ORIGINAL ARTICLE

Comparison of Immunohistochemical Coloring, Hematoxyline and Eosin Staining for Detecting of Lymphatic Metastasis in patients with Breast Cancer and Negative Lymph Node Involvement

MASOUMEH JAFAR AGHAEI, MOHSEN DEHGHANI ZAHEDANI, AMIN SHAFIZAD, ALIA ATASH ABPARVAR
Faculty of Medical Sciences, Hormozgan University of Medical Sciences, Bandar Abbas, Iran.
Correspondence to Dr. Masoumeh Jafar Aghaei Email: amamali110@gmail.com

ABSTRACT

Background: The patients with breast cancer are examined for detecting lymph node involvement and metastasis by Hematoxyline and Eosin staining which is routinely used and may have fase negative.
Aim: To compare immunohistochemical detection of lymph node metastasis with Hematoxyline and Eosin staining with EMA and Cytokeratin (CK) antigens in patients with breast cancer and Negative lymph node involvement. .
Methods: In this observational retrospective cross-sectional study, 64 consecutive patients with breast cancer who had negative H and E results were enrolled and the EMA and CK results were determined and compared with H and E.
Results: The EMA and CK results were negative in all patients showing 100% congruence with H & E results for lymph node metastasis.
Conclusion: there is high congruence rate for detection of lymph node metastasis with Hematoxyline & Eosin staining and EMA and CK.
Keywords: Hematoxyline & Eosin, EMA, CK, lymphatic metastasis

INTRODUCTION

Breast cancer is the most common cancer after lung and stomach cancers and the most common among women, consisting of one third of all cancers. It is the second leading cause of cancer deaths in women. According to medical records in Iran, breast cancer has increased among the women and consists of 11/3%) of all cancers in women.

The lymph node status, classification by coloring with hematoxylin and eosin (H & E), is one of the most promising predictors of breast cancer[1,2]. Before the introduction of screening mammography, 60% of newly diagnosed patients with breast cancer had negative nodes[3]. After its introduction, with the detection of smaller and smaller lesions, its percentage increased to 65%[4]. Although the absence of node involvement has a better prognosis than its presence, 20-30% of the patients with negative nodes progress to the metastases that ultimately decrease.

But hematoxylin and eosin method can have false negative results.in some studies, up to 50% of the cases have been reported false negative[6]. Therefore, using more precision and diagnostic sensitivity methods, such as immunohistochemical evaluation (IHC), can help in the more accurate diagnosis of lymphatic metastases[10,7] and reduce false negative ones.

Recent studies demonstrated that pathologic evaluation of the sentinel node has evolved from routine evaluation with bisection of the lymph node to ultrastaging with serial sections stained with hematoxylin and eosin (H&E). Some investigators have added immunohistochemical (IHC) staining of SLN to help detect micrometastases. However, the value of IHC staining in addition to ultrastaging has yet to be determined. As well, the clinical significance of micrometastases as defined as a focus of metastatic cells with a size less than or equal to 2mm to an inguinal node is unknown.

Yadav and colleagues carried out a study in 2014 in India. They assessed 30 patients with breast cancer and negative lymph node involvement by H and E coloring of IHC method. The results showed that five cases were micro-metastasis[9].

Gujam and colleagues, in 2014 in England, studied 360 patients with breast cancer and negative lymph node involvement by H and E coloring of IHC method. They observed micrometatasis in 16%-35% of cases[16].

Richard G. Moore and colleagues studied pathologic evaluation of inguinal sentinel lymph nodes in vulvar cancer patients: a comparison of immunohistochemical staining versus ultrastaging with hematoxylin and eosin staining in 2003. They found that the addition of immunohistochemical staining to ultrastaging with H&E staining in the pathologic evaluation of inguinal sentinel lymph nodes does not increase the detection of micrometastasis in patients with primary squamous cell carcinoma of the vulva.According to the importance of H and E coloring of IHC method in predicting the metastasis, we decided to compare the IHC and H & E staining for the diagnosis of lymph node metastasis in patients with breast cancer and negative lymph nodes involvement .

METHOD

This cross-sectional study was conducted to compare IHC with H & E staining for diagnosing the lymph node metastasis in patients with breast cancer and negative lymph node involvement. the patients with breast cancer referring to the pathology department of Shahid Mohammadi Hospital until 2016, who had breast cancer and negative H & E staining were enrolled in the study. Patients whose records were incomplete and male genders were excluded.

The sampling method was convenient in the duration of 2010 to 2016 and the sample size was calculated 64 according to the Cochran formula. The variables were

included age, type of tumor, number of lymph nodes, tumor size, H & E staining,anti-genes of epithelial membrane antigen, and Cytokeratin. The data were recorded in a checklist.

Negative lymph nodes samples were selected, evaluated, and the presence or absence of Epithelial membrane antigen (EMA) and Cytokeratin (CK) antigens, indicating the frequency of occult micrometastasis, was determined by the IHC method (based on H & E staining, which is the most common histological coloring method using for medical diagnosis, tumor detection and other pathologies. In this method, thin sections are made from the tissue and stained with hematoxylin and eosin, which making the cell nucleus purple and cytoplasm pink). IHC is a color scheme used to determine the antigen in tissues. The basis of this method is the binding antibody and antigen to the target. Antibodies are labeled in this method with specific colors such as fluorescein or Rhodamine and attached to the peroxidase enzyme. in the presence of antigen, brown color is created by enzymatic reactions.

After collecting the required data, SPSS software version 13 was used to analyze the data. For qualitative variables and frequencies, mean and standard deviations were calculated. The statistical test used was Kappa.

RESULT

In this cross-sectional study, the mean age of patients was 44.1±7.2. The maximum age was 59 and the minimum age was 28 years. The median age was 43.5± 2.7.

The mean size of the lesion in the patients was 3.05± 0.89 centimeters. The maximum size was 5 cm and the minimum was 1.2 cm. The median size was 3±0.89.

The mean number of lymph nodes in the patients under study was 2.13 with a standard deviation of 3.8. The maximum number was 22 and the minimum age was 4. The median number was 13 and the standard deviation was 3.8. The tumor type was invasive ductal in 75.73% and invasive lobular in 25.26%.

The frequency of tumor type is shown in Table 1 based on age, lesion size and number of lymph nodes. there was a significant difference type of tumor and age (P = 0.017). There was no significant difference between the type of tumor and the size of lesion (P = 0.223) and the number of lymph nodes (P = 0.570).

Table 1 Frequency distribution of tumor type based on age, size of lesion and number of lymph nodes

Variable	invasive ductal	invasive lobular	P Value
age	43/6 ± 6/9	52/3 ± 4/9	223/0
Lesion size	3 ± 0/9	3/6 ± 0/8	570/0
lymph nodes number	13/1 ± 3/9	14/3 ± 1/5	017/0

EMA and CK results were negative in all patients.

DISCUSSION

The standard method of staging breast cancer, which is clinically in the lower stage (I-II), is to find a lymph node[4] patients with breast cancer are routinely examined by a vagrant lymph node until a lymphatic metastasis is detected. After detecting a lymph node, H & E staining is used routinely[4,5], but this method can have false negative cases, and in some studies, up to 50% of the cases have been reported as false negative. Therefore, further diagnostic procedures such as evaluating the accuracy and sensitivity of IHC can help in more accurate diagnosis lymph node metastasis[7,8,9,10] and reduce false negative ones. According to the importance of detecting lymph node metastases as soon as possible, we compared the IHC and H & E staining for the diagnosis of lymph node metastasis in the patients with breast cancer and negative lymph nodes.

In this study, epithelial membrane antigen and Cytokeratin were negative in all patients. According to the negative lymph nodes based on H & E staining, the two methods had 100% sensitivity. In our study, the mean size of the lesion was 3.25. The clinical relevance of micrometastasis to a regional lymph node or sentinel node is controversial. Multiple investigators have examined the prognostic implication for breast cancer patients found to have micrometastasis (less than 2 mm in size) in an isolated axillary node and have reported conflicting results[18,19,20].

Nasser and colleagues found that occult metastases are more common in greater tumor[18]. McGuckin and colleagues showed that there is a significant relationship between lymph node metastasis detected by IHC and tumor size. In their study, it was shown that there is metastasis in 35% of tumors larger than 2 cm and in 20% of tumors smaller than 2 cm[19]. In the study of Cote and colleagues, 26% of cases with a tumor size greater than 2 cm and 16% with tumor size less than or equal to 2 cm were positive for lymph node metastases detected by IHC[15]. Umekita et al. showed that the highest rate of metastasis was found in tumors larger than 2 cm[20].

In our study the tumor type was invasive ductal in 75.73% and invasive lobular in 25.26%. Hainsworth et al. Found that percentage of lymph node metastasis in invasive lobular carcinoma and invasive ductal carcinoma was 1.90% and 11.49%[21]. Grabau and colleagues showed that there is a significant relationship between the type of tumor and the lymph node metastases detected by IHC, so that the rate of detection of lymph node metastasis was higher in invasive ductal carcinoma[22]. In the study of McGuckin and Cote, no significant relationship was found between the type of tumor and metastasis of lymph nodes, but the rate of lymph node metastasis was higher in invasive lobular carcinoma[15,19].

However, Hainsworth and Grabau found significant statistical associations between the number of lymph nodes and lymph node metastases by IHC[21,22]. But similar studies need to perform with a large number of samples.

In a study by Kohlberger et al. in Austria, the results of which were published in 2001, 101 patients with breast cancer were investigated and 11 of them were negative in H & E coloring that were diagnosed by IHC method And the IHC method increased the detection rate of lymph node

micro metastases[7], which, as seen, was also good in our research.

In a study by Choudhury et al. in India, published in 2015, 32 patients with negative lymph nodes were screened for H & E staining, and it was reported that according to IHC, an increase of 18.7% was found in cases of occult micro metastasis[8], however, in our study, H & E staining did not differ from the IHC method.

In a study by Yadav et al. in India, the results of which were published in 2014, 30 patients with breast cancer with negative lymph nodes in H & E staining were examined by IHC and found that 5 of them were secret micro metastasis[9] which in our research did not show any secret micro metastasis.

In the study of Wells et al. in England in 1984, 45 patients with breast cancer with negative lymph nodes were diagnosed with H & E staining. In the IHC study, 15% of the cases were diagnosed as secret micro metastasis[10], which was 0 percent in our research.

In a study by Cote et al in the United States in 1999, 736 patients with breast cancer with negative lymphatic cancers were diagnosed with H & E staining, of which 39% were IHC based on lymphatic micro metastasis[15], which were all negative in our study.

In a study by Gujam et al in the UK in 2014, 360 patients with breast cancer with negative lymph nodes were diagnosed with H & E staining, based on the type of marker used in the IHC method, lymphatic micro-metastasis was detected in 16 to 35% of patients. In our study[16], the antigens of epithelial membrane antigen and Cytokeratin were negative in all cases.

In a study by Turner et al in the United States in 1997, 103 patients with breast cancer with negative lymph nodes were diagnosed with H & E staining, according to the IHC method, lymphoma micro-metastatic was found in 14.3% of patients[17], Which is higher than our zero-percent statistic.

CONCLUSION

Emerging technology has allowed for the detection and sampling of selected lymph nodes from lymphatic basins deemed to be at risk for metastatic disease. Based on all aspects, it is concluded that immunohistochemical staining and staining of hematoxylin and eosin are well suited for detecting lymphatic metastasis in the negative lymph node complex in patients with breast cancer. At the end, multicenter studies with higher sample sizes and comparisons with other diagnostic methods are suggested in patients with malignant mammary tumors suspected of lymph node metastasis.

REFERENCES

1. Harirchi I, Ebrahimi M, Zamani N, Jarvandi S, Montazeri A. Breast cancer in Iran: a review of 903 case records. Public Health. 2000; 114(2):143-5.
2. Vahdaninia M, Montazeri A. Breast cancer in Iran: a survival analysis. Asian Pac J Cancer Prev. 2004 Apr-Jun;5(2):223-5.
3. Radice D, Redaelli A. Breast cancer management: quality-of-life and cost considerations. Pharmacoeconomics. 2003;21(6):383-96.
4. Salem A. Sentinel lymph node biopsy in breast cancer: a comprehensive literature review. J Surg Educ. 2009 Sep-Oct;66(5):267-75.
5. Motomura K, Komoike Y, Nagumo S, et al. Sentinel node biopsy to avoid axillary lymph node dissection in breast cancer. Breast Cancer. 2002;9(4):337-43.
6. Cohen LF, Breslin TM, Kuerer HM, Ross MI, Hunt KK, Sahin AA. Identification and evaluation of axillary sentinel lymph nodes in patients with breast carcinoma treated with neoadjuvant chemotherapy. Am J Surg Pathol. 2000 Sep;24(9):1266-72.
7. 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 Jan-Feb;21(1B):697-9.
8. 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 Jan-Mar;4(1):28-31.
9. Yadav R, Singh S, Marwah N, Kataria K, Aggarwal G, Sen R. Immunohistochemical detection of axillary lymph node micrometastases in breast cancer patients: Increasing the accuracy of detection and decreasing labor intensive serial sectioning. Indian J Cancer. 2014 July-September;51(3):267-71.
10. Wells CA, Heryet A, Brochier J, Gatter KC, Mason DY. The immunocytochemical detection of axillary micrometastases in breast cancer. Br J Cancer. 1984 Aug;50(2):193-7.
11. Berek JS, Adashi EY, Hillard PA. Novak's Gynecology. 14th Baltimore, MD: Lippincott Williams & Wilkins; 2006.
12. American Cancer Society. Breast Cancer Facts & Figures 2009-2010. Available at http://www.cancer.org
13. Andreoli TE, Carpenter CJ, Griggs Bengamin IJ. Cecil Essentials of Medicine. 8th edition. W.B. Saunders. 20011.
14. Fauci AS, braumwald E, Kasper DL, et al. Harrison's Principles of Internal Medicine. 18th edition. McGraw-Hill. 2012.
15. Cote RJ, Peterson HF, Chaiwun B, et al. Role of immunohistochemical detection of lymph-node metastases in management of breast cancer. International Breast Cancer Study Group. Lancet. 1999 Sep 11;354(9182):896-900.
16. Gujam FJ, Going JJ, Mohammed ZM, Orange C, Edwards J, McMillan DC. Immunohistochemical detection improves the prognostic value of lymphatic and blood vessel invasion in primary ductal breast cancer. BMC Cancer. 2014 Sep 18;14:676.
17. Turner RR, Ollila DW, Krasne DL, Giuliano AE. Histopathologic validation of the sentinel lymph node hypothesis for breast carcinoma. Ann Surg. 1997 Sep;226(3):271-8.
18. Nasser IA, Lee AK, Bosari S, Saganich R, Heatley G, Silverman ML. Occult axillary lymph node metastases in "node-negative" breast carcinoma. Hum Pathol 1993;24:950-7.
19. McGuckin MA, Cummings MC, Walsh MD, Hohn BG, Bennett IC, Wright RG. Occult axillary node metastases in breast cancer: Their detection and prognostic significance. Br J Cancer 1996;73:88-95.
20. Umekita Y, Ohi Y, Sagara Y, Yoshida H. Clinical significance of occult micrometastases in axillary lymph nodes in "node-negative" breast cancer patients. Jpn J Cancer Res 2002;93:695-8.
21. Hainsworth PJ, Tjandra JJ, Stillwell RG, Machet D, Henderson MA, Rennie GC, *et al*. Detection and significance of occult metastases in node-negative breast cancer. Br J Surg 1993;80:459-63.
22. Grabau D, Jensen MB, Rank F, Blichert-Toft M. Axillary lymph node micrometastases in invasive breast cancer: National figures on incidence and overall survival. APMIS 2007;115:828-37.