Journal of Cardiovascular Disease Research

ISSN: 0975-3583, 0976-2833 VOL15, ISSUE 1, 2024

ORIGINAL RESEARCH

TUMOR ANGIOGENESIS AND METASTASIS – CORRELATION IN TRIPLE NEGATIVE BREAST CARCINOMA

Senthil Ponnusamy[1], Kavitha Manoharan[2], Bhuvaneswari K.A[3], Chetna Sharma[4], Kasthuri Thilagam.K[5], Sridhar Shanmugam[6]

[1]M.D, Assistant Professor, Department of Pathology, Government medical college & ESIC Hospital, Coimbatore, Tamil Nadu, India.
[2]M.D, Assistant Professor, Department of Pathology, Government medical college & ESIC Hospital, Coimbatore, Tamil Nadu, India.
[3]M.D.R.D, Assistant Professor, Department of Radio diagnosis, Government medical college, Coimbatore, Tamil Nadu, India.
[4]M.D, Professor, Department of Pathology, Government medical college & ESIC Hospital, Coimbatore, Tamil Nadu, India.
[5]M.D, Professor, Department of Pathology, Government medical college & ESIC Hospital, Coimbatore, Tamil Nadu, India.
[6]M.D, Assistant Professor, Department of Pathology, Government medical college & ESIC Hospital, Coimbatore, Tamil Nadu. India.

Corresponding Author: Dr. **Chetna Sharma**, Professor, Department of Pathology, Government medical college & ESIC Hospital, Coimbatore, Tamil Nadu, India.

ABSTRACT

Background: The breast cancer is the most common cancer in India. The triple negative breast duct carcinoma [TNBC] cases behave as aggressive subtype with poor prognosis. The neovascularization is important mechanism in tumor development, invasion and metastasis. Aim: To study CD34 expressions as vascular marker in TNBC in females to analyses the microvascular count(MVC) and comparison of the metastatic breast tumor cells with non-metastatic behavior of the breast tumor cells

Methods: CD34 expression was studied in 53 cases of TNBC in South Indian female patients over a period of 3 years. Sections from paraffin blocks were stained with Silver Stain method to find out basement Membrane (BM) integrity status of tumor lobules and immune-stained for CD34 to study angiogenesis. Radiologically patients were followed up for visceral metastasis.

Results: In our study lymph node metastasis was positive in 51 cases out of total 53 cases. In the metastasizing cases MVC was observed to be 62 MV/HPF, compared to 42 MV/HPF in the non-metastatic TNBC case. The MVC showed lower count (25/ HPF) in type 1 (intact BM) TNBC compared to >40 / HPF in Type 2 and 3 (breached BM) TNBC. MVC in TNBC cases was found to be higher in the metastasizing cases compared to non-metastasizing cases (P value – < 0.001 was significant).

Conclusion: The micro vascular density in TNBC cases was found to be higher in metastasizing cases compared to non-metastasizing cases. In the treatment of TNBC with CD34 positive, high MVC, combining antiangiogenic therapy to the usual therapeutic agents may be useful

Keywords: TNBC, CD34, Metastasis, Micro vascular count

ISSN: 0975-3583, 0976-2833 VOL15, ISSUE 1, 2024

INTRODUCTION

The incidence of breast cancer is increasing worldwide. It is the most common cancer in India. The prevalence of TNBC which is greater in India when compared to western literature with increase in the number of the cases of triple negative breast duct carcinoma [TNBC].[1-6] Prognosis of the breast carcinoma depends on the biological or molecular cell-types of the carcinoma; hence, immuno-histochemical markers studies have become important in the final diagnosis of breast carcinoma. Estrogen Receptors (ER), Progesterone Receptors (PR), and Human epidermal growth factor receptor 2 (HER2-neu) markers have become routine diagnostic techniques for identification of various types of breast duct carcinoma. Luminal cell breast duct carcinoma which does not express the ER, PR and EGFR2 markers are identified as TNBC and have poor prognosis.[6-9] Very few studies have been conducted so far to assess the micro vessel density in breast carcinoma. Since no studies on CD34 expression have been undertaken so far in Indian female population, hence the present work was planned to study CD34 expressions and their utility in micro vessel density of TNBC in the South Indian patients in the Coimbatore region.

MATERIALS & METHOD

In this study CD34 expression was studied in 53 cases of TNBC at Department of Pathology, Government Medical College and ESI Hospital, Coimbatore over a period of 3 years from 2019 to 2021. From among 404 breast carcinoma cases submitted for histo-pathological diagnosis and immuno-histochemical [ER, PR, Her 2 neu] expressions, total 53 cases showing triple-negativity. Metadata of the patient and clinical findings were recorded, and the paraffin tissue blocks after histopathology diagnosis were processed for 1) Reticulin Silver impregnation to find out integrity status of basement membrane of malignant lobules in TNBC, and 2) immune staining of sections to find out CD34 expression in the blood vessels and endothelial cells. Radiologically patients were investigated for visceral metastasis.

SILVER IMPREGNATION: Sections from the tumor-blocks were Silver Stain impregnated applying the Gomori's method. Basement Membrane integrity status was recorded in each case applying the criterion that; 1) TNBC type 1 lesion showed in-situ duct carcinoma with well-defined intact BM, 2) TNBC Type 2 presented in-situ carcinoma with focally or partially breached BM, and 3) TNBC Type 3 lesion showed invasive duct carcinoma with surrounding fibrosis without any basement membrane structure.

CD34 STAINING: CD34 (Clone QBEnd/10) antibody and immune stain reagents obtained from Thermo scientific company and the staining protocol, were used for immune-staining of the sections for CD34 expression. The staining of endothelial cells in blood vessels was taken as internal control. Cellular localization of CD34 was in the cell membrane or cytoplasm and the tissue cells expressing antibody labeled CD34 displayed brown granular staining of the cytoplasm of endothelial cells.[10]

The tumors were frequently heterogeneous in their vessel density, however, the area of highest neovascularization was found by scanning the tumor section at low power and identifying the areas of invasive carcinoma with the highest number of discrete microvessels staining for CD34. The method used for micro-vessel counting was modified from Weidner et al, the individual microvessels were counted on a 400x microscopic field in 3 microscopic fields. Average number of non-canalized micro-vessels count per field was calculated. This was called the microvessel count. Any brown staining endothelial cell or endothelial cell cluster that was clearly separate from adjacent micro-vessels was considered a single, countable micro-vessel. Vessel lumina were not necessary for a structure to be defined as a micro-vessel.[11]

ISSN: 0975-3583, 0976-2833 VOL15, ISSUE 1, 2024

RESULTS

Age incidence of TNBC in the present south Indian population is shown in the table-1. Higher incidence of TNBC was observed in the age groups 30–50 years. The incidence of metastasis observed in the present 53 cases of TNBC is shown in the table-2. Nearly half of the total TNBC cases presented with metastasis mostly to the lymph nodes. Basement membrane integrity status observed in present 53 cases of TNBC is shown below in the table-3. In present TNBC series, Type 3 basement status was the most common pattern observed in 48 (90.5%) cases, followed by less common patterns in remaining TNBC type 1 and TNBC Type 2 cases. CD34 stained sections in 53 cases of TNBC were studied for angiogenesis (micro-vessel count). The results are shown in table 4, Figures 1-2.

Table 1: Showing age group distribution in 53 cases of TNBC

Age in years	No. of cases	Percentage
21-30	4	7.5%
31-40	17	32.1%
41-50	22	41.5%
>50	10	18.9%
21 - >50	53	100%

Table-2: Showing metastasis pattern in 53 cases of TNBC.

Site	No. of cases	Percentage cases
Lymph node	24	45.28%
L.N. + Lung	2	3.8%
L.N +Liver	1	1.9%
No metastasis	26	49.05%
Total cases	53	100%

Table 3: Showing the BM Status in 53 cases of TNBC.

TNBC - BM patterns	No of cases	Percentage
Type 1	2	3.8%
Type 2	3	5.7%
Type 3	48	90.5%
Total	53	100%

Table 4: Showing micro vessel count of in metastasizing and non-metastasizing 53 TNBC cases

TNBC	No of Metastasizing cases	Metastasizing cases (Micro vessel count) Mean ±S.D per Hpf	No of non-metastasizing cases	Non-metastasis cases (Micro vessel count)Mean ±S.D/ Per Hpf
Type-1TNBC (intact BM)	Nil	Nil	2	25.5±**13.43**
Type-2 TNBC	2	59±**1.41**	1	48

ISSN: 0975-3583, 0976-2833 VOL15, ISSUE 1, 2024

(Partial breach BM)				
Type-3 TNBC (Complete breach BM)	25	63.85±**10.60**	23	45.22±**9.89**
Total	27	62±**10.6**	26	42.16± **0.7**

P value – (< 0.001) chi square 29.75 p

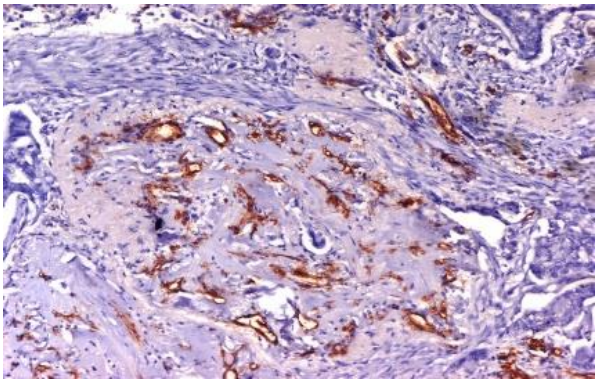

Figure-1: CD34 expressions in micro-vessel in areas of high (CD34- x-200)

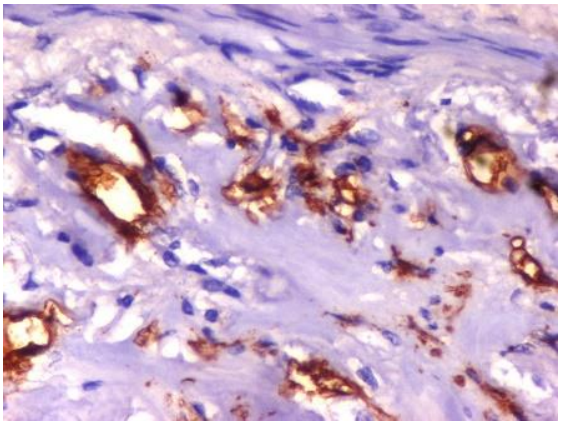

Figure-2: CD34 expressions in micro-vessels in areas of high vascularization (CD34- x 400)

DISCUSSION

The incidence of TNBC in the present study in south Indian population, was observed to be 53/404 (13.12%) of the total female breast duct carcinoma. Previous studies showed TNBC incidences of ∼10–26% of invasive breast carcinomas.[2,6,8,12-15] In the present study common age group involved by TNBC was observed to be 40 to 50 years. Other studies reported that women with triple-negative breast cancers were significantly more likely to be under age of 40 years.[16] Suresh et al reported median age for highest TNBC incidence in Central India to be 49 years in the age group 22-75 years.[17] Sharma et al, in North Indian Women, found highest TNBC incidence in the age group 21-30 years.[8] Present study findings are in agreement with that of Suresh et al.[17]

In our study lymph node metastasis was observed to be positive in 51 percent cases. Suresh et al, Dent et al, and Lakshmaiah et al in their studies on TNBC breast carcinoma cases, observed the lymph node metastasis in 35%, 55% and 63% cases respectively.[5,17,18] The present findings showed that the rate of lymph node metastasis in TNBC cases did not differ from the breast

ISSN: 0975-3583, 0976-2833 VOL15, ISSUE 1, 2024

carcinoma in general, which also presented with lymph node metastasis in more than 50 per cent cases.[17] Dent et al, however, in their study suggested an excess risk of distant metastasis in the triple negative breast cancer (TNBC) compared to other forms of breast cancer. Suresh et al in their cases reported low rate of metastasis in the viscera only in 6.4% cases.[17,19] The differences could be due to smaller sample sizes.

Van de Rijn suggested that CD34 is a good marker for the human hematopoietic cells and vascular endothelial cells.[20] In the present study tumour angiogenesis density was studied on CD34 stained sections in 53 TNBC cases. In the metastasizing cases of TNBC the mean micro-vessel (MV) count was observed to be 62 MV/HPF, compared to 42 MV/HPF in the non-metastatic TNBC case. The MV /HPF showed lower count (25/ HPF) in type 1 (intact BM) TNBC compared to >40 / HPF in Type 2 and 3 (breached BM) TNBC Groups. Weidner et al, Salih et al in their study, in general breast carcinoma, observed similar pattern of MV counts.[11,21]

Micro vascular density in TNBC cases was found to be higher in the metastasizing cases compared to non-metastasizing cases (P value – < 0.001 was significant), the pattern was similar to other types of breast carcinoma in the women. Vascular density increased with the increase of the grade of the breast carcinoma.

The increased levels of vascular endothelial growth factor (VEGF) in TNBC patients had shorter relapse free survival. The antiangiogenic therapy improves the treatment efficacy in TNBC patients.[22] VEGF-2 has been reported as a prognostic factor in TNBC. It suggests that vascular pathway is an essential mechanism for targeting in TNBC Basal Cell subtype.[23] The therapy with monoclonal anti- VEGF antibody bevacizumab with weekly paclitaxel caused a significant increase in response rates and progression-free survival in the metastatic TNBC Basal Cell subtypes.[24]

CONCLUSION

The micro vascular count in TNBC cases was found to be higher in the metastasizing cases compared to non-metastasizing cases and micro-vessels can be better visualized by the CD34 Staining. In the treatment of TNBC, CD34 positive with high MVC, combining antiangiogenic therapy to the usual therapeutic agents may be useful. However, further studies on a larger scale are needed for confirmation.

Conflict of Interest: None
Funding: Nil
Acknowledgement:
Special thanks to Professor- Mohammed Naim

REFERENCES

1. Bordoloi D, Kunnumakkara AB (2018) Alarming burden of triple-negative breast cancer in India. Clin Breast Cancer 18: e393-e399.
2. Statistics of Breast Cancer in India 2012. http://www.breastcancerindia.net/statistics/stat_global.html
3. Gluz O, Liedtke C, Gottschalk N, et al. Triple-negative breast cancer: current status and future directions. Annals of Oncology 2009; 20: 1913–27.
4. Thakur KK, Bordoloi D, Kunnumakkara AB (2018) Alarming burden of triple-negative breast cancer in India. Clin Breast Cancer 18: e393-e399.

ISSN: 0975-3583, 0976-2833 VOL15, ISSUE 1, 2024

5. Dent R, Trudeau M, Pritchard KI, Hanna WM, Kahn HK, et al. (2007) Triple-negative breast cancer: Clinical features and patterns of recurrence. Clin Cancer Res 13: 4429-4434. 40]
6. Alteri R, Barnes C, Burke A,et al. Breast cancer facts & figures 2013-2014. American Cancer Society, Surveillance and Health Services Research, 2013.
7. Khan RI, Marilyn M, et al. A Review of Triple-Negative Breast Cancer. Cancer Control ,July 2010;17;No.3
8. Sharma B, Satyanarayan, Kalwar A, et al. Five year retrospective survival analysis of triple negative breast cancer in north-west India. Indian journal of cancer. 2013;50:330-32.
9. Naim M, Kumar A, Gaur K, John VT. Pattern of oestrogen, progesterone and Her2neu receptors expression in a heterogeneous carcinoma of the breast in a lactating woman.BMJ Case Rep.2010 Nov23.
10. Cîmpean Am, Raica M, Narița D. Diagnostic significance of the immunoexpression of CD34 and smooth muscle cell actin in benign and malignant tumors of the breast. Romanian Journal of Morphology and Embryology.2005; 46:123–129.
11. Weidner N, Semple JP, Welch WR, Folkman J. Tumor angiogenesis and metastasis– correlation in invasive breast carcinoma.N Engl J Med 1991;324:1– 8.
12. Carey LA, Perou CM, Livasy CA et al. Race, breast cancer subtypes, and survival in the Carolina Breast Cancer Study. JAMA 2006; 295: 2492–2502.
13. Cancello G, Maisonneuve P, Rotmensz N et al. Prognosis and adjuvant treatment effects in selected breast cancer subtypes of very young women (<35 years) with operable breast cancer. Ann Oncol 2010; 21: 1974–1981.
14. Azim HA, Jr, Michiels S, Bedard PL et al. Elucidating prognosis and biology of breast cancer arising in young women using gene expression profiling. Clin Cancer Res 2012; 18: 1341–1351.
15. Lund MJ, Trivers KF, Porter PL et al. Race and triple negative threats to breast cancer survival: a population-based study in Atlanta, GA. Breast Cancer Res Treat 2009; 113: 357–370.
16. Mohapatra M, S Satyanarayana. Evaluation of clinico-pathologic findings of breast carcinoma in a general hospital in southern India. Indian J Cancer 2013; 50 (4): 297 – 301.
17. Suresh P, Ullas Batra, Doval DC. Epidemiological and clinical profile of triple negative breast cancer at a cancer hospital in North India, Indian J Med PaediatrOncol. 2013; 34: 89–95.
18. Lakshmaiah KC, Das U, Suresh TM, Lokanatha D, Babu GK, Jacob LA, and Babu S. A study of Triple Negative Breast Cancer at a Tertiary Cancer Care Center in Southern India. Ann Med Health Sci Res. 2014; 4: 933–37.
19. Dent R, Hanna WM, Trudeau M, Rawlinson E, Sun P, Narod SA. Pattern of metastatic spread in triple negative breast cancer. Breast Cancer Res Treat. 2009 May;115(2):4238.
20. Van de Rijn M, Rouse RV. CD34. A review. Appl Immunohistochem 1994;2:71–80
21. Salih RF, Hussein AG, Qasim BJ. Immunohistochemical Expression of CD34, Smooth muscle Actin and Type IV collagen in Breast carcinoma. A clinicopathological study. IRAQI J Med:2012:10(2);148-152.
22. Linderholm BK, Klintman M, Grabau D et al. Significantly higher expression of vascular endothelial growth factor (VEGF) and shorter survival after recurrences in premenopausal node negative patients with triple negative breast cancer.Cancer Res 2009; 69:
23. Ryden L, Ferno M, Stal O et al. Vascular endothelial growth factor receptor 2 is a significant negative prognostic biomarker in triple-negative breast cancer: results from a controlled randomised trial of premenopausal breast cancer. Cancer Res 2009; 69:
24. Miller K, Wang M, Gralow J et al. Paclitaxel plus bevacizumab versus paclitaxel alone for metastatic breast cancer. N Engl J Med 2007; 357:2666–2676