Original Article

Role of immunohistochemical markers CD31 and D2-40 for lymphovascular invasion in invasive breast carcinoma

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Introduction: Breast cancer is one of the leading causes of cancer death in females worldwide. Prognosis in patients of breast cancer depends on various biological and morphological criteria. A well-known and not yet explored parameter is lymphovascular invasion (LVI). LVI can be confused with the stromal artifacts. Hence, its specific confirmation by immunohistochemical (IHC) markers is highly beneficial. **Aims and Objectives:** The aim of the study was to analyze LVI in invasive breast carcinoma (No special type) by means of IHC stains CD31 and D2-40. **Material and Method:** A total of 45 cases of invasive breast carcinoma were analyzed in the Department of Pathology, Rohilkhand Medical College and Hospital, Bareilly. Evaluation of formalin-fixed paraffin embedded sections was done using H and E and IHC markers, namely, CD31 and D2-40, estrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2-NEU. **Results:** Statistical data showed that CD 31 IHC marker showed a significant advantage over H and E staining in detection of blood vessels invasion by the tumor cell (P = 0.001) and D2-40 also showed a statistically significant detection rate as compared to H and E staining (P = 0.03).

KEY WORDS: CD31, D2-40, immunohistochemical, lymphovascular invasion

INTRODUCTION

Breast cancer is one if the leading cause of cancer death in female's worldwide, breast carcinoma is one of the leading causes of cancer death in females worldwide. Management of breast carcinoma needs to be tailored for each patient according to their prognosis, which depends on various biological and morphological factors.

Traditional prognostic and predictive factors include number of positive axillary lymph nodes, tumor size, tumor grade, lymphatic and vascular invasion, estrogen receptor (ER), and progesterone receptor (PR) status. A well-known and not yet explored parameter is lymphovascular invasion (LVI). Lymphatic vessels are considered the main route by which tumor cells reach the axillary lymph nodes. LVI is considered individual predictor of lymph node metastasis in breast carcinoma.

The diagnosis of LVI is made based on the presence of tumor emboli within vascular channels lined by a single layer of endothelial cells. Lymphatic vessels are flattened channels or open spaces lined by a single layer of endothelial cells whose lumen is sometimes filled with lymphocytes. D2-40 marker is helpful in identification of lymph vessel invasion and CD31 marker is helpful in identification of blood vessel invasion.

The present study tries to carefully evaluate LVI using immunohistochemical (IHC) markers CD31 and D2-40.

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MATERIALS AND METHODS

This was an observational study carried out in the Department of Pathology, Rohilkhand Medical College and Hospital, a tertiary care hospital in Rohilkhand region Bareilly, UP. The study duration was 1 year from November 1, 2020, to October 31, 2021.

A total of 45 cases of diagnosed cases of invasive breast cancer (NST) were included in our study. All the samples were processed as per the routine histopathology laboratory practice, histopathological interpretation was done and tumor was graded using Modified Nottingham Histologic Score. LVI, PNI, ER, PR, HER2 NEU, LNR, pN, and LNR status were identified in all the cases.

All cases reported as invasive breast cancer (NOS) was subjected to IHC analysis using CD31 and D2-40 markers. LVI was diagnosed on microscopy as per CAP protocol [Table 1].

The findings of LVI in IHC section were evaluated and compared with those of H and E stained sections. The findings of LVI detection were detected in both H and E stained slide and IHC stained slide, were correlated.

Statistical analysis

Data were filled in predesigned pro forma. The data were entered into MS Excel sheet and analyzed using Statistical Package for the Social Sciences version 22 software and result was tabulated.

IEC Clearance

Study was conducted after taking informed consent and approval from Institution's Ethical Committee.

OBSERVATIONS AND RESULTS

The present study was conducted to analyze the LVI in invasive breast cancer (NST) by means of IHC stains CD31 and D2-40 in 45 consecutive cases of invasive breast carcinoma.

DISCUSSION

The study was conducted with the aim to analyze LVI in invasive breast carcinoma by means of IHC stains CD31 and D2-40. A wide range of distribution of age was seen in our study, ranging from 20 years to 75 years of age. Maximum number of patients (31.1%) was seen in age group 30–39 years [Table 2]. The mean age was 46.3±12.8. Our findings were comparable with the study done by Agarwal *et al*.[1] where age ranged from 32 to 75 years, mean age being 48, majority of them were <50 years. Findings similar with our study were also seen in study by Lee *et al*.[2] were age ranged from 28 to 76 years mean age being 49.4±10.7, majority of them were <50 years. Similar findings were seen in the study done by Rahman *et al*.[3] and Marinho *et al*.[4]

In our study, all the subject presented with complaint of breast lump, affecting predominantly in right breast 26 (57.7%), and rest 19 (42.2%) affected in left breast. In majority of patient's upper outer quadrant 30 (66.6%) was involved followed by the lower inner quadrant 6 (13.3%), upper inner quadrant 5 (11.1%), lower outer quadrant 3 (6.6%), and with retro areolar area 1 (2.2%) [Table 3]. As per the study conducted by Rummel *et*

Table 1: Criteria for lymphovascular invasion (lvi) (cap protocol)

1	LVI must be diagnosed outside the border of the invasive carcinoma. The most common area to find LVI is within 1 mm of the edge of the carcinoma.
2	The tumor emboli usually do not confirm exactly to the contours of the space in which they are found. In contrast, invasive carcinoma with retraction artifacts mimicking LVI will have exactly the same shape
3	Endothelial cell nuclei should be seen in the cells lining the space
4	Lymphatic's are often found adjacent to blood vessels and often partially encircle a blood vessel

Table 2: Age-wise distribution of the studied population

Age groups	*n*	%
20–29 years	2	4.4
30–39 years	14	31.1
40–49 years	8	17.8
50–59 years	11	24.4
60–69 years	7	15.6
70–79 years	3	6.7
Total	45	100

Table 3: Distribution of studied population according to chief complaint

Chief complaint	Frequency	Percent
Presenting complaint		
Breast lump	45	100.0
Site		
Lt breast	19	42.2
Rt breast	26	57.7
Quadrant		
Upper outer quadrant	30	66.6
Lower inner quadrant	6	13.3
Upper inner quadrant	5	11.1
Lower outer quadrant	3	6.6
Retroaleolar Area	1	2.2
Lump size<2 cm, 2–5 cm, >5 cm		
<2 cm	5	11.1
2–5 cm	2	2.2
>5 cm	38	86.6
Skin involvement- present/absent		
Absent	36	80
Present	9	20

Table 4: Distribution of studied population according to IHC panel CD31 and D240

Marker	Sensitivity	Specificity	PPV	NPV	Accuracy
CD31	82.1	70.6	82.1	70.6	77.8
D2-40	80.0	52.0	57.1	76.5	64.4

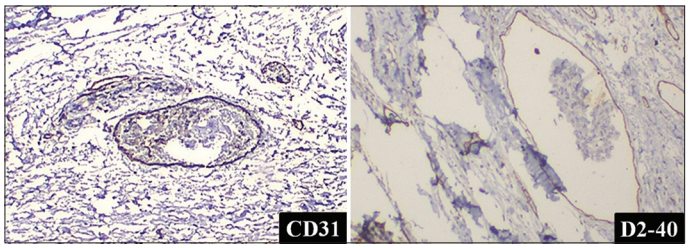

Figure 1: Microphotograph showing CD31(right) and D2-40 positivity(left)

al.[5] and Babu and Ahmad,[6] in majority of their patients upper outer quadrant of the breast were affected, which was similar to our findings.

We found sensitivity, specifity, PPV, NPV & accuracy of CD31 to be 82.1%, 70.6%, 82.1%, 70.6% & 77.8%respectively. We found sensitivity, specifity, PPV, NPV & accuracy of D2-40 to be 80 52 %, 57.1%, 76.5% & 64.4% respectively [Table 4].

In terms of size of breast lump, patient's predominantly presented with >5 cm breast lump (86.6%), followed by <2 cm (11.1%) and 2–5 cm (2.2%) size. Skin involvement was in 20% of patient whereas 80% did not show skin involvement. In the study done by Mohammed *et al*.,[7] 558 (55.6%) patient presented with breast lump size >1.5 cm, whereas 446 (44.4%) patient presented with <1.5 cm breast lump size, their findings where similar to our study.

The lymph node status was assessed as the LNR, which is defined as the ratio of positive lymph nodes to total number of lymph nodes recovered on grossing. In our study, maximum numbers of patients had LNR of <0.25–0.65 (46.7%) intermediate risk factor, <0.25 (44.5%) low risk factor and >0.65 (8.8%) high risk factor, which contradicts with the study done by Agarwal *et al*.[1] in which 26% cases fell in low risk, 24% in intermediate risk, and 50% in high risk.

Majority of patients in our study, 51.1% presented with Grade 3 tumor stage, followed by 42.2% of patients with Grade 2 and 6.7% with Grade 1. Similar findings were observed in the study done by Lee *et al*.[2] showed 44.9% patients presented with Grade 3, 35.9% with Grade 2 and 19.2% with Grade 1, study done by Rahman *et al*.[3] showed 42.2% patients presented with Grade 3, 40% with Grade 2, and 18% with Grade 1. Agarwal *et al*.[1] study showed that 44% patients presented with Grade 3, 30% with Grade 2, and 26% with Grade 1.

In our study, 39 (86.7%) cases did not show perineural invasion whereas 6 (13.3%) showed Perineural Invasion. Study done by Narayan *et al*.[8] had similar findings showed 84.3% patients with the presence of perineural invasion whereas 15.6% with absence of Perineural invasion.

From our data collected 22 (48.8%) patient showed ER positivity and 23 (51.1%) patient showed ER negativity, 18 (40%) patient showed PR positivity, and 27 (60%) patient showed PR negativity, and 36 (80%) patient showed HER 2NEU positivity whereas 9 (20%) showed HER 2 NEU negativity. The findings were accordance with the study done by Agarwal *et al*.[1] where 15 (30%) ER positivity and 35 (70%) ER negativity patient, 14 (28%) showed PR positivity, 36 (72%) showed PR negativity, 30 (60%) showed Her 2 Neu negativity, and 20 (40%) showed Her 2 Neu positivity, whereas study conducted by Lee *et al*.,[2] had a contrasting observation where the ER positive patient were 51 (63.7%), ER negative patient were 29 (36.3%), PR positive patient were 45 (56.3%), PR negative patient were 35 (43.7%), Her 2 Neu positive patient were 24 (30%), and negative were 56 (70%).

In our study, triple negative breast carcinoma where present in 18 (40%) Luminal B subtype in 11 (24.4%) and Her 2 Neu Enriched in 7 (15.6%) Luminal A subtype 9 (20%). The study done by Al-Thoubaity,[9] showed molecular subtypes of breast cancer, had a strong prognostic and predictive factor in invasive breast cancer patients. Their study showed 58.5% patient with Luminal A subtype, 16% with Triple negative, 14% with Luminal B subtype, and 11.5% with Her 2 Neu Enriched.

In our study on H and E slides 34 (75.6%) presented with LVI, whereas 11 (24.4%) had absence of LVI on H and E. Our study findings were accordance with the study done by Agarwal *et al*.,[1] 36 (72%) presented LVI whereas 14 (28%) had absence of LVI, Our study contradicts with the finding of study done by Dileep and Prasad[10] where 24 (40%) showed LVI, whereas 36 (60%) showed absence of LVI.

In our study, 28 (62.3%) showed positive CD31 and 17 (37.7%) showed negative CD31, Our findings is accordance with the study done by Dileep and Prasad,[10] where they showed CD31 positivity in 45 (75%) and negative in 15 (25%), our study contradicts with the study done by Lee *et al*.[2] where CD31 positive in 18 (22.5%) and negative in 62 (77.5%).

In our study, 20 (44.5%) showed positive D2-40 whereas 25 (55.5%) showed negative D2-40, our study findings were accordance with the study done by Lee *et al*.[2] where 7 (8.8%) were positive for D2-40 and 73 (91.2%) were negative, our study contradicts with the study done by Dileep and Prasad[10] were 40 (66.7%) showed positive D2-40 and 20 (33.3%) showed negative D2-40 result.

In this study, we found that CD 31 (P = 0.001) and D2-40 (P = 0.03) had a distinct advantage over H and E staining in detection of LVI [Figure 1]. Similar results were also observed by Agarwal *et al*.,[1] Kahn *et al*.,[11] and Dileep and Prasad.[10]

CONCLUSION

Breast cancer is one of the leading causes of cancer death in female worldwide as well as in India. The prognosis of patients of breast cancer depends on various biological and morphological criteria. A well-known and not yet explored parameter is LVI.

LVI is considered as an individual predictor of lymph node metastasis in breast carcinoma. LVI can be diagnosed on H and E stained histopathological sections within 1mm of the edge of the carcinoma. But sometimes LVI can be confused with the stromal artifacts, and also there is often inter-observer variation, hence, necessitating IHC confirmation.

In our study, 28 patients showed presence of LVI (H and E), whereas 17 showed absence of LVI (H and E), after applying IHC marker CD31, 5 patients showed positive result which were absent on LVI (H and E) which may be due to stromal artifact. Furthermore, IHC marker D2-40 showed that only 20 slides were truly having LVI positivity out 28 (on H and E) and 25 slides were negative for LVI instead of 17 as per H and E staining examination. D2-40 also showed a statistically significant detection rate as compared to H and E staining ($P = 0.03$). Thus, D2-40 stain helped in detecting the true LVI which could have been misinterpreted due to stromal artifact or inter-observer variation on H and E staining.

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