Scholars Journal of Applied Medical Sciences
Abbreviated Key Title: Sch J App Med Sci
ISSN 2347-954X (Print) | ISSN 2320-6691 (Online)
Journal homepage: https://saspublishers.com

OPEN ACCESS

Pathology

Determination of Microvessel Density by CD34 Immunoreactivity in Female Breast Cancer and its Relation with Grading and Staging

Fateha Nur[1*], Shah Md. Badruddoza[2], S. M. Asafudullah[3], Dr. Saifeen Parvin[4]

[1]Assistant Professor (MD), Department of Pathology, Marks Medical College, Dhaka, Bangladesh
[2]Professor & Ex-Head of the Department, Department of Pathology, Rajshahi Medical College, Rajshahi, Bangladesh
[3]Professor & Head of the Department, Department of Pathology, Rajshahi Medical College, Rajshahi, Bangladesh
[4]Assistant Professor, Department of Pathology, Diabetic Association Medical College, Faridpur, Dhaka, Bangladesh

DOI: 10.36347/sjams.2022.v10i10.041 | **Received:** 11.09.2022 | **Accepted:** 15.10.2022 | **Published:** 30.10.2022

***Corresponding author:** Fateha Nur
Assistant Professor (MD), Department of Pathology, Marks Medical College, Dhaka, Bangladesh

Abstract **Original Research Article**

Background: Angiogenesis is a basic process that enables neoplasm to thrive. Microvessel density (MVD) evaluation is an accepted parameter for assessing the angiogenesis process within a tumour. The research was focused to evaluate angiogenesis by using CD34 immunomarker in invasive breast cancer and to correlate the microvessel density with grades and stages. ***Objective:*** To determine microvessel density by CD34 immunoreactivity in female breast cancer and its relation with grading and staging. ***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:*** In this study, most of the patients (71.1%) belonged to age $\leq$ 50 years where the mean age was found 47.5 $\pm$ 12.4 years. In 45 cases, tumour sizes ranged from 1-8 cm. 86.7% (39 cases) ranges from 0-5 cm, where only (6 cases) 13.3% were more than 5 cm. About 30 cases (66.7%) lymph nodes were positive for metastasis. 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:*** As a new prognostic marker it would be of great value in diagnosis and in identifying patients at high risk of tumor recurrence more accurately. By this way breast cancer patients might be benefited from adjuvant therapies.

Keywords: Angiogenesis, microvessel density, tumor, adjuvant therapies.

INTRODUCTION

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 (Breast cancer facts and figures 2019-2020). Between 2008 and 2012, there was a 20% increase in breast cancer incidence worldwide [1]. According to the report of Globocan by Ferlay *et al.*, (2012) the cancer incidence and mortality rate are uprising in developing countries like Bangladesh [2].

Angiogenesis is the formation of new blood vessels from the existing vasculature. It is important in a variety of physiological processes, such as growth and differentiation, ovulation; wound healing [3, 4]. Pathological angiogenesis is also a component of much diverse pathology ranging from diabetes and atherosclerosis to cancer, a disease that cannot progress without the formation of new blood vessels [5]. Angiogenesis is important in the growth, invasion and metastasis of the tumor [6]. It provides increased availability of oxygen and nutrients to the tumour as well as the most important route of exit from the primary tumour into the blood stream [7]. New proliferating capillaries have leaky basement membranes, making them more accessible to tumor cells than mature vessels [8]. One potential indicator of adverse prognosis in breast cancer is tumour induced angiogenesis [9]. Angiogenesis inhibitors slow and inhibit tumour growth and metastasis *via* different mechanism [10]. It has an important role in the control of cancer progression [11].

The numerical value of tumor angiogenesis is defined as microvessel density (MVD). MVD is measured by counting small and tortuous vessels in the tumor tissue [12]. Microvessel density (MVD) evaluation is an accepted parameter for assessing the angiogenesis process within a tumour. MVD can be immunohistochemically evaluated by using anti-CD34 antibodies (Popiela *et al.*, 2008) [13]. MVD can be quantitated by counting the expression of diverse molecules such as VEGF, CD31, CD34 and von Willebrand Factor (vWF). Among them CD34 stained micro vessels greater and more intense than others [14–16].

CD34 is an endothelial cell-specific marker and have close association with the process of angiogenesis [17]. CD34 is particularly sensitive to tumour angiogenesis, as it can clearly represent the state of neovascularization during the growth of a tumour [18]. Solid tumours require neovascularization to grow beyond about 1 mm [19].

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 [20, 21]. Using anti-angiogenic drugs with chemotherapeutic agent are more effective in treating breast cancer [10]. Expression of MVD can predict biological behavior, rate of growth and metastasis of breast cancer. This marker will help in understanding the metastatic process [22, 23]. At a time, it will act as prognostic marker (Weidner *et al.*, 1992) and predictive marker for targeted therapy [24, 25].

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.

OBJECTIVES

General Objectives

Determination of microvessel density by CD34 immunoreactivity in female breast cancer and its relation with grading and staging.

Specific Objectives

1. To evaluate MVD by using CD34 immune marker.
2. To see the relationship of MVD with grades and stages of breast cancer.

METHODOLOGY

Type of Study

This was cross-sectional type of descriptive study.

Place of Study

Was conducted at Department of Pathology, Rajshahi Medical College.

Study Period

Was carried out from 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.

Sample Selection Criteria

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 was conducted by applying chi-square test. The level of significance was set at 95% and p value < 0.05 was considered significant.

RESULT

A total number of 45 patients underwent total mastectomy with axillary clearance for breast cancer and diagnosed as invasive breast carcinoma in the Department of Pathology, Rajshahi medical college during July 2019 to June 2021 were included in this study. Histopathology followed by immuno histochemistry (CD34) expression was done. All the results were recorded in predesigned data sheet.

Table I: 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 + SD = 47.5 + 12.4 years.

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 < 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 + SD was found 47.5 + 12.4 years.

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.

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 < 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 + SD of tumor size was found 3.53+ 1.7 cm.

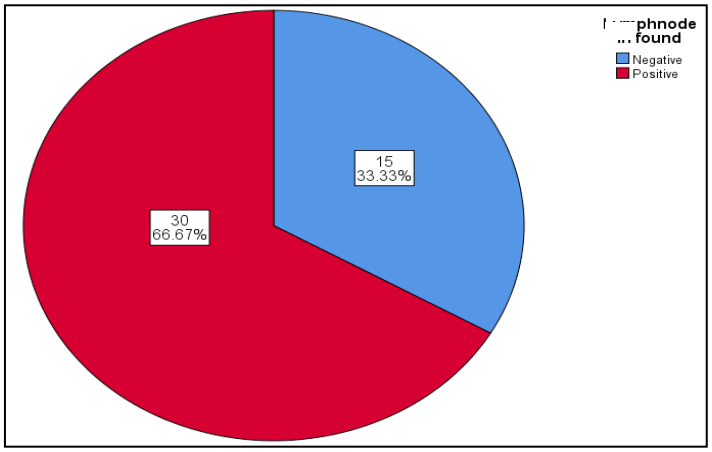

Figure 1: Lymph node metastases in study cases (n=45)

Figure 1 Pie chart represents the lymph node metastasis of the patients. Total lymph nodes found in 45 cases, range from 0 to 13. About 30 cases (66.7%) of samples had positive lymph node metastasis and 15 cases (33.3%) were free from metastasis.

Table III: Distribution of the samples with positive lymph node metastases

Number of lymph node metastases	Number of samples	Percentage
1-2	18	60%
>3-5	8	26.7%
>5	4	13.3%

Table III. In 30 positive cases, 18 LN (60%) ranged from 1 to 2, 8 LN (26.7%) ranged from 3 to 5 and 4 (13.3%) LN was >5 in number.

Table IV: Morphologic type of breast carcinoma

Morphologic type	Low MVD (%)	High MVD (%)	Total
Invasive ductal carcinoma (IDC)	14 (36.8%)	24 (63.2%)	38
Invasive lobular carcinoma (ILC)	5 (71.4%)	2 (28.6%)	7
Total	**19**	**26**	**45**

$X^2 = 3.25$, df = 1, P value 0.089

Table IV. In our study, we found 38 cases (84.4%) of invasive ductal carcinoma and 7 cases (15.6%) of invasive lobular carcinoma. Other morphologic type of breast cancer was not found within this study period. Average MVD in IDC was 18.9 + 5.3 and ILC was 13.4 + 7.0. 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.

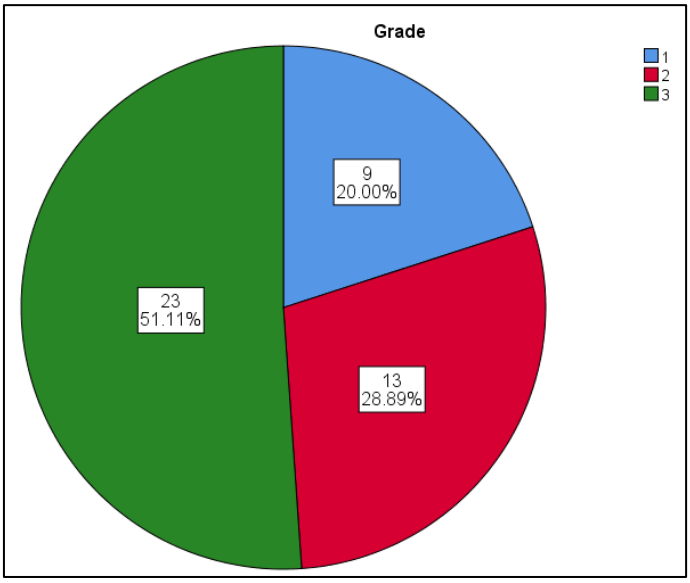

Figure 2: Histological grade of tumours in study cases

Figure 2 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.

Table V: 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 V. 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 VI: 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 VI 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 VII: 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 VII 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.

DISCUSSION

Breast cancer is the leading cause of cancer deaths among women. Results from experimental studies suggest that tumor progression and metastasis in breast cancer is angiogenesis dependent. Angiogenesis has attracted growing interest as a prognostic indicator in tumor progression [8]. The theory is that the number of micro vessels within a tumor provides an estimate of the angiogenic potential of tumor cells. In this way the probability of tumor growth, invasion, and metastasis can be measured.

Regarding age distribution, it was observed that about 44.4% of patients belonged to the age group of 41-50 years. The mean + 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 [26, 27]. 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 [28, 29]. 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) [30]. 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 [31]. 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 + SEM tumor size was found 3.53+ 0.257 cm ranging from 1 to 8 cm. Fattahi *et al.,* (2014) observed mean tumor size was 2.65 ± 1.1cm[26]. In another study, Wang *et al.,* (2012) found tumor size of ≤ 2 cm was 50.9% and of > 2cm was 43.6% [32]. 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 [28].

Lymph node metastasis is one of the strongest predictive factors for local recurrence. In present study, the number of total lymph nodes in 45 cases ranged from 0 to 13. About (30) 66.7% of samples had positive lymph node metastasis and (15) 33.3% were free from tumor. Verma *et al.,* (2013) showed 81.25% of lymph node involvements and Agnani *et al.,* (2020), showed 61.5% of lymph node involvement which is comparable with present study. In 30 positive cases, 18 LN (60%) range from 1 to 2, 8 LN (26.6%) range from 3 to 5 and 4 (13.3%) LN was >5 in number [9, 33].

In accordance with Agnani *et al.,* (2020) the MVD and lymph nodes metastasis were found to be correlated in this study with a significant P value 0.001 [9]. These findings were related with the studies done by Horak *et al.,* (1992), Weidner *et al.,* (1991), Bosari *et al.,* (1992) and Sener *et al.,* (2016) who found a significant relationship between increased MVD and metastasis to lymph nodes [24, 34, 35]. However, there are also some other studies found no relationship between MVD and axillary lymph node metastases [21, 36].

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) [8, 9, 37, 38].

Miliaras *et al.*, (1995) found no relationship to vessel count with histologic grades even though grade I tumours had lower values (45.94 + 16.54) than grade II (53.13 + 23.22) and grade III tumours (51.71 + 20.64). 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 + 18.4) and lobular (43.5 + 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 [36]. 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.

CONCLUSION

MVD stained by CD34 correlated with lymph node metastasis, histologic grades and stages. Higher MVD associated with higher tumor grade, stage and lymph node metastasis were indirectly predicting poor prognosis. The marker can be used with triple assessment in routinely preserved breast cancer tissue to diagnose and to find out relevant risk groups for the development of metastasis in breast cancer. From our study we would like to emphasize that, the quantitative determination of microvessel density may be important, for not only prognostic value but also it may help to expect responses to angiogenesis inhibiting drugs. That will increase the life-span as well as be cost effective for the patients.

REFERENCES

1. Garbee, D., Danna, D., & Lemoine, C. (2014). The impact of side effects on adherence and persistence with oral anti-cancer agents in women diagnosed with early stage breast cancer: a systematic review of quantitative evidence protocol. *JBI Evidence Synthesis*, *12*(10), 27-39.
2. Ferlay, J. (2012). F. 2012. *GLOBOCAN*, 2012.
3. Folkman, J., & Klagsbrun, M. (1987). Angiogenic factors. *Science*, *235*(4787), 442-447.
4. Folkman, J., & Shing, Y. (1992). Angiogenesis. *The Journal Biological Chemistry*, 267(16), 10931–10934.
5. Folkman, J. (1972). Anti-angiogenesis: new concept for therapy of solid tumors. *Annals of surgery*, *175*(3), 409-416.
6. Hanahan, D., & Folkman, J. (1996). Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. *cell*, *86*(3), 353-364.
7. Zetter, B. R. (1998). Angiogenesis and tumor metastasis. *Annual review of medicine*, *49*(1), 407-424.
8. Pyakurel, D., Karki, S., & Agrawal, C. S. (2014). A study on microvascular density in breast carcinoma. *Journal of Pathology of Nepal*, *4*(7), 570-575.
9. Agnani, B., Solanki, R., Ansari, M., & Agnani, S. (2020). Prognostic Significance of Microvessel Density as Assessed by anti CD34 Monoclonal Antibody in Invasive Ductal Carcinoma of Breast. *Asian Pacific Journal of Cancer Biology*, *5*(3), 75-79.
10. Patel, A., & Hielscher, A. (2015). Angiogenesis inhibitors in the treatment of breast cancer: Exploring avenues of new therapeutic targets. *Journal of Cancer Prevention & Current Research*, *2*, 6.
11. Folkman, J. (2002, December). Role of angiogenesis in tumor growth and metastasis. In *Seminars in oncology* (Vol. 29, No. 6, pp. 15-18). WB Saunders.
12. Craft, P. S., & Harris, A. L. (1994). Clinical prognostic significance of tumour angiogenesis. *Annals of Oncology*, *5*(4), 305-311.
13. Popiela, T., Sikora, J., Klimek, M., Basta, P., Niemiec, T., Dobrogowski, J., ... & Dutsch-Wicherek, M. (2008). The analysis of CD34 antigen immunoreactivity level in invasive ductal breast cancer with respect to the presence of lymph node metastases. *Neuro-endocrinology Letters*, *29*(4), 443-446.
14. Hasan, J., Byers, R., & Jayson, G. C. (2002). Intra-tumoural microvessel density in human solid tumours. *British journal of cancer*, *86*(10), 1566-1577.
15. Martin, L., Green, B., Renshaw, C., Lowe, D., Rudland, P., Leinster, S. J., & Winstanley, J. (1997). Examining the technique of angiogenesis assessment in invasive breast cancer. *British journal of cancer*, *76*(8), 1046-1054.
16. Da Silva, B. B., Lopes-Costa, P. V., Dos Santos, A. R., de Sousa-Júnior, E. C., Alencar, A. P., Pires, C. G., & Rosal, M. A. (2009). Comparison of three vascular endothelial markers in the evaluation of microvessel density in breast cancer. *European journal of gynaecological oncology*, *30*(3), 285-288.
17. Satterthwaite, A. B., Burn, T. C., Le Beau, M. M., & Tenen, D. G. (1992). Structure of the gene encoding CD34, a human hematopoietic stem cell antigen. *Genomics*, *12*(4), 788-794.
18. Chen, Z., Xu, S., Xu, W., Huang, J. I. A. N., Zhang, G. U., Lei, L. E. I., ... & Wang, X. (2015). Expression of cluster of differentiation 34 and vascular endothelial growth factor in breast cancer, and their prognostic significance. *Oncology Letters*, *10*(2), 723-729.
19. Charpin, C., Dales, J. P., Garcia, S., Carpentier, S., Djemli, A., Andrac, L., ... & Bonnier, P. (2004). Tumor neoangiogenesis by CD31 and CD105 expression evaluation in breast carcinoma tissue microarrays. *Clinical cancer research*, *10*(17), 5815-5819.

20. Nielsen, D. L., Andersson, M., Andersen, J. L., & Kamby, C. (2010). Antiangiogenic therapy for breast cancer. *Breast Cancer Research*, *12*(5), 1-16.
21. Bharti, J. N., Rani, P., Kamal, V., & AgARwAL, P. N. (2015). Angiogenesis in breast cancer and its correlation with estrogen, progesterone receptors and other prognostic factors. *Journal of Clinical and Diagnostic Research: JCDR*, *9*(1), EC05.
22. Weidner, N., Semple, J. P., Welch, W. R., & Folkman, J. (1991). Tumor angiogenesis and metastasis—correlation in invasive breast carcinoma. *New England Journal of Medicine*, *324*(1), 1-8.
23. Fox, S. B., Generali, D. G., & Harris, A. L. (2007). Breast tumour angiogenesis. *Breast cancer research*, *9*(6), 1-11.
24. Weidner, N., Folkman, J., Pozza, F., Bevilacqua, P., Allred, E. N., Moore, D. H., ... & Gasparini, G. (1992). Tumor angiogenesis: a new significant and independent prognostic indicator in early-stage breast carcinoma. *JNCI: Journal of the National Cancer Institute*, *84*(24), 1875-1887.
25. Hlatky, L., Hahnfeldt, P., & Folkman, J. (2002). Clinical application of antiangiogenic therapy: microvessel density, what it does and doesn't tell us. *Journal of the National Cancer Institute*, *94*(12), 883-893.
26. Fattahi, A. S., Tavassoli, A., Rohbakhshfar, O., Sadeghi, R., Abdollahi, A., & Forghani, M. N. (2014). Can methylene blue dye be used as an alternative to patent blue dye to find the sentinel lymph node in breast cancer surgery?. *Journal of Research in Medical Sciences: The Official Journal of Isfahan University of Medical Sciences*, *19*(10), 918-922.
27. Özdemir, A., Mayir, B., Demirbakan, K., & Oygür, N. (2014). Efficacy of methylene blue in sentinel lymph node biopsy for early breast cancer. *The journal of breast health*, *10*(2), 88-91.
28. Reda, M., & Hendawy, A. (2008). Sentinel Node Biopsy Using Methylene Blue for Diagnosis of Breast Cancer Node Metastasis. It Is Simple and Safe, But is it Reliable Method?. *Kasr El Aini Journal of Surgery*, *9*(2), 35.
29. Younis, L. K., El Sakka, H., & Haque, I. (2007). The prognostic value of E-cadherin expression in breast cancer. *International journal of health sciences*, *1*(1), 43-51.
30. Abd ElMoneim, H. M., & Zaghloul, N. M. (2011). Expression of E-cadherin, N-cadherin and snail and their correlation with clinicopathological variants: an immunohistochemical study of 132 invasive ductal breast carcinomas in Egypt. *Clinics*, *66*(10), 1765-1771.
31. Suciu, C., Cîmpean, A. M., Mureşan, A. M., Izvernariu, D., & Raica, M. (2008). E-cadherin expression in invasive breast cancer. *Romanian Journal of Morphology and Embryology*, *49*(4), 517-23.
32. Wang, M., Zhou, W., Zhao, Y., Xia, T., Zha, X., Ding, Q., ... & Wang, S. (2012). A novel finding of sentinel lymphatic channels in early stage breast cancer patients: which may influence detection rate and false-negative rate of sentinel lymph node biopsy. *PLoS One*, *7*(12), e51226.
33. Verma, K., Kumar, S., & Srivastava, A. N. (2013). Prognostic significance of microvessel density in breast cancer of Indian population. *Int J Sci Eng Res*, 4(2).
34. Horak, E. R., Klenk, N., Leek, R., Lejeune, S., Smith, K., Stuart, N., ... & Stepniewska, K. (1992). Angiogenesis, assessed by platelet/endothelial cell adhesion molecule antibodies, as indicator of node metastases and survival in breast cancer. *The Lancet*, *340*(8828), 1120-1124.
35. Bosari, S., Lee, A. K., DeLellis, R. A., Wiley, B. D., Heatley, G. J., & Silverman, M. L. (1992). Microvessel quantitation and prognosis in invasive breast carcinoma. *Human pathology*, *23*(7), 755-761.
36. Miliaras, D., Kamas, A., & Kalekou, H. (1995). Angiogenesis in invasive breast carcinoma: is it associated with parameters of prognostic significance?. *Histopathology*, *26*(2), 165-169.
37. Safwat, M. D., Habib, F., Elayat, A., Oweiss, N., Reffat, S., & Algaidi, S. (2009). Morphometric and immunohistochemical study of angiogenic marker expressions in invasive ductal carcinomas of the human breast. *Folia Morphologica*, *68*(3), 144-155.
38. Kwon, G. Y., Lee, S. D., & Park, E. S. (2005). Mast cell and macrophage counts and microvessel density in invasive breast carcinoma-comparison analysis with clinicopathological parameters. *Cancer Research and Treatment: Official Journal of Korean Cancer Association*, *37*(2), 103-108.