



Comparing the Detection of Lymph Nodes Micrometastasis in Breast Cancer by the Hematoxylin and Eosin Staining Method (H&E) and the Immunohistochemical Method (IHC)

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Abstract

Background: Breast cancer is the most common cancer in women. The most important factor in determining the prognosis and treatment of invasive breast cancer is axillary lymph nodes involvement. It is possible not to detect micrometastasis with applying staining tissues by the Hematoxylin and Eosin (H & E) staining method. Therefore, this study was conducted to determine and compare the detection of axillary lymph node micrometastasis in breast cancer by H & E and immunohistochemical (IHC) method.

Methods: A cross sectional analytical study was carried out. By using census method, 80 female patients diagnosed with breast cancer and lymph node non-involvement were selected in Shahid Sadoughi General Hospital pathology ward, Yazd, up to 2016. IHC was performed to re-detect lymph node micrometastasis. The checklist was used to collect data of demographic, clinical, and pathological characteristics of the study population. The data collection was analyzed, using statistical software version SPSS-18, descriptive statistics, and Chi-Square analysis test. P value less than 0.05 was considered statistically significant.

Results: Of the 80 samples using H & E staining method, no case was diagnosed with lymph node micrometastasis, but using IHC, 50 cases (62.5%) were found negative and 30 cases (37.5%) were found positive. The majority of tumors in the positive group had poorly differentiated grade and the difference between the tumor grade in both positive and negative group was statistically significant ($P = 0.001$). Also, the majority of tumors in the positive group were located in the upper-outer and lower-outer quadrant of breast and the difference between tumor locations in both positive and negative group was statistically significant ($P = 0.001$).

Conclusions: According to the results, IHC is more beneficial than the H&E method to detect micrometastatic cells and to examine tissues that have high-grade tumors and also tumors located in upper-outer or lower-outer more possibility of metastasis.

Keywords: Breast Cancer, Micrometastasis, Lymph Node

1. Background

Breast cancer is the most common cause of cancer-related deaths in women (1). According to the world health organization (WHO), more than 1.2 million patients are diagnosed with breast cancer each year (2). This cancer is the cause of 21.4% of all malignancies in Iran and is the most common cancer among Iranian women (3). The highest incidence rates of breast cancer are seen in the developed countries (4). Prognostic factors for breast cancer have increased dramatically in recent years, including clinicopathological characteristics, such as tumor size, histologic grade, and axillary lymph node involvement status, which are effective in breast cancer prognosis (5, 6). One of the most important factors of determining prog-

nosis in breast cancer recurrence is the lymph node involvement status, the axillary lymph nodes, which receive 75% of breast lymph (7, 8). The detection of metastatic lymph node, especially in the early stages and micrometastasis (clusters of tumor cells ranging between 0.2 mm and 2 mm) and isolated tumor cell (ITC) (not larger than 0.2 mm), is not often detectable with regular Hematoxylin-Eosin staining method (9). Various studies have reported different results; so, given that one of the most important factors in determining prognosis, recurrence in breast cancer and lymph node involvement status in breast cancer are the axillary lymph nodes that receive 75% of breast lymph (8-10). The detection of metastatic lymph node, especially in the early stages and limited to a small number

of cells (micrometastasis), is not often detectable with regular Hematoxylin-Eosin staining method (9). Therefore, IHC is used to accurately diagnose individual metastasis of a cell to lymph nodes (10). According to the great importance of the type of treatment for the survival of the individual and considering that the involvement or non-involvement of lymph nodes around the tumors is directly effective on the disease stage (11, 12), this study was carried out to determine the diagnostic accuracy of IHC method to identify the individual axillary metastasis of a cell in patients with breast cancer, who were reported by negative H & E method.

2. Methods

The present cross sectional analytical study was conducted on paraffin embedded blocks of patients with breast carcinoma with negative axillary lymph nodes status. The required sample size in this study was estimated 80 specimens, based on the significance level of 5% and power of 80%, according to the kappa coefficient of 0.4, and based on previous studies. After obtaining permission from the ethics committee of the school of medicine (N = 140625) and visiting the pathology department of Shahid Sadoughi General hospital, by using census method a total of 80 archived samples of women with breast cancer and no lymph node involvement have been prepared until 2016. The inclusion criteria for tumor type were an infiltrative ductal carcinoma, infiltrative lobular carcinoma, and medullary carcinoma. Other breast malignant tumors that are less common were excluded from the study. Then, all H&E prepared glass slides of their lymph nodes were removed from the file and were re-studied by 2 independent pathologists. All specimens with negative lymph nodes in re-study were selected and their paraffin embedded blocks were extracted from pathology archives. IHC tests were conducted on the paraffin embedded blocks by using 2 anti-bodies composed of epithelial membrane antigen (EMA) and Pan-cytokeratin (Dako, Denmark). Stained glass slides were studied by 2 separate pathologist and positive results were reported when clusters of tumor cells ranged between 0.2 mm and 2 mm and Isolated Tumor Cell (ITC) (not larger than 0.2 mm) were detected. The results were entered in the checklist and, then, were compared with initial results. Data composed of a type of breast carcinoma, patients' age, the location of the tumor, the size of tumor, and IHC results were entered in the prepared checklist. Necessary statistical comparisons were made by the Chi-square test, using Statistical Package for the Social Sciences (SPSS) version 18 for statistical differences in 2 detection methods H & E and IHC. Also, using descriptive statistical indices, such as frequency, mean and results standard

deviation were analyzed. P value less than 0.05 was considered statistically significant.

3. Results

Of the 80 patients, the frequency of lymph node involvement positivity by IHC method divided into 2 positive and negative groups; 37.5% were positive and 62.5% were negative. The results showed that more than half of the samples were negative by IHC method (Table 1).

Table 1. The Frequency of Positive Involved Lymph Nodes by IHC Staining Method^a

Results	The Frequency of the Test
Negative	50 (62.5)
Positive	30 (37.5)
Total	80 (100)

^aValues are expressed as No. (%).

Using IHC, 80 patients under study for the frequency of lymph node involvement were divided according to tumor size into 3 categories of ≤ 2 cm, 2 - 5 cm, and > 5 cm. The negative percentages were 69.2, 61.8, and 58.3, respectively and the total was 62.5%; Positive percentages were 30.8, 38.2, and 41.7, respectively and the total was 37.5%, which means that most tumors in both positive and negative group are in 2 cm to 5 cm size category and the difference between tumor size in the 2 positive and negative groups was not statistically significant ($P = 0.839$) (Table 2). Of 80 patients under study, the frequency of lymph nodes involvement, according to the tumor grade and using IHC method, was divided into 3 groups: poorly, moderately, and well differentiated. Fifteen cases were in the negative group and 30 cases were in the positive group, which in negative group showed 25%, 73.3%, and 80% in poorly, moderately, and well-differentiated groups, respectively and in total 62.5%, and positive group showed 75%, 26.7%, and 20%, respectively and in total 37.5%. The results showed that the correlation between tumor grade and presence or absence of micrometastasis was statistical significance ($P = 0.001$) (Table 3). The frequency of lymph node involvement using IHC and according to the tumor location was divided into 3 groups of upper-outer, lower-outer, upper-inner, lower-inner, and central. The percentages of the positive and negative group were 71.8 to 28.2, 0 to 100 and 69.2 to 30.8, 40 to 60 and 78.6 to 21.4 and in total 62.5 to 37.5, respectively. The results showed that the majority of tumors in positive group were at the upper-outer and lower- outer areas and the difference between tumor location in the positive and negative groups was statistically significant ($P = 0.001$) (Ta-

ble 4). Depending on the tumor type, 3 groups of infiltrative lobular, infiltrative ductal and medullary carcinoma have been determined. The percentages of both positive and negative group were 60 to 100, 57.7 to 100 and 100 to 100 and in total 37.5 to 62.5, respectively. The results showed that the majority of tumors in both negative and positive group were infiltrative ductal and the difference between tumor type in the positive and negative group was not statistically significant ($P = 0.068$) (Table 5). A total of 80 patients under study in terms of age range of > 20 were divided into 5 groups of 20 to 30, 31 to 40, 41 to 50, 51 to 60, and > 60 ; the percentages of both positive and negative groups were 0 to 100, 20 to 80, 36.4 to 63.6, 45 to 55, and 47.4 to 52.6 and in total 37.5 to 62.5, respectively, The results showed that most negative patients were categorized into 41 to 50 age range group and in most positive patients were categorized into 51 to 60 and more than 60 age range group, and there was no significant difference between the positive and negative groups in this respect ($P = 0.152$) (Table 6).

Table 2. The Comparison Frequency of Lymph Node Involvement According to the Tumor Size by IHC Staining Method^a

Results	Tumor Size				P Value ^b
	≤ 2	2 - 5	5 <	Total N (100)	
Negative	9 (69.2)	34 (61.8)	7 (58.3)	50 (62.5)	0.839
Positive	4 (30.8)	21 (38.2)	5 (41.7)	30 (37.5)	
Total	13 (100)	55 (100)	12 (100)	80 (100)	

^a Values are expressed as No. (%).

^b Chi-square test.

4. Discussion

The aim of the present study was comparing the detection of axillary lymph nodes micrometastasis in patients with breast cancer by 2 different staining methods, one conventional staining method (H&E) and the other one, specific staining method (IHC) by using 2 immunohistochemistry anti-bodies (EMA and Pancytokeratin). In our study, there was significant differences between negative group and positive group with tumor location and tumor grade, but the correlation between negative and positive groups with tumor type, tumor size, and patients age were not statistically significant. In this study, out of 80 samples, no one was diagnosed with the lymph nodes involvement, but by using IHC method, 50 samples (62.5%) were found negative and 30 samples (37.5%) were found positive. The results were similar to the results of other studies in

this field (10, 11). Also, in a study conducted by Weaver et al. among 157 negative samples belonging to 70 patients, only 4.6% of the samples were detected as hidden metastasis (9). In our study, a significant correlation was found between tumor grade and micrometastasis that is similar to study by Amir et al. (13) and Choudhury M's study (10). In the present study, the majority of negative results belonged to tumors in 2cm to 5cm size category. The difference between tumor size in both positive and negative groups was not significant that was similar to other studies (10-12), but non-aligned with the study conducted by Dabbs et al. (14), which reported that by increasing the tumor size, the rate of micrometastasis increased. Also, in a study conducted by Gobardhan et al. (15), it was shown that by increasing the tumor size, micrometastasis decreased and macrometastasis increased. However, this difference could be due to the small sample size studied by our study. In the present study, the majority of tumors were in the age group of 41 to 50 and 61 and there was no significant difference between the positive and negative groups in this respect. In a study by Aledavood et al. (13), the median patient age was 49 years; in the study by Viale et al. (16), the median patient age was 58 years. In the present study, the results showed that the majority of tumors in the negative group were moderately differentiated and in the positive group were poorly differentiated that was non-aligned with the study of Amir et al. (13) and most of the positive groups belonged to moderately differentiated tumors. The majority of tumors in the positive group were in the upper-outer and lower-outer location; the difference between the 2 groups was significant. It seems that these results may be helpful in the differential diagnosis and identification of the cases. In addition, the majority of tumors in the 2 groups were of the infiltrative ductal type and there was no significant difference. Unfortunately, about the results cited by the researcher, similar studies were not found.

In conclusion, this study aimed at comparing the results of H & E and IHC staining's methods in the detection of lymph nodes micrometastasis. We found that 37.5% of negative lymph nodes in H & E method were positive in IHC method and there was a significant correlation between tumor grade and tumor location with micrometastasis, but no significant correlation with tumor size, patients' age, and tumor type with lymph node micrometastasis. Therefore, we recommended to detection micrometastasis by IHC method in patients with negative lymph nodes breast carcinoma, who have a higher tumor grade and also tumors located in upper-outer or lower-outer areas of the breast.

Table 3. Comparison the Frequency of Lymph Node Involvement According to Tumor Grade by Using IHC Staining Method^a

Results	Tumor Grade				P Value ^b
	Poorly Differentiated	Moderated Differentiated	Well Differentiated	Total	
Negative	5 (25)	33 (73.3)	12 (80)	50 (62.5)	0.001
Positive	15 (75)	12 (26.7)	3 (20)	30 (37.5)	
Total	20 (100)	45 (100)	15 (100)	80 (100)	

^aValues are expressed as No. (%).

^bChi-square test.

Table 4. Comparison the Frequency of Lymph Nodes Involvement According to the Tumor Location by Using IHC Method^a

Location/Results	Upper-Outer	Lower-Outer	Upper-Inner	Lower-Inner	Central	Total	P Value ^b
Negative	28 (71.8)	0 (0)	9 (69.2)	2 (40)	11 (78.6)	50 (62.5)	0.001
Positive	11 (28.2)	9 (100)	4 (30.8)	3 (60)	3 (21.4)	30 (37.5)	
Total	39 (100)	9 (100)	13 (100)	5 (100)	14 (100)	80 (100)	

^aValues are expressed as No. (%).

^bChi-square test.

Table 5. Comparison the Frequency of Lymph Nodes Involvement According to the Tumor Type by Using IHC Method^a

Results	Tumor Type				P Value ^b
	Infiltrative Lobular Carcinoma	Infiltrative Ductal Carcinoma	Medullary Carcinoma	Total	
Negative	12 (60)	30 (57.7)	8 (100)	50 (62.5)	0.068
Positive	8 (40)	22 (42.3)	0 (0)	30 (37.5)	
Total	20 (100)	52 (100)	8 (100)	80 (100)	

^aValues are expressed as No. (%).

^bChi-square test.

Table 6. Comparison the Frequency of Lymph Nodes Involvement According to the Age of the Participants by Using IHC Method^a

Results	Age of Participants						P Value ^b
	20 - 30	31 - 40	41 - 50	51 - 60	61 <	Total	
Negative	4 (100)	12 (80)	14 (63.6)	11 (55)	9 (47.4)	50 (62.5)	0.152
Positive	0 (0)	3 (20)	8 (36.4)	9 (45)	10 (52.6)	30 (37.5)	
Total	4 (100)	15 (100)	22 (100)	20 (100)	19 (100)	80 (100)	

^aValues are expressed as No. (%).

^bChi-square test.

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Footnotes

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References

1. Ferlay J, Héry C, Autier P, Sankaranarayanan R. Springer; 2010. Global burden of breast cancer. *Breast cancer epidemiology*; p. 1-19.
2. Siegel R, DeSantis C, Virgo K, Stein K, Mariotto A, Smith T, et al. Cancer treatment and survivorship statistics, 2012. *CA Cancer J Clin*. 2012;62(4):220-41. doi: [10.3322/caac.21149](https://doi.org/10.3322/caac.21149). [PubMed: 22700443].

3. Taghavi A, Fazeli Z, Vahedi M, Baghestani AR, Pourhoseingholi A, Barzegar F, et al. Increased trend of breast cancer mortality in Iran. *Asian Pac J Cancer Prev*. 2012;**13**(1):367-70. [PubMed: [22502702](#)].
4. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer*. 2015;**136**(5):E359-86. doi: [10.1002/ijc.29210](#). [PubMed: [25220842](#)].
5. Stankov A, Bargallo-Rocha JE, Silvio AN, Ramirez MT, Stankova-Ninova K, Meneses-Garcia A. Prognostic factors and recurrence in breast cancer: experience at the national cancer institute of Mexico. *ISRN Oncol*. 2012;**2012**:825258. doi: [10.5402/2012/825258](#). [PubMed: [22830047](#)].
6. Sinha S, Nath J. Predictive and Prognostic Factors in Breast Cancer and their Association with ER PR HER2/neu Expression. *J Carcinogenesis Mutagenesis*. 2016;**7**(2). doi: [10.4172/2157-2518.1000263](#).
7. Tjan-Heijnen VC, Buit P, de Widt-Evert LM, Ruers TJ, Beex LV. Micrometastases in axillary lymph nodes: an increasing classification and treatment dilemma in breast cancer due to the introduction of the sentinel lymph node procedure. *Breast Cancer Res Treat*. 2001;**70**(2):81-8. [PubMed: [11768607](#)].
8. Garg M, Nagpal N, Sidhu DS, Singh A. Effect of Lump Size and Nodal Status on Prognosis in Invasive Breast Cancer: Experience from Rural India. *J Clin Diagn Res*. 2016;**10**(6):PC08-11. doi: [10.7860/JCDR/2016/20470.8039](#). [PubMed: [27504343](#)].
9. Weaver DL, Krag DN, Manna EA, Ashikaga T, Waters BL, Harlow SP, et al. Detection of occult sentinel lymph node micrometastases by immunohistochemistry in breast cancer. An NSABP protocol B-32 quality assurance study. *Cancer*. 2006;**107**(4):661-7. [PubMed: [17024757](#)].
10. Choudhury M, Agrawal S, Pujani M, Thomas S, Pujani M. Immunohistochemical detection of axillary lymph node micrometastases in node negative breast cancer patients using cytokeratin and epithelial membrane antigen. *South Asian J Cancer*. 2015;**4**(1):28-31. doi: [10.4103/2278-330X.149946](#). [PubMed: [25839017](#)].
11. Kohlberger P, Gantert M, Volk-Orlowska T, Kieback DG, Gitsch G. Immunohistochemical detection of lymph node metastases in node-negative breast cancer patients. *Anticancer Res*. 2001;**21**(1B):697-9. [PubMed: [11299828](#)].
12. Rutledge H, Davis J, Chiu R, Cibull M, Brill Y, McGrath P, et al. Sentinel node micrometastasis in breast carcinoma may not be an indication for complete axillary dissection. *Mod Pathol*. 2005;**18**(6):762-8. doi: [10.1038/modpathol.3800394](#). [PubMed: [15761490](#)].
13. Amir AS, Azar FP, Sahram N, Naser FM, Na'ma MR, Farid F, et al. Prevalence of occult metastases in axillary sentinel lymph nodes of breast carcinoma. *Pol J Pathol*. 2012;**63**(1):40-4. [PubMed: [22535605](#)].
14. Dabbs DJ, Fung M, Landsittel D, McManus K, Johnson R. Sentinel lymph node micrometastasis as a predictor of axillary tumor burden. *Breast J*. 2004;**10**(2):101-5. [PubMed: [15009035](#)].
15. Gobardhan PD, Elias SG, Madsen EV, van Wely B, van den Wildenberg F, Theunissen EB, et al. Prognostic value of lymph node micrometastases in breast cancer: a multicenter cohort study. *Ann Surg Oncol*. 2011;**18**(6):1657-64. doi: [10.1245/s10434-010-1451-z](#). [PubMed: [21153885](#)].
16. Viale G, Maiorano E, Mazzarol G, Zurrida S, Galimberti V, Luini A, et al. Histologic detection and clinical implications of micrometastases in axillary sentinel lymph nodes for patients with breast carcinoma. *Cancer*. 2001;**92**(6):1378-84. [PubMed: [11745213](#)].