



Evaluation of Lymphatic Vessel Invasion by Immunohistochemistry in Breast Carcinoma

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Background and Objectives: Breast carcinoma, the most common malignant tumor and the leading cause of cancer death in women, affects Iranian women at least one decade earlier. Lymphatic vessel invasion (LVI) is one of the most important prognostic factors in breast carcinoma. The aim of this study was to compare the results of detecting lymphatic invasion in immunohistochemistry (IHC) method with routine histologic evaluation. **Materials and Methods:** A total of 116 breast cancer specimens were evaluated. IHC staining for D2-40 marker was performed on paraffin-embedded blocks of tumor samples to detect LVI. **Results:** Sensitivity, specificity, positive, and negative predictive values of H and E staining for detection of the lymphatic invasion were 41%, 66%, 54.8%, and 53.5%, respectively. Kappa coefficient between H and E and D2-40 stainings was 0.078. The most common sites of lymphatic invasions were intratumoral area and tumor periphery. **Conclusions:** It seems that D2-40 staining is necessary for accurate diagnosis of LVI in breast cancer. Furthermore, evaluation of intratumoral area and tumor periphery can probably increase the chance of finding lymphatic invasion.

Key words: Breast carcinoma, lymphatic invasion, D2-40

INTRODUCTION

Breast cancer is the most common malignancy and the leading cause of cancer death in women with more than 1,000,000 new cases and 519,000 deaths annually worldwide.¹⁻³

Although breast carcinoma is relatively less common in Iran, its occurrence has been increased recently.^{3,4} Breast cancer involves Iranian women at least one decade earlier^{3,4} more than 30% of Iranian patients are younger than 30 years, while in western countries, only 6% of patients are younger than 40.³ On the other hand, almost 70% of Iranian women present in advanced noncurable stages.³

Breast cancer prognosis is related to different factors including age, tumor size, grade, histologic type, lymphatic vessel invasion (LVI), lymph node metastasis, and expression of hormone receptors.^{1,2} Among them, LVI and lymph node metastasis are the main prognostic factors in patient's survival.^{1,5} Furthermore, LVI plays a critical rule in cancer

metastasis.² It is considered as an independent prognostic factor in lymph node-negative patients with mortality of breast cancer increases from 29% to 53% in node-negative patients with LVI.^{2,6,7} This emphasizes the importance of correct detection of LVI.

Lymphatic invasion is characterized as tumor emboli within an endothelial-lined lymphatic space,⁶ but it should be noted that lymphatic capillaries have no pericytes, smooth muscle, or basement membrane, makes it very hard and challenging to distinguish them from retraction artifact in breast cancer.^{5,8-10} This problem can be solved by applying specific lymphatic endothelial markers such as LPVE-1, Prox, and D2-40.^{5,10}

The aim of this study was to use specific markers to detect LVI in invasive breast carcinomas and compare the results with routine H and E evaluation of the specimens.

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MATERIALS AND METHODS

This retrospective study was conducted on 116 consecutive cases of invasive breast carcinoma underwent excisional biopsy, lumpectomy, or mastectomy with axillary lymph node dissection in Urmia University of Medical Sciences, Urmia, Iran, during March 2011 to June 2014. The research was approved by the Ethics committee of the university.

Histopathology slides of all 116 cases were retrieved from archive of the pathology department. Histological type of tumor, LVI, and lymph node metastasis were confirmed by reviewing the H and E stained slides. To evaluation of LVI, according to Aishima *et al.*, three different areas including intratumoral area, tumor periphery (a 0.2 cm wide band of tumor adjacent to the invasive fronts), and peritumoral area (a 0.2 cm wide band of nontumoral tissue adjacent to the invasive front) were studied.¹¹

IHC staining was performed on 4 µm thick sections obtained from paraffin blocks. D2–40 (clone D2–40, DAKO Corporation, Glostrup, Denmark) was used to detect LVI.

All IHC stained slides were blindly studied by the same pathologist without any knowledge of the related H and E staining results.

Statistical analysis

The results are expressed as the mean ± standard deviation (SD). Statistical analysis was performed using SPSS software version 16 (SPSS Inc., Chicago, IL, USA). Normality of data was evaluated with the Kolmogorov–Smirnov, Mann–Whitney U test, Chi-squared, and Fisher's Exact tests were used for the evaluation of the relationship between variables. A value of $P < 0.05$ were considered as statistically significant. Cohen's Kappa test was also used to evaluate interrater agreement for qualitative (categorical) items.

RESULTS

The age of the patients ranged from 20 to 86 years (mean ± SD: 48.96 ± 11.09). Of 116 cases, three of them had no residual tumor in mastectomy specimen because the whole tumor was removed in the previous lumpectomy. Hence, the study was conducted on 113 mastectomy samples. Of these 113 specimens, 100 cases (88.49%) were invasive ductal carcinoma, 11 were (9.74%) invasive lobular carcinoma, and 2 were (1.77%) unclassified.

LVI was detected in 42/113 (37.17%) cases by H and E staining, and 56/113 (49.55%) by D2–40 IHC staining method. In 23 (41.1%) of these 56 cases, LVI was also detected by H and E staining, but the other 33 cases (58.9%) were reported as negative in H and E slides. Thus, the false-negative result of

H and E staining in the detection of LVI was 58.9%. Of 57 cases described as LVI negative in D2–40 staining, LVI was reported as positive in 19 cases (33.3%) in H and E staining. Thus, the false positive rate of H and E staining was 33.3% [Table 1].

Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of H and E staining method in detecting LVI were 41%, 66%, 54.8%, and 93.5%, respectively.

Kappa coefficient factor for H and E and D2–40 IHC staining was 0.078. The most common sites of LVI documented by D2–40 staining were intratumoral and tumor periphery areas [Figure 1].

No relationship was observed between patient's age, tumor size, or histologic grade and LVI in IHC method [Table 2].

DISCUSSION

In this study, we used IHC staining method to identify lymphatic invasion (using D2–40 marker) in breast cancer specimens. We tried to compare the results of IHC staining with routine histopathologic evaluation method, H and E staining, to find whether further detection methods are needed during our routine pathologic evaluation.

In this study, H and E and D2–40 IHC staining methods detected LVI in 42/113 (37.17%) and 56/113 of cases (49.55%), respectively. Similarly, de Mascarel *et al.* found 39 LVI using IHC staining and 14 by H and E in their 94 evaluated breast cancer specimens.¹²

Sensitivity, specificity, PPV, and NPV of H and E staining in the detection of LVI in this study were 41%, 66%, 54.8%,

Table 1: Evaluation of lymphatic invasion in breast carcinoma by H and E and D2-D40 staining

D2-D40	H and E		Total (%)
	Positive, n (%)	Negative, n (%)	
Positive	23 (41.1)	33 (58.9)	56 (100)
Negative	19 (33.3)	38 (66.7)	57 (100)
Total	42 (37.2)	71 (62.8)	113 (100)

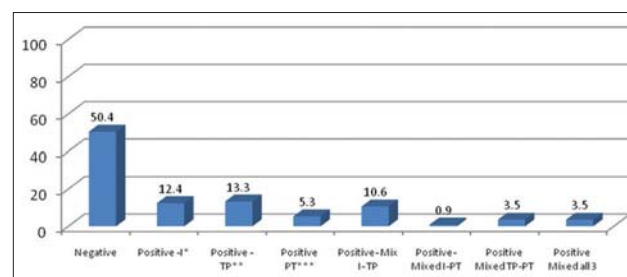


Figure 1: Distribution of lymphatic invasion according to involved area. *Intratumor, **Tumor periphery, ***Peritumor

Table 2: Lymphatic vessel invasion correlation with the age, tumor size, and histologic tumor grade

Parameter	Number and percentage of positive cases	P
Age (years)		
<50	37 (52.9)	0.24
≥50	19 (42.2)	
Tumor size (cm)		
<2	14 (53.8)	0.39
≥2	42 (48.3)	
Histologic grade		
I	9 (16.4)	0.09
II	33 (60)	
III	13 (23.6)	

and 53.5%, respectively. These results were reported as 20%, 97%, 50%, and 88.6%, respectively, by Debal *et al.*, showing the insufficiency of H and E staining in the detection of LVI in breast cancer.¹³

Comparable with our results, Marinho *et al.*, found increased LVI detection from 17/123 cases (13.8%) by H and E staining to 35/123 cases (28.5%) using D2–40 IHC staining. Although their Cohen's Kappa factor for diagnostic agreement between these two methods was 0.439 (moderate) comparing to 0.078 (poor) obtained in this study.⁵

False-negative and false-positive results of H and E staining were 59.8% and 33.3% in our results which were frankly higher than 10.8% false-negative and 2.7% false-positive results reported by Debal *et al.*¹³ This may be related to the expertise of the pathologists reporting the samples.

We also found intratumoral and tumor periphery areas as the most common sites for detection of LVI (55.5% and 62.5% of cases, respectively).

Only 27% of cases, LVI was found in the peritumoral area, while in the study of Mohammad RAA *et al.*, the most common area of LVI was peritumoral area (76%). Although in this study, tumor periphery was not evaluated.¹⁴ Evaluation of other tumoral organs such as bladder and biliary tract also revealed different results. In bladder tumors, the peritumoral area was the most common site,¹⁵ but in biliary tract tumors, LVI was observed most commonly in tumor periphery.¹¹

In contrast to other studies, we did not find a significant relationship between LVI and tumor grade ($P=0.9$).^{5,14} Reports on the correlation between LVI and tumor size is also controversial as some studies same as ours have found no correlation and some others have observed significant correlation.^{5,6,14}

In summary, we found a significant increase in identification of LVI using IHC staining besides routine pathologic evaluation of breast cancer specimens. As the LVI

is an important prognostic factor in breast cancer, we suggest using IHC staining beside routine evaluation of breast cancer specimens to better detect LVI.

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Conflicts of interest

There are no conflicts of interest.

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