma and 7 cases (15.6%) of invasive lobular carcinoma[27]. Other morphologic type of breast cancer was not found within this study period. About 14 (36.8%) showed low MVD and 24 (63.2%) showed high MVD in IDC. In case of ILC, 5 (71.4%) was low MVD and 2 (28.6%) was high MVD. There is no significant relation (P value 0.089) of MVD with these two different morphologic types.

Higher mean microvessel count was found in patients of breast carcinoma with increasing AJCC staging of tumour. In the study, stage I showed, 10 (83.3%) cases had low MVD. In stage II, there were 9 (41.0%) cases with low MVD and 13 (59.0%) cases with high MVD. In stage III all 11 cases (100%) had high MVD. There was a statistically significant positive correlation with p value 0.001 between high histologic stage and high MVD. Verma *et al.* (2013) and Agnani *et al.* (2020) also found higher mean microvessel count in patients of breast carcinoma with increase in TNM stage of tumour[3,9].

Low microvessel count gave the impression to be an excellent marker to identify patients with good prognosis. In the study, high MVD was found to be a significant unfavorable prognostic factor in breast cancer such as, lymph node metastasis, high histologic grade, and stage. It had been suggested that higher microvessel count obtained by immunohistochemistry using antibody against CD34, are associated with higher tumor grade and stage which indirectly predict poor prognosis. Thus, detection of CD34 expression may provide a novel potential tumour marker for the clinical diagnosis and prognosis of patients with breast cancer.

We could not however show a significant correlation with tumour size and MVD probably because of small sample size. Other known variables like ER, PR and Her-2-neu status was not done in this study.

If these results are confirmed in larger studies, it might be possible to add microvessel count to other prognostic factors to identify patients at high risk for recurrence and to guide decisions on adjuvant systemic therapy. It could be a likely therapeutic target for antiangiogenic therapy. Therefore, inhibition of angiogenesis is an attractive strategy for treatment of cancer.

Additional work on anti-angiogenic factors needs to be done so as to explore better targeted therapies which will have profound effect on reducing tumor burden and prevent metastasis. For establishing microvessel density as prognostic marker, long term follow-up studies and calculation of overall survival and relapse free survival is needed.

CONCLUSIONS

It also estimated the relation between the percentage of area covered by MVD with different histologic grades and stages. This study estimated the density of newly formed micro vessels by CD34 expression in all the above cases. Then was tried to find out the relation between angiogenic vessels density with tumour size, lymph node metastasis, histologic grade and stage. According to histopathological grade 51.1% belonged to grade III followed by 28.9% grade II and 20% grade I. Histological stage showed most of the cases was stage II followed by stage I then stage III.

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