Section: Pathology

Original Research Article

CD34-POSITIVE STROMAL CELLS PAUCITY IN THE STROMA OF MICRO-INVASIVE AND INVASIVE TRIPLE NEGATIVE BREAST DUCTAL CARCINOMA

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

[1]Assistant professor, Department of Pathology, Government medical college & ESIC Hospital, Coimbatore, Tamil Nadu, India.
[2]Professor, Department of Pathology, Government medical college & ESIC Hospital, Coimbatore, Tamil Nadu, India.
[3]Assistant professor, Department of Pathology, Government medical college & ESIC Hospital, Coimbatore, Tamil Nadu, India.
[4]Assistant professor, Department of Pathology, KAP Vishwanathan Government medical college, Trichy, Tamil Nadu, India.
[5]Assistant professor, Department of Pathology, Government medical college & ESIC Hospital, Coimbatore, Tamil Nadu, India.
[6]Professor, Department of Pathology, Government medical college & ESIC Hospital, Coimbatore, Tamil Nadu, India

Received : 09/11/2023
Received in revised form : 12/01/2024
Accepted : 31/01/2024

Keywords:
CD34, TNBC, BM.

Corresponding Author:
Dr. Anitha Chellam,
Email: senthiljune07@gmail.com

DOI: 10.47009/jamp.2024.6.1.406

Source of Support: Nil,
Conflict of Interest: None declared

Int J Acad Med Pharm
2024; 6 (1); 2050-2054

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 importance of the stromal microenvironment that the stromal loss of CD34 expression and acquisition of SMA myofibroblastic features might constitute a prerequisite for the tumor invasiveness. **Objectives:** The present work was planned to study CD34- fibrocytic expressions and their utility in invasive property and metastasis of TNBCs. **Material and Methods:** In this study done in 53 TNBC patients, over a period of 3 years. From the paraffin tissue blocks submitted for silver staining method to find out basement membrane(BM) integrity to identify the status of tumor lobules and CD34 immune staining of sections and scoring done. **Results:** On silver staining 53 TNBC cases only 2 (3.8 %) cases showed malignant ducts with intact BM (TNBC type-1), tumour ducts with focal breached BM (TNBC type-2) in 3 (5.7%) cases and invasive duct carcinoma without BM (TNBC type-3) in 48 (90.5%) cases. The stromal scoring of CD34 done in 100 normal control sections all ducts showed mostly +3 (87%) or +2 score (17%). CD 34 scores observed in TNBC type-1, 60% ducts showed +2 score and 37% duct units were +3. In the TNBC type 2 and type 3 groups were most of the ducts observed score that 0 and +1. These findings showed that CD 34 score decreased in TNBC invasive carcinomatous groups, compared to normal and intact BM. **Conclusion:** Similar loss of CD34 expression (score) had been reported in the otherwise invasive breast duct carcinoma in female. Hence, CD34 expression did not appear to be TNBC type specific. Loss of CD34 score may be useful in initial investigation to identifying and confirming the infiltrating carcinoma in tissue core biopsies.

INTRODUCTION

The breast cancer is the most common cancer in women and also the leading cause of cancer-related mortality in the worldwide. The breast cancer were 2.3 million women newly diagnosed in the globe at 2020. [1-4] Also the breast cancer is most common cancer in India with 13.6% of all new cancer cases in 2020 and prevalence rate was 25.8 cases per 100,000 people[5]. In India prevalence of TNBC (ER-/PR-/HER2-) phenotype was 25.04% which is higher in comparison to the western literature. Findings showed an alarmingly inclining trend of TNBC in India.[6] Already known fact that TNBC having poor prognosis[7-9] and metastases is reported to be more aggressive compared to other breast cancer sub-types and involving viscera.[10,11] The importance of the stromal microenvironment has been suggested to play a major role in breast carcinoma by promoting tumor growth, progression and invasion [12-15].
Recently few studies have reported on CD34 stromal expressions and their utility in diagnosis and care in

ISSN (O): 2687-5365; ISSN (P): 2753-6556

various breast lesions of western females. Since no studies on CD34 expression were so far undertaken in the TNBC cases Indian female population, the present work was planned to study CD34-fibrocytic expressions and their utility in invasive property of TNBC in the south Indian patients.

MATERIALS AND METHODS

In this study CD34 expressions was studied in 53 cases of TNBC patients at Department of Pathology, Government medical college and ESIC Hospital, Coimbatore over a period of 3 years from 2020 to 2022. Among 404 breast carcinoma cases submitted for histopathological diagnosis and ER, PR, Her-2neu expressions, total 53 cases showing triple negativity. Metadata of the patient and clinical findings were recorded, and paraffin tissue blocks were processed for Reticulin Silver impregnation to find out integrity status of basement membrane of malignant lobules in TNBC, and CD34 immune staining of sections to find out CD34 expressions in the stroma around the normal and malignant glands.

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; TNBC type-1 lesion showed in-situ duct carcinoma with well-defined intact BM, TNBC Type-2 presented in-situ carcinoma with focally or partially breached BM and 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 expressions.

Scoring CD34 Expression

In this study microscope Olympus CX21i was used to examine the findings. The duct and lobular units were identified and CD34 expression grading was performed for the duct and lobular units separately applying the method of Chauhan et al [16] and Catteau et al. [17] Grading was done from 0 to 3+, as below-grade 0 means up to 5% stromal cells positive for CD34 expressions, grade 1+ means = >5 and up to 25% stromal cells showing +ve immune-reactivity, grade 2+ means = >25 and up to 50% stromal cells presenting immune-reactivity, 3+ = >50% stromal cells immune-reactive. One high power microscopic field was assumed to harbor 100 stromal cells. (Catteau et al) [17].The staining of endothelial cells in blood vessels was taken as internal control (Cimpean et al). [18]

The 100 duct-fields were studied in normal control-breast-tissue from normal-margins of excised tumor-breast. CD34 score was recorded to find out how many duct units showed 0, +1, +2, +3 scores respectively.

Interpretation of Immune Expression of CD34:

Grade 0- was interpreted as complete loss of CD34

Grade 1+ was interpreted as reduced expression,

Grade 2+ and 3+ were interpreted as retained expression of CD34.

RESULTS

Among 53 cases of TNBC in the present study the highest incidence of TNBC was observed in 22 (41.5%) cases in the age group 41-50 years. Among 53 TNBC cases of silver staining showed only 2 (3.8 %) cases presented with TNBC type-1 patterns, 3 (5.7%) cases showed TNBC type-2 patterns and TNBC type-3 patterns was observed mostly in 48 (90.5%) cases. (Table-1) and figures 1-3.

Table 1: Showing reticular staining patterns in TNBC

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

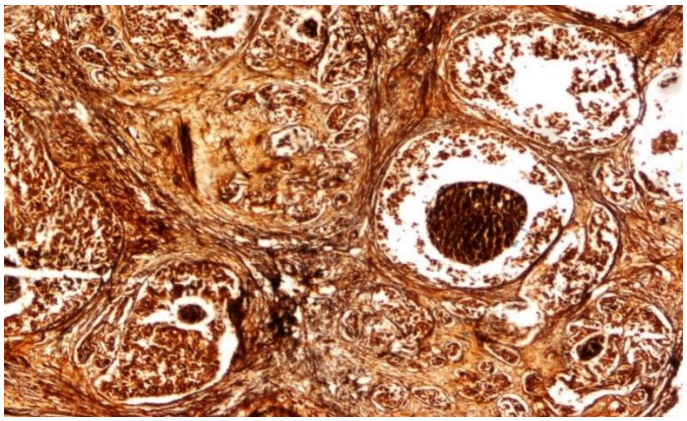

Figure- 1: Micro-photograph showing malignant ducts with intact BM (arrows) in TNBC type-1 (Silver impregnation x 50)

ISSN (O): 2687-5365; ISSN (P): 2753-6556

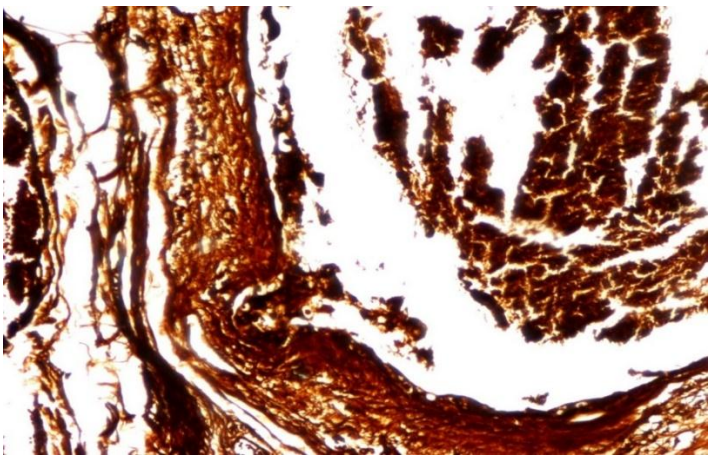

Figure- 2: Micro-photograph showing tumor ducts with focally or partially breached BM (arrow) in TNBC type-2 (Silver impregnation x 400)

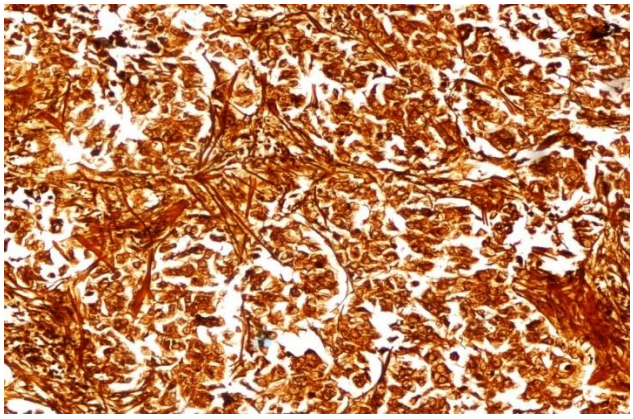

Figure- 3: Micro-photograph showing cancer cell sheets with out BM and extensive reticulin producing fibrosis around-TNBC type-3 (Silver x 200)

CD34 Expression

In 53 TNBC cases were studying the CD34 expression applying semi quantitative assessment. In the test group TNBC type 1 (intact BM group), 50 duct units were studied per case in two cases. In the test group TNBC type 2, 50 duct units were studied per case in three cases. In the test group type 3 (complete breach BM group) TNBC, 10 duct units were studied per case in 48 cases. The number and percentage of cases showing 0, +1, +2, +3 scores respectively, in all the test and control groups are shown in the table 2 and figures 4-6.

Table 2: Number of duct units showing various CD34 score in the control and the test groups

Groups	No of cases	Total no duct units studied	Number duct-units (%) Showing various CD34 score			
			0	1+	2+	3+
Control	2	100	0	0	17 (17%)	83 (83%)
TNBC type-1 ducts group	2	100	0	3 (3%)	60 (60%)	37 (37%)
TNBC type-2 ducts group	3	150	102 (68%)	42 (28%)	6 (4%)	0
TNBC type-3 ducts group	48	480	472 (98.3%)	8 (1.7%)	0	0

ISSN (O): 2687-5365; ISSN (P): 2753-6556

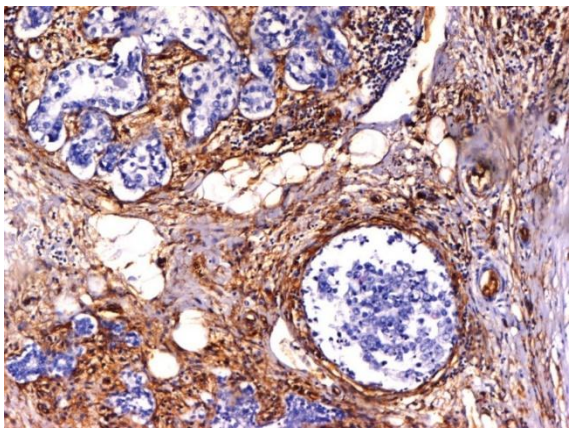

Figure- 4: Micro-photograph showing (+3) stromal positive CD34 expression in Type 1 TNBC Test group (CD34 x 200).

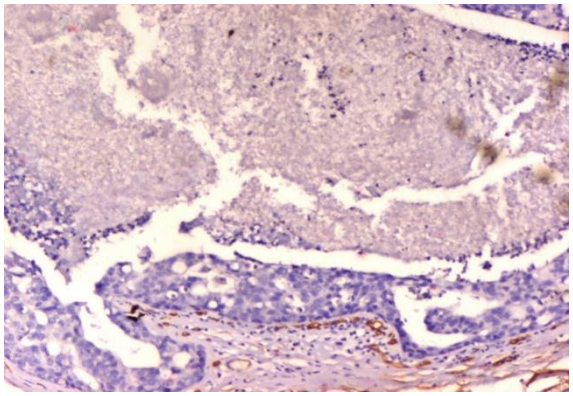

Figure- 5: Micro-photograph showing 0 CD34 score in Type 2 TNBC group (CD34 x 200)

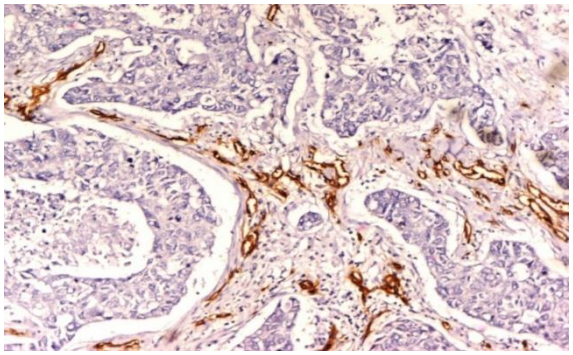

Figure- 6: Micro-photograph showing 0 score CD34 expression in Type 3 TNBC group (CD34 x 200).

DISCUSSION

The incidence of TNBC in the present study in south Indian populations was observed to be 53/404 (13.12%) of the female breast duct carcinoma. Previous studies showed TNBC incidences of ~10–26% of invasive breast carcinomas. [6,7 19-24] In this study in 53 TNBC cases of CD34 expressions and assessment in 100 normal control sections all ducts showed mostly +3 (87%) or +2 grade (17%). In the TNBC type-1, 60 percent ducts showed +2 grade and 37% duct units presented +3 grade. In the test TNBC type 2 and type 3 groups most of the ducts presented 0 and +1 CD 34 grades. These findings showed that CD 34 score decreased in TNBC group, compared to normal; but, in early TNBC duct units also presented high +3 and +2 scores, hence, dividing line could not be possible between individual early TNBC versus normal breast. In the Advanced infiltrating TNBC, however, score reached +1 and zero levels, which distinguished infiltrating TNBC from the early TNBC and normal breast. CD4 score, hence, may be useful as marker of breach of ductal BM and infiltrative activity of carcinoma breast.

In previous study of Catteau et al, however high loss of CD 34 expression in their cases was reported in high grade in-situ carcinoma. In present study CD 34 score in intact BM TNBC was +2 and +3 close to normal, but, number of cases in this group in present study was very low (only 2 cases).[17] Hua et al in their study also reported high CD34 loss in In-situ carcinoma.[25] In another study by Chauhan et al loss in low grade carcinoma reported to be lower grade. They also suggested that loss of CD34 could be related to invasive potential of breast carcinoma.[16] Findings suggested that loss of CD 34 score may be useful in identifying and confirming the infiltrating carcinoma ducts, for the diagnosticians not able to identify infiltrating ducts in the routine H & E stained sections.

Barth P.J et al, Kuroda et al, Yazhou et al, Cimpean et al in their studies also reported loss of CD 34 expressions in the invasive cases of female breast duct carcinoma. Results in the present study showed that invasive TNBC presented loss of CD34 expression score alike of the cases of otherwise invasive breast duct carcinoma in female.[18,26-28] The stromal loss of CD34 expression and acquisition of SMA myofibroblastic features might constitute a prerequisite for the tumor invasiveness[26]. The loss of CD34 expression was significantly more frequent in high nuclear grade DCIS[16,17] and specially in invasive breast malignant lesions [16-18,25-29]

Loss of CD34 expressions in infiltrative TNBC groups was severe (0 to 1), as compared to the normal and early TNBC cases, hence, could be useful as diagnostic marker of high grade carcinoma. However, no dividing line is possible between an early case of TNBC and normal breast CD34 for the diagnosis of carcinoma.

CONCLUSION

Similar loss of CD34 expression (score) had been reported in the otherwise invasive breast duct carcinoma in female. Hence, CD34 expression did not appear to be TNBC type specific. Loss of CD 34 score may be useful in identifying and confirming the infiltrating carcinoma, for the Pathologist may not able to identify infiltrating ducts in the core biopsies of routine H & E stained sections and further studies needed for further evaluation.

REFERENCES

1. WHO statistics breast cancer 12 July 2023

ISSN (O): 2687-5365; ISSN (P): 2753-6556

2. Ferlay J, Ervik M, Lam F, Colombet M, Mery L, Pineros M. Lyon: International Agency for Research on Cancer; [May; 2019]. 2019. Global Cancer Observatory: Cancer Today. [Google Scholar]
3. Hortobagyi GN, de la Garza Salazar J, Pritchard K, et al. The global breast cancer burden: variations in epidemiology and survival. Clin Breast Cancer. 2005;6:391–401. [PubMed] [Google Scholar]
4. Varughese J, Richman S. Cancer care inequity for women in resource-poor countries. https://pubmed.ncbi.nlm.nih.gov/21364864/ Rev Obstet Gynecol. 2010;3:122–132. [PMC free article] [PubMed] [Google Scholar]
5. Park JE. Textbook of preventive and social medicine. Jabalpur: Banarsidas Bhanot; 2019. Textbook of Preventive and Social Medicine, 25th ed; pp. 433–434. [Google Scholar].
6. Sarkar and Akhtar. Triple Negative Breast Cancer Prevalence in Indian Patients over a Decade: A Systematic Review. Int J Clin Biostat Biom 2022, 8:045
7. 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.
8. 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.
9. Naim M, Mayank S, Khan A. Breast cancer classification, Serum adiponectin and SNPrs2241766.Lap Lambert Academic Publication, Germany 2014.
10. Heitz F, Harter P, Lueck HJ et al. Triple-negative and HER2-overexpressing breast cancers exhibit an elevated risk and an earlier occurrence of cerebral metastases. Eur J Cancer 2009; 45: 2792–2798.
11. Liedtke C, Mazouni C, Hess KR et al. Response to neoadjuvant therapy and long term survival in patients with triple-negative breast cancer. J Clin Oncol 2008; 26: 1275–1281.
12. Bhowmick NA, Neilson EG, Moses HL: Stromal fibroblasts in cancer initiation and progression.Nature 2004, 432:332337.PubMed Abstract | Publisher Full Text
13. Cortez E, Roswall P, Pietras K: Functional subsets of mesenchymal cell types in the tumor microenvironment.Semin Cancer Biol 2014, 25:39.PubMed Abstract | Publisher Full Text
14. De Wever O, Van Bockstal M, Mareel M, Hendrix A, Bracke M: Carcinoma associated fibroblasts provide operational flexibility in metastasis.Semin Cancer Biol 2014, 25:3346.PubMed Abstract | Publisher Full Text
15. Hinz B, Phan SH, Thannickal VJ, Prunotto M, Desmouliere A, Varga J, De Wever O, Mareel M, Gabbiani G: Recent developments in myofibroblast biology:paradigms for connective tissue remodeling.Am J Pathol 2012, 180:13401355.PubMed Abstract | Publisher Full Text]
16. Chauhan H, Abraham A, et al. There is more than one kind of myofibroblast: analysis of CD34 expression in benign, in situ, and invasive breast lesions. J ClinPathol. 2003;56;271-276.
17. Catteau X, Simon P, Vanhaeverbeek M, Noël J. Variable Stromal Periductular Expression of CD34 and Smooth Muscle Actin (SMA) in Intraductal Carcinoma of the Breast. PLoS ONE 2013: 8(3): e57773
18. 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.
19. Alteri R, Barnes C, Burke A, et al. Breast cancer facts & figures 2013-2014. American Cancer Society, Surveillance and Health Services Research, 2013.
20. Statistics of Breast Cancer in India 2012.http://www.breastcancerindia.net/statistics/stat_global.html
21. 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.
22. 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.
23. 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.
24. 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.
25. Hua X, Yu L, Huang X, Liao Z ,Xian Q. Expression and role of fibroblast activation protein alpha in microinvasive breast carcinoma. Diagnostic Pathology 2011, 6:111.
26. Barth P.J, Ebrahimsade S, Ramswamy A, Moll R. CD34(+) fibrocytes in invasive ductal carcinoma, ductal carcinoma in situ, and benign breast lesions. Virchows Arch.2002; 440: 298-303.
27. Kuroda N, Jin YL, Hamauzu T, Toi M, Miyazaki E, Hiroi M, Moriki T, Enzan H. Consistent lack of CD34-positive stromal cells in the stroma of malignant breast lesions. HistolHistopathol. 2005;20: 707-71.
28. Yazhou C, Wenlv S, Weidong Z, Licun W. Clinicopathological significance of stromal myofibroblasts in invasive ductal carcinoma of the breast. Tumour Biol. 2004;25 (56):290-5.
29. Ramaswamy A., Moll R., Barth P.J., CD34+ fibrocytes in tubular carcinomas and radial scars of the breast. Virchows Arch, 2003, 443(4):536-540.

ISSN (O): 2687-5365; ISSN (P): 2753-6556