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A Study of Microvascular Density in Carcinoma of Breast with CD34 Immunostaining

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Introduction: Tumor agiogenesis is generally classified in to two types, namely, sprouting and intussusceptive angiogenesis. The formation of new blood vessels from the already existing is termed as sprouting angiogenesis. Many growth factors including endothelial growth factor (VEGF) contribute the formation of new vessels at the tumor sites. On other hand, the tumor able to split the existing blood vessels, this type is termed as intussuscepted angiogenesis and discovered in human colon adenocarcinoma xenograft. In intussuscepted angiogenesis, an existing blood vessel splits into two new blood vessels by formation of trans vascular pillars.
Objective: The present study aimed to analyse the nicrovascular density on breast cancer patients using CD 34 immunostaining method.
Results: This mis-regulation may lead to the development of cancers. Histological grading with Grade II were more with 19 (63.3%) cases followed by Grade I with 4 (13.3%) and Grade III with 3 (10%) cases. There was increase in mean vascular density in Grade II when compared with Grade I and Grade III. However no significant correlation was observed statistically with a P value of 0.176. Using different antibodies such as CD34, CD31, Factor VIII and CD105 to microvessels differentiation was highlighted.
Conclusion: The results showed that the anti-CD34 monoclonal antibody is more sensitive than the anti- CD31 antibody in calculation of MVD in breast cancer as mentioned in previous studies.

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Keywords: Vascular endothelial growth factor (VEGF); human colon adenocarcinoma; angiogenesis; mammary glands; milk line differentiation.

1. INTRODUCTION

Breast cancer is the most common type of cancers found in females and overall, it is the second most common reason for cancer related deaths in females. Over 2 million new cases were detected in 2018 and its incidence increases with the age. In India, its incidence rates start to rise in the early 30's and usually peaks during 50-64 years. The literatures showed that 1 in 28 women is likely to develop breast cancer during her lifetime [1,2]. The breast cancer incidence found to be rose in developing countries due to several factors including increased rate of life expectancy, urbanization and changed habitation along with westernized lifestyle. Invasive ductal carcinoma is the predominant type of malignancy that accounts for nearly 65% - 80% of breast carcinoma cases [3-5].

Due to lack of awareness, in India and few underdeveloped, the patients only represent them at advanced stages of the diseases and this delayed diagnosis eventually lead to the poor prognosis and diminished five year survival rate. Meanwhile, since last decades, much betterment came in to existing and despite of increased incidence, there is a reduced mortality rates, particularly, in last 20 years. This was achieved by the creating awareness about breast cancer screening among the individuals and introduction of adjuvant systemic chemotherapy [6-8].

Various factors contribute to beginning and progression of breast cancer. The patients with high risk could be identified by a number of significant prognostic factors such as their age, tumor grade, lymph node involvement, menopause status and expression of the type of predominant hormonal receptor. Several other predictive factors include oestrogen and progesterone receptor, HER2/neu amplification, cell cycle markers *viz.,* Ki-67 and angiogenesis. The angiogenesis become an emerging predictive and prognostic factor in tumor grading particularly for breast cancers. Angiogenesis is characterized by the formation of new blood vessels from the epidermal layer of the existing vasculature. Recent research in angiogenesis revealed it potential role in cancer related phenomenon such as carcinogenesis, tumor invasion and its metastasis [9-11].

Folkman discovered the angiogenesis and proved its essential in cancer therapeutics. He hypothesized that growth of tumour and metastasis are inter dependent each other and supported by tumor Angiogenesis *.ie.,* growth of new vessels towards and within the tumor site. The onset of cancers (benign to malignant transformation) and metastatic cascade of events predominately rely on the tumor angiogenesis [12,13].

Quantification of microvascular density is the most common method to analyze the grade of tumoral angiogenesis in breast cancer. It was first developed by Weidner et al. [5] and they stained the blood microvessels using immunohistochemical stain with panendothelial marker [5]. They used few markers such as CD31, Factor VIII antigen (Von Willebrand's factor), PECAM-1 or CD34. According to Weidner's approach, the initial step is hot spot detection *ie.,* identifying the neovessel density rich area scanning the whole tumoral section at low power light microscopy. Then the identified spots are counted using high power (x200field) fields. The stained endothelial cell or clusters which was separated from others or adjacent vessels are counted as a single microvessel irrespective of present or absent of lumen [6]. CD34 are considered to be a valuable immunostaining markers that accountable for highlighting the microvessel variations [14-17].

The tumour derived myoepithelial cells are differed from their normal counterparts by their capacity to interact luminal breast epithelial cells for changing the polarity and basement membrane deposition. The present study aimed to measure the microvascular density labelled immunohistochemistry using CD34 among patients with breast carcinoma and to analyse the possible correlation with other clinicopathological factors.

2. MATERIALS AND METHODS

With the approval of the Ethical committee, SBMCH (Sree Balaji Medical college and Hospital), the study was carried out in the Department of Pathology, for about 33 months duration from January 2017 to September 2019. The present study used to analyse the retrospective and prospective data of 30 breast

carcinoma cases diagnosed by clinical and histopathological method. Totally, 74 breast specimens were included in the study [18-20].

Patient details such as age, clinical history, disease stage, diagnosis details and pathological details were noted and analysed. The patients were selected based on the following inclusion and exclusion criteria.

2.1 Inclusion Criteria

Patients with invasive breast carcinoma irrespective of type, grade diagnosed by histopathology in the department. Invasive breast carcinoma irrespective of nodal metastasis or not. Patient irrespective of whether axillary dissection done for lymph node status or not.

Mastectomy, Modified Radical Mastectomy and Lumpectomy specimen received in the department of pathology.

2.2 Exclusion Criteria

Breast carcinoma in situ irrespective of type diagnosed by Histopathology Male patients. Samples from post radiation therapy, post chemotherapy patients and core biopsy specimens. The final study population included 30 patients, who are diagnosed with breast carcinoma who has undergone mastectomy procedure after obtaining informed written understandable consent.

The specimen was submerged in 10% neutral buffered formalin. After adequate fixation, the gross morphological details of the specimens were recorded with total submission of breast samples and representative bits were taken from mastectomy specimens. After tissue processing, 3-5 micrometer thickness sections were stained with Hematoxylin and eosin H&E stain. Then they were analysed microscopically. Clinical data relevant to the case was collected from the hospital and laboratory records.

2.3 Statistical Analysis

SPSS (IBM, v21) was used for statistical analysis. Descriptive statistical method was adopted to find out the correlation of microvascular density with the prognostic factors-tumour size, tumour grade and lymph node metastasis. $p < 0.05$ was considered to be significant in the study.

3. RESULTS AND DISCUSSION

The present study was conducted in the Department of Pathology in collaboration with the Department of Surgery, SBMCH, Chennai for the duration of 33 months beginning from January 2017. A sum of total 30 number of Breast Carcinoma cases were selected for studying CD34 immunostaining to measure microvascular density (Table 1). The present study was enrolled with only women patients between 38 to 79 years old. The mean age of the study was 52.93 years. Out of the 30 cases, 12 (40%) were lesser than 50 years old and 18 (60%) were > 50 years old.

Table 1. Age-wise distribution of breast carcinoma

Age Group (years)	Number of carcinoma	Percentage (%)
30-39	01	3.3
40-49	10	33.3
50-59	10	33.3
60-69	08	26.8
>70	01	3.3

3.1 Age of the Patients

Further dividing the age group of patients into five sub-categories, it is observed that breast carcinomas were equally common in the age groups of 40-49 years and 50-59 years, each group constituting 33.3% of total cases, followed by 60-69 years (26.8%) and next to follow is the age group of 30-39 years and 70-79 years constituting 3.33% each. The young patient in our study was 38 years and oldest patient was 79 years of age. The mean age of our patients having breast carcinoma was 52.93 years.

3.2 Tumour Size

On comparing the tumour sizes it is observed that 18(60%) cases had tumour size measuring <5.0 cm in diameter and the remaining 12(40%) had tumours of >5.0 cm in diameter.

3.3 Histomorphology

The majority of the patients in our study group showed morphological features of Infiltrating Ductal Carcinoma (IDC) comprising 25 (83.3%) cases, followed by Metaplastic Carcinoma 3 cases and 1 case each of Mucinous Carcinoma and Cystic Papillary Carcinoma of breast.

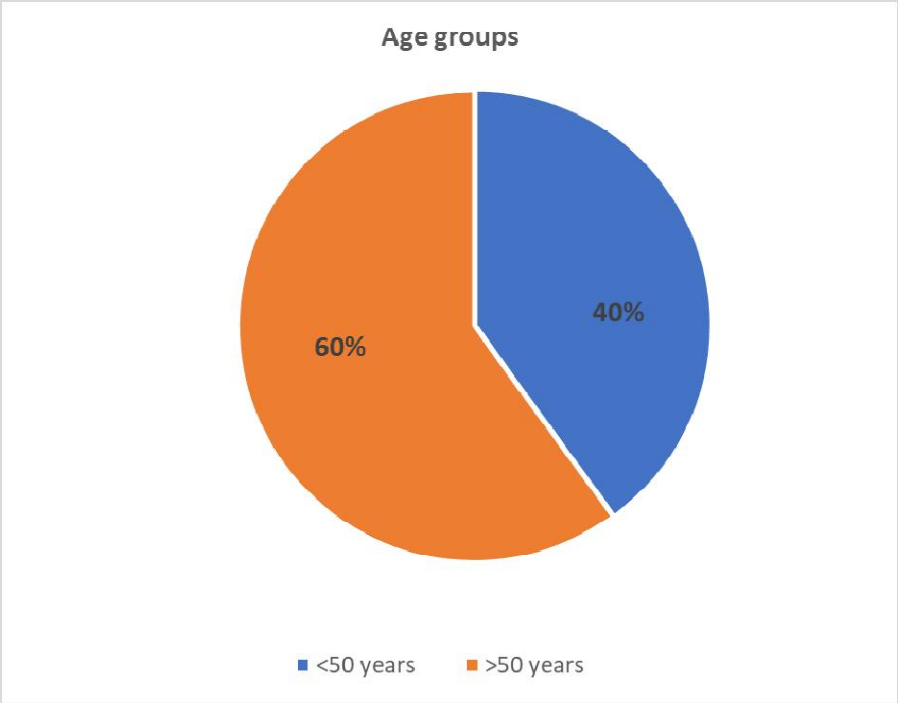

Fig. 1. Pie chart showing broad age group distribution

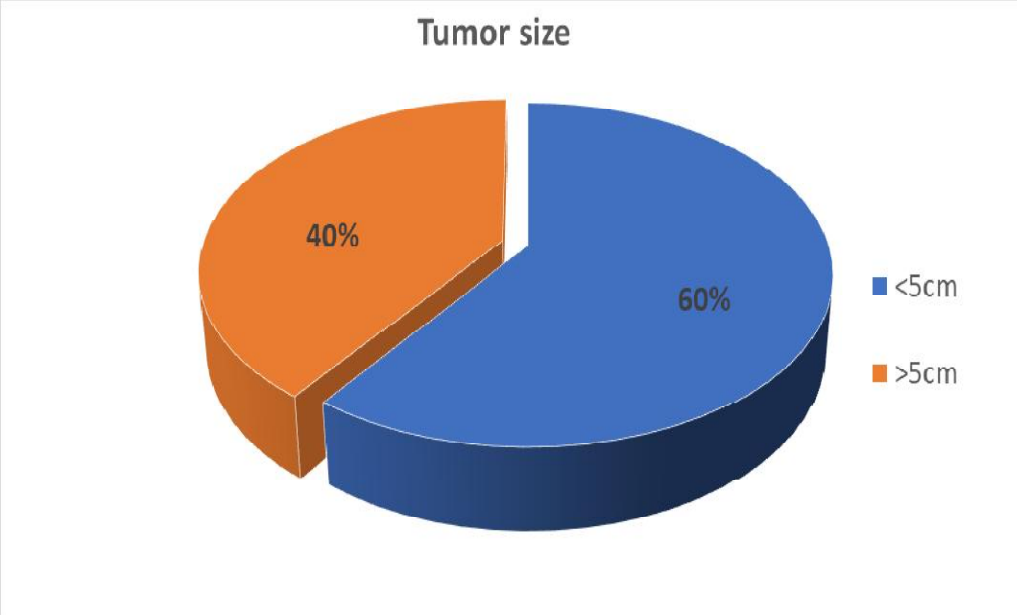

Fig. 2. Pie chart showing tumour size distribution

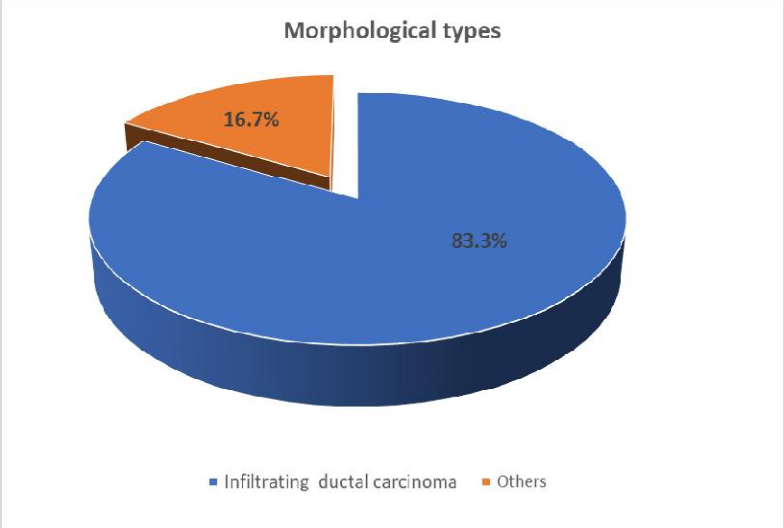

Fig. 3. Pie chart showing morphological spectrum of Breast carcinoma

Table 2. Showing Histological grade of 26 cases

Tumor Grade	No of cases	Percentage (%)
I Grade (3-5) score	4	15.4%
II Grade (6-7) score	19	73.07%
III Grade (8-9) score	3	11.53%
Total	26	100%

3.4 Histological Grading

Nottingham modification of Bloom Richardson system was used for grading the specimen. In our study, 19 (73.07%) cases showed Grade II disease, followed by 4(15.4%) cases with Grade I lesion. Three (11.53%) cases were diagnosed as Grade III disease.

3.5 Lymphovascular and Perineural Invasion

In our study, only 4 (13.3%) cases show lymphovascular tumour emboli, three being IDC, Grade-II and one (3.33%) case of Metaplastic carcinoma. No perineural tumour cell invasion was detected (Figs. 4- 14).

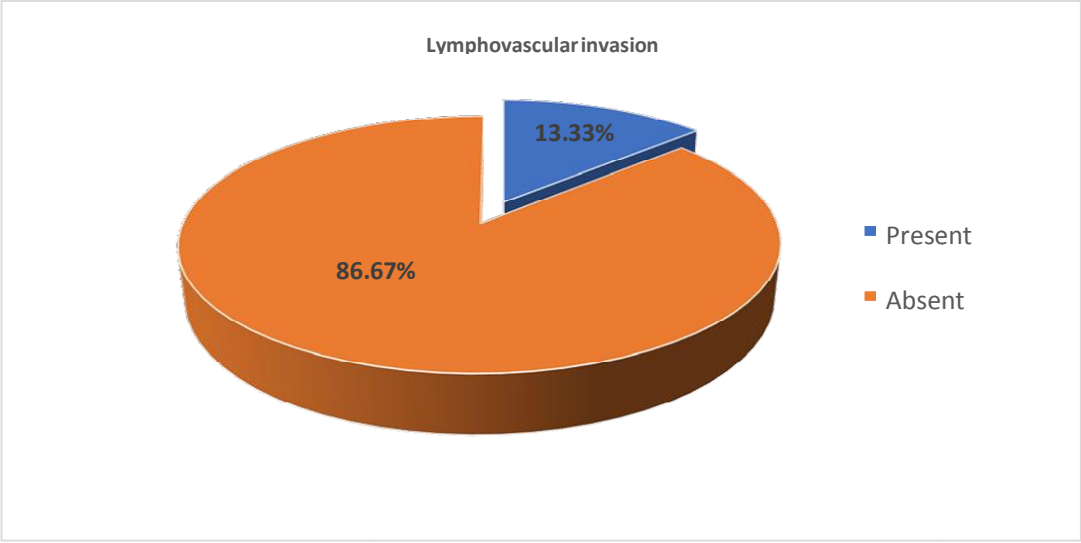

Fig. 4. Pie chart showing lymphovascular tumour cell embolization

Fig. 5. Modified Radical Mastectomy showing a well circumscribed tumour

Fig. 6. Modified Radical Mastectomy showing a well circumscribed tumour

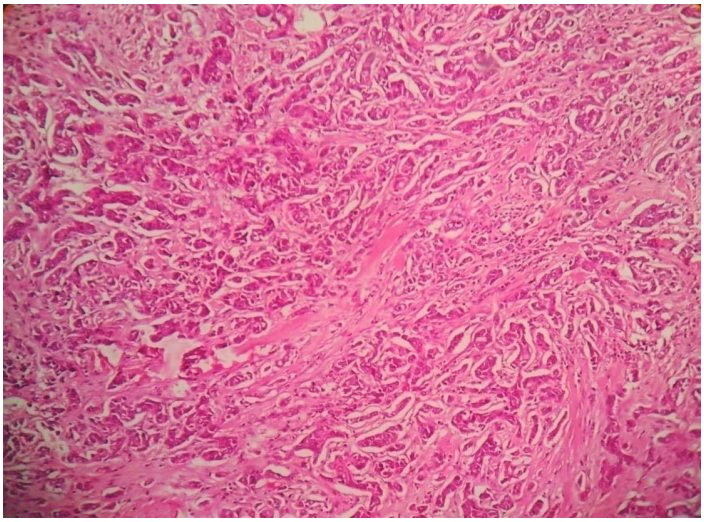

Fig. 7. NOS - IDC Grade II (H & E, 100X)

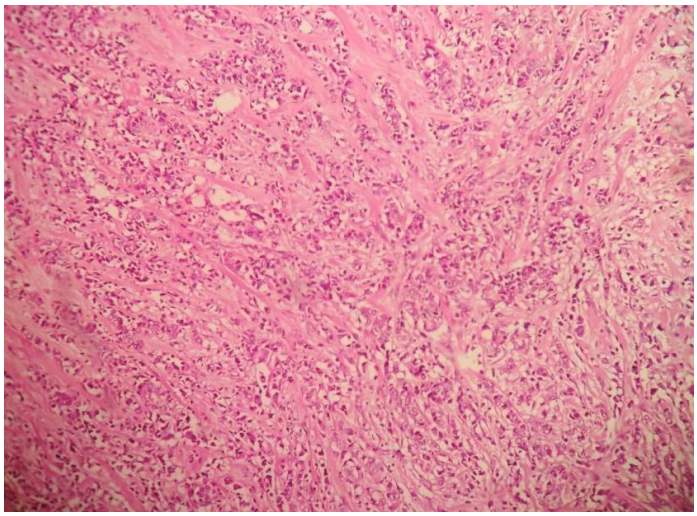

Fig. 8. NOS - IDC Grade III (H & E, 100X)

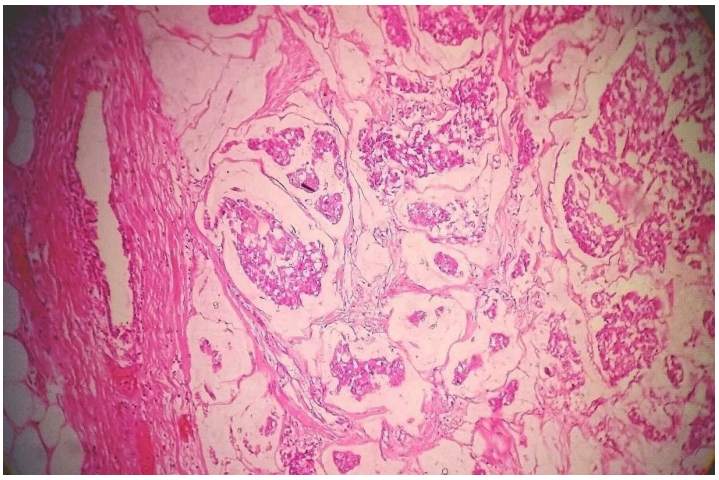

Fig. 9. Mucinous Carcinoma specimen from Breast tissue (H &E, 100X)

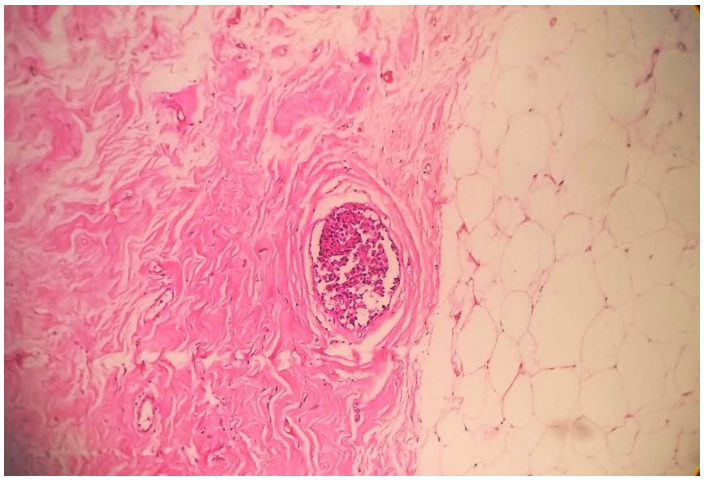

Fig. 10. IDC showing vascular invasion (H & E, 100X)

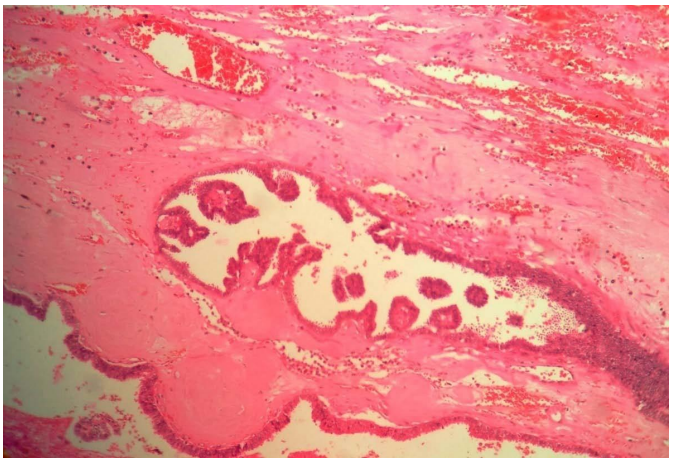

Fig. 11. Cystic papillary specimen from Breast tissue (H & E, 100X)

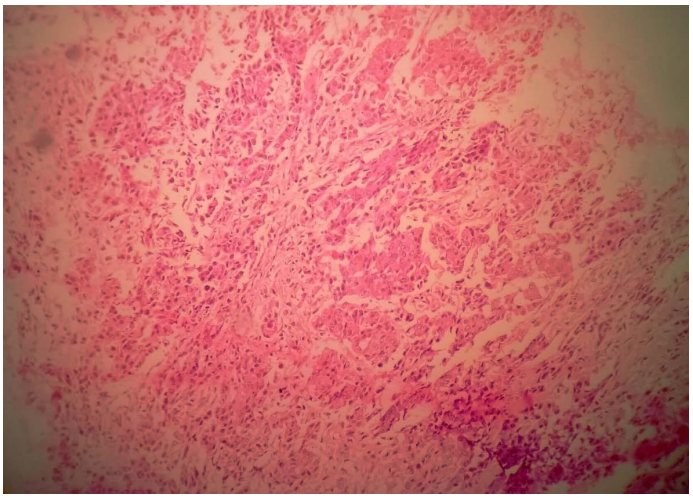

Fig. 12. Metaplastic Carcinoma from Breast tissue with squamous differentiation (H & E, 100X)

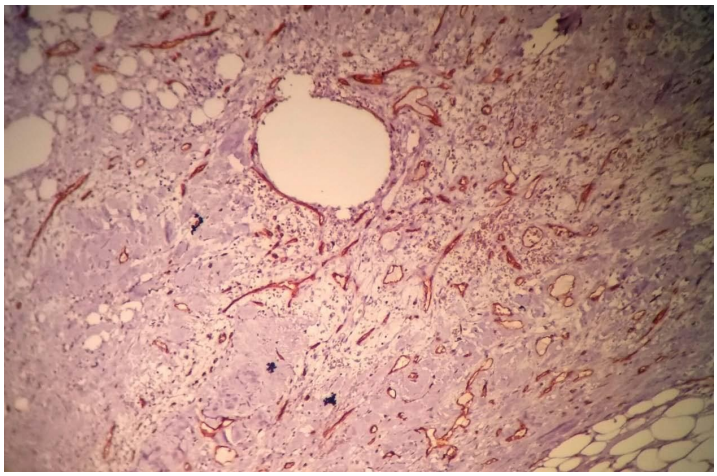

Fig. 13. Photograph showing maximum microvessels (Hot spot) area in the tumor section of IDC (CD 34 stain, 100X)

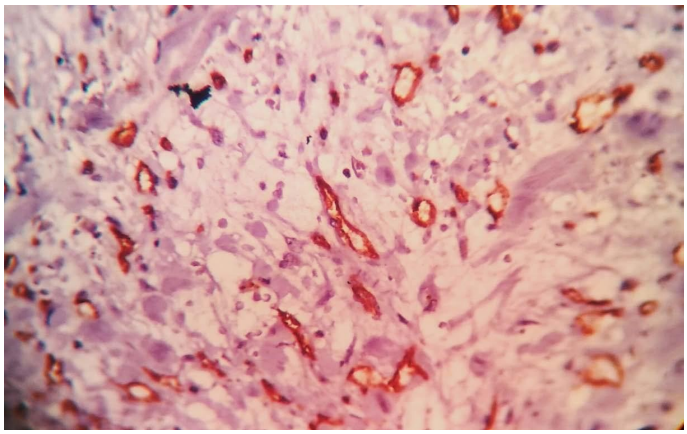

Fig. 14. Photograph showing maximum microvessels in IDC (Hot spot area)(CD 34 stain, 400X)

Angiogenesis is emerged as an important prognostic indicator in determining the tumor progression. The number and density of the micro vessels within a tumor is directly proportional to the potential of tumor cells and used to calculate the probability of tumor growth, its invasion ratio and metastasis stage. Several studies confirmed the practical applications of microvessel density in determining the stages many solid tumour types such as melanoma, prostate cancer and breast cancer [21-24]. Further, the tumor angiogenesis is usually quantified microvessels along with other important immunohistochemical markers such as Factor VIII, CD31 and CD 34. The present study analysed the microvascular density by immunohistochemical staining using CD 34 in 30 cases of confirmed breast cancer patients [25-28] In this study the age of the patient were ranged from 38 years to 79 years with mean age of 52.93 years. 40% of the cases belonged to be lesser than 50 years and 60% of patients were higher than 50 years. The present study did not show any relationship between MVD and patient's age (p value >0.05). Our results were concordant with the previous studies [29-32].

The present study showed 20.37 ± 7.83 as mean vascularization count and this value was slightly higher than the previously report (< 19). Horak et al. [32] reported the mean vascularization counts of tumour with size less than 5.0 cm as 23.12 ± 6.34 could be compared with the present study.

In this study, infiltrating ductal carcinoma NST type was predominant among the cases (25 samples, 83.3%) cases. The remaining 5 (16.7%) cases include Metaplastic carcinoma 3 cases,. Mucinous carcinoma 1 case and Cystic Papillary carcinoma 1 case. Histological grading was done in all IDC using Modified Bloom Richardson grading system and Mucinous carcinoma cases and the study also showed that increase of mean vascular density in Grade II tumor (Table 2) when compared to Grade I and Grade III with a mean MVD of 23.76 and standard deviation of 7.41. Theses results could be attributed to the findings of Miliaras et al. [30] and Biesaga et al. [31]. However a significant correlation with mean vascular count and histological grade was observed in a study by Dhakal et al. [33]. Contrast to our studies, significant relationship between increased MVD and metastasis to lymph node was reported previously by Weidner et al. [5] and Bosari et al. [34]. For micro vessels counting, the selection of antibody for immunostaining was critical. The choice of antibody determines the range of sensitivity and the specificity and there was no universal agreement in selection of the particular set of antibodies for detecting the microvessels. Further, choice of fixative, tissue- processing regime, antigen-retrieval system and visualization method also need to be considered while carry out the study. Adopting the method by, Weidner et al. [4], we counted the microvessels density by finding the vascular hotspots at 100x and point count at 400x magnification. In contrast, Martin et al. evaluated the single field with 200x for finding out the microvessel density.

Cluster of differentiation (CD) 34 is a specific marker of vascular endothelial cells and known as human hematopoietic progenitor cell antigen. It consists of a single-chain trans membrane glycoprotein with 105-120kDa and located on the long arm of chromosome [1]. In fact, CD 34 is

particularly sensitive to tumor angiogenesis since it is particularly sensitive to the tumor induced neoangiogenesis. CD 34 usually highly expressed in tumors with a higher MVD.

Several studies showed that existence of no relationship between MVD and above mentioned prognostic factors. In contrast with, few studies could correlate them. Due to small sample size, our study could not attribute to either of the above statements as conclusive.In addition, number of studies maight use more than one antibodies as markers and this might also be a reason not determining accurate association of prognostic factors and micro vessel density. In this case, few studies already showed that the anti-CD34 monoclonal antibody is more sensitive than anti-CD31 antibody and anti-factor VIII related antigens in the calculation of MVD in breast cancer as shown [34]. Since we did not use any other antibodies in the calculation of MVD in breast carcinoma for comparison with any of the other antibodies, we could not elucidative the role of MVD selection with microvessel density. Thus increased sample size and designing the study with more antibodies could give elusive results. Our sample size was only thirty. The density of microvessel varied with the dirrent stages of the tumor, particularly, several studies showed that their density was higher initial stage and non-comparable at the later stage *i.e.,* metastasis. At present, microvessel density as a prognostic marker for breast cancer is unreliable meanwhile, microvessel count seems to be an excellent marker to identify patients with good prognosis [35]. The reasons for variation could be attributed to differences in the investigation methods, technical problems (e.g. variability in immune staining) and the subjectivity with selection process and counting. However, in the future, antibodies specific to proliferating endothelium combined with automated analysers could be very useful in angiogenesis-induced microvessel density.

4. CONCLUSION

The exact identification and quantification of MVD is very useful technique in estimating the impact of the anti-angiogenic drugs used in breast cancer treatment. The microvessel counting method and antibodies used to count intra-tumoral microvessels are being used for high risk patients with adjuvant therapy. But, selection of high-risk patients who are likely to receive adjuvant therapy. The present study showed that methods need to be further standardization based on individual nature of prognostic factor.

CONSENT

As per international standard or university standard, patients' written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

The study was approved by the Institutional Ethics Committee, Sree Balaji Medical College and Hospital.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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