

Discordance of Human Epidermal Growth Factor Receptor-2 Expression in Primary Breast Cancer and Related Axillary Lymph Node Metastasis

Azar Naimi¹, Fereshteh Mohammadizadeh², Bahar Saffar³

Department of Pathology, School of Medicine, Isfahan University of Medical Sciences, Isfahan

Abstract

Introduction: Difference in human epidermal growth factor receptor-2 (Her-2) expression in metastatic axillary lymph nodes and primary breast tumor may affect treatment response, as studies have shown that change of therapy according to lymph node metastasis biomarker expression, improved patient prognosis. The aim of current study is to evaluate the Her-2 expression in primary breast cancer (BC) and its accordance with metastatic axillary lymph nodes. **Materials and Methods:** This is a cross-sectional study on 70 patients with BC conducted in Isfahan University of Medical Sciences. Age, number of involved lymph nodes, Her-2, estrogen receptor (ER), and progesterone receptor expression and in case of Her-2 expression, its grade in primary BC were recorded. Her-2 expression was evaluated in the metastatic axillary lymph node by immunohistochemistry method. **Results:** The mean age of patients who had concordance was 51.2 ± 13.1 and those who did not have this association was 48.9 ± 12.1 ($P = 0.45$). 48.6% of samples showed discordance of primary BC and lymph node metastasis. In ER-positive patients, 42.9%, and in ER-negative group, 60%, revealed concordance. In discordance group, 50% revealed Grade 1, 43.2% Grade 2 and 61.5% Grade 3. **Discussion:** Lower number of metastatic lymph nodes was significantly associated with Her-2 expression concordance. Our study found positive conversion in 12.5% of patients who have primary BC negative results with positive metastatic lymph nodes and 42.1% of negative conversion. In the current study, we found a high rate of discordance of Her-2 expression in primary BC with metastatic lymph nodes. The number of metastatic lymph nodes affected Her-2 expression concordance diversely.

Key words: Breast cancer, discordance, human epidermal growth factor receptor-2, immunohistochemistry, lymph node metastasis

INTRODUCTION

Breast cancer (BC), as the most prevalent malignancy of females, accounts for more than 10% of all malignancies worldwide.^[1] Over 2,00,000 females with BC were diagnosed in the United States in 2013.^[2] Half million female deaths are occurring due to BC worldwide per year.^[3] Prevalence of BC, as the most prevalent female malignancy and important concern of health in Iran, accounts for about 32% of Iranian females' malignancies. Furthermore, a number of 7000 new cases are diagnosed each year.^[4]

Several risk factors have been explained for BC developing, including genetic background, environmental factors, family history, menstrual and pregnancy status, early menarche, age of menopause, alcohol consumption, and also the previous history of cancer.^[4]

BC grading and staging play an important role in the prognosis of BC and have been discussed about for many years. Further investigations led to the discovery of human epidermal growth factor receptor-2 (Her-2) marker that acts as an important therapeutic and prognostic factor.^[5]

Her-2 marker is a member of epidermal growth factor receptors. Overexpression of Her-2 may act as an oncogene that can be detected in 25–30% of BCs. Patients with Her-2/neu overexpression may respond to treatment with trastuzumab appropriately.^[6]

Address for correspondence:

Azar Naimi, Department of Pathology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran. E-mail: azar.naimi@med.mui.ac.ir

Received: 10-05-2018

Revised: 18-06-2018

Accepted: 23-06-2018

The incidence of biomarkers in metastatic lymph nodes in cases with unknown tumor primary origin provides important information about origin of cancer and its prognosis. Her-2 overexpression in auxiliary lymph nodes of BC patients can affect disease management significantly, as studies have shown that change of therapy according to lymph node metastasis biomarker expression, improved patient prognosis.^[7]

Some studies have assessed the association between Her-2 expression in metastatic lymph node with primary BC and found even up to 95% of accordance.^[8]

The aim of current study is to evaluate the Her-2 expression in primary BC, its accordance with metastatic axillary lymph nodes and also to assess this association with some prognostic factors.

MATERIALS AND METHODS

This is a cross-sectional study on 70 patients with BC conducted in pathology department of Al Zahra Hospital (affiliated to Isfahan University of Medical Sciences).

The experiment protocol was approved by the Research Committee and Ethics Committee of Isfahan University of Medical Sciences.

Inclusion criteria in current study included; (1) existence of breast carcinoma paraffin blocks in archive section of Pathology Department of Al Zahra Hospital, (2) coincidence of axillary lymph node metastasis, (3) axillary metastatic lymph node block accessibility, (4) acceptable quality of lymph node block for sampling, and (5) also estrogen receptor (ER), progesterone receptor (PR), and Her-2 expression evaluation on primary breast carcinoma sample. Low-quality samples, suspicious ones, and samples with a report of two-plus Her-2 were excluded.

The BC samples existed in pathology department archive were assessed by a resident of pathology. All information including age, number of involved lymph nodes, Her-2, ER, and PR expression positivity/negativity and in case of Her-2 expression, its grade in primary BC were recorded.

Among metastatic lymph nodes of chosen BC samples, one block was selected randomly, and Her-2 expression was evaluated in the metastatic axillary lymph node by immunohistochemistry (IHC) method. The used antibody in our study is Rabbit Anti-human c-erbB-2 Oncoprotein, DAKO Brand, made by Denmark.

Two pathologists studied Her-2 slides and reported the results using this approved scaling:^[9]

0+(negative): Non-staining or mild membranous staining of tumoral cells ($\leq 10\%$).

1+(negative): Extremely mild and incomplete membranous staining of $\geq 10\%$ of tumoral cells.

2+(equivocal): Mild to moderate incomplete membranous staining of $\geq 10\%$ of tumoral cells.

3+(positive): Intense complete membranous staining of $\geq 10\%$ of tumoral cells.

Her-2 slides of primary BC were reviewed by two pathologists and rescored by mentioned scale.

In order of consistency with fluorescence *in situ* hybridization methods, 3+ results of IHC evaluation were considered positive, and 0 and 1+ results were negative. Then, the Her-2 expression results of lymph nodes were compared with primary tumors.

Primary BC s were also graded using Nottingham histologic grade.^[10]

Obtained data were analyzed with IBM SPSS 22 United States. Descriptive data are reported in mean \pm standard deviation. For analytics, *t*-test, Chi-square, and ANOVA were used. $P < 0.05$ was considered significant.

RESULTS

This study was conducted on 70 patients with BC with a mean age of 50.10 ± 12.60 . Number of 38 (54.3%) patients had metastasis to one lymph node, while 17 (24.3%) had two, and 15 (21.4%) had three lymph node involvement. Half of the patients were ER and PR positive.

Expression of Her-2 in primary BC and also metastatic lymph nodes are shown in Table 1.

The concordance of Her-2 expression in primary BC and metastatic lymph nodes is shown in Table 2.

Nearly 48.6% of samples showed discordance of primary BC and lymph node metastasis. The most discordance is seen in equivocal (2+) results.

Table 3 is demonstrating the association of Her-2 expression concordance in metastatic lymph nodes and ER-PR expression and number of metastatic lymph nodes.

Table 1: Her-2 expression in primary BC and metastatic lymph node

Her2 IHC score	Her-2 expression	
	Breast (%)	Metastatic lymph node (%)
IHC result		
0+	31 (44.3)	26 (37.1)
1+	9 (12.9)	12 (17.1)
2+	11 (15.7)	14 (20.0)
3+	19 (27.1)	18 (25.7)

Her-2: Human epidermal growth factor receptor-2, BC: Breast cancer

Grading of primary BC and association with Her-2 expression concordance/discordance is shown in Table 4.

The mean age of patients who had concordance was 51.2 ± 13.1 , and those who did not have this association was 48.9 ± 12.1 ($P = 0.45$).

DISCUSSION

Overexpression of Her-2 can be an underlying reason for BC. Her-2 expression is in association with poor prognosis, rapid tumor growth and also better response to adjuvant chemotherapy and Herceptin. 8–20% of BC patients are Her-2 positive worldwide and results of a study in Iran was in accordance with other regions.^[11]

Although Her-2 expression status in primary BC is expected not to change in lymph node metastasis, several studies have reported contrary results. This discordance has been ranged

even up to 34%.^[8,12] The discordance of Her-2 expression has been reported in both positive and negative direction.^[13,14] This study has been conducted in Iran to assess this concordance in BC cases of Isfahan province.

The discordance of Her-2 expression may be associated with some factors including the method of Her-2 status assessment, evaluation of Her-2 in tissue section or serum protein levels, and administration of anti-Her2 regimen before second specimen obtaining.^[13] These confounders were omitted by matching.

In current study, 48.6% of primary BC s were in discordance with metastatic lymph nodes while various inconsistent rates have been reported in other studies. Leni *et al.* have presented concordance of 95.28%^[8] and Falck *et al.* mentioned 97%.^[15]

18.5% of samples showed discordance in negative direction (Table 2): 5 negative primary BC reveal positive metastatic lymph node and 8 positive primary BC reveal negative metastatic lymph node. This discordance in negative direction has been assessed in previous studies and even up to 37% has been reported.^[13]

While our study found positive conversion in 12.5% of patients who have primary BC negative results with positive metastatic lymph nodes and 42.1% of negative conversion, Aurilio *et al.* mentioned 13% of negative conversion in comparison with the lower rate of positive conversion with 5%.^[14] Of course, both studies have reported a higher rate of negative conversion, but in the current study, it is considerably higher.

In the current study, there was no statistically significant association between ER/PR receptor status and Her2 concordance. Previous studies have presented that Her2 discordance status may not affected by hormone receptors status, whether positively or negatively, although Her2 expression has been mentioned as a factor for Tamoxifen resistance.^[12] In general, this topic has not been explained deeply and can be of interest in further studies.

In this study, Her2 concordance was not in association neither with patients' age nor primary tumor grade. Barresi *et al.* reported similar results about primary tumor grading for both concordance and discordance of Her2.^[8]

Number of metastatic lymph nodes was statistically in accordance with Her2 expression concordance, as the lower

Table 2: Concordance of Her-2 expression in primary BC and metastatic lymph nodes

Breast lymph node	Negative (%)	Equivocal (%)	Positive (%)
Negative	25 (62.5)	10 (25)	5 (12.5)
Equivocal	5 (45.5)	2 (18.2)	4 (36.4)
Positive	8 (42.1)	2 (10.5)	9 (47.4)

Her-2: Human epidermal growth factor receptor-2

Table 3: Association of Her-2 expression in metastatic lymph nodes and ER-PR expression

ER-PR/lymph node	Total number	Number of concordance (%)	P
ER-PR receptor			
Positive	35	15 (42.9)	0.08
Negative	35	21 (60)	
Number of metastatic lymph nodes			
1	38	24 (63.2)	0.01
2	17	8 (47.1)	
3	15	4 (26.7)	

Her-2: Human epidermal growth factor receptor-2.
ER-PR: Estrogen receptor-progesterone receptor

Table 4: Association of BC grading with concordance/discordance of Her-2 expression in axillary metastasis

Concordance/discordance	Grading			Total	P
	G1	G2	G3		
Concordance	10 (50)	21 (56.8)	5 (38.5)	36 (51.4)	0.66
Discordance	10 (50)	16 (43.2)	8 (61.5)	34 (48.6)	

Her-2: Human epidermal growth factor receptor-2, BC: Breast cancer

number of metastatic lymph node was associated with higher probability of concordance. In the study of Barresi *et al.* there was no significant relationship between number of metastatic lymph node and Her2 expression concordance.^[8]

CONCLUSION

In the current study, we found a high rate of discordance of Her2 expression in primary BC with metastatic lymph nodes. The number of metastatic lymph nodes affected Her-2 expression concordance diversely. Furthermore, no association between Her-2 expression concordance/discordance and hormonal receptors, grade or patient age was found.

REFERENCES

1. Abdollahi A, Etemadi M. Pathological characteristics of triple-negative breast cancer at main referral teaching hospital, April 2014 to April 2015, Tehran, Iran. *Int J Hematol Oncol Stem Cell Res* 2016;10:200.
2. Rubovszky G, Udvarhelyi N, Horváth Z, Láng I, Kásler M. Triple-negative breast carcinoma--review of current literature. *Magy Onkol* 2010;54:325-35.
3. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A, *et al.* Global cancer statistics, 2012. *CA Cancer J Clin* 2015;65:87-108.
4. Soleimani A, Rahmani Y, Farshchian N, Delpisheh A, Khassi K, Shahmohammadi A, *et al.* The evaluation of p53 polymorphism at codon 72 and association with breast cancer in Iran: A systematic review and meta-analysis. *J Cancer Prev* 2016;21:288.
5. Weigelt B, Peterse JL, van 't Veer LJ. Breast cancer metastasis: Markers and models. *Nat Rev Cancer* 2005;5:591-602.
6. Esteva FJ, Hortobagyi GN. Prognostic molecular markers in early breast cancer. *Breast Cancer Res* 2004;6:109.
7. Falck A-K, Fernö M, Bendahl P-O, Rydén L. St Gallen molecular subtypes in primary breast cancer and matched lymph node metastases-aspects on distribution and prognosis for patients with luminal a tumours: Results from a prospective randomised trial. *BMC Cancer* 2013;13:558.
8. Leni A, Barresi V, Zeppa P, Franco V. Discordance rate of HER2 status in primary breast carcinomas versus synchronous axillary lymph node metastases: A multicenter retrospective investigation. *OncoTargets Ther* 2014;7:1267-72.
9. Wolff AC, Hammond ME, Hicks DG, Dowsett M, McShane LM, Allison KH, *et al.* Recommendations for human epidermal growth factor receptor 2 testing in breast cancer: American society of clinical oncology/college of american pathologists clinical practice guideline update. *J Clin Oncol* 2013;31:3997-4013.
10. Galea MH, Blamey RW, Elston CE, Ellis IO. The Nottingham prognostic index in primary breast cancer. *Breast Cancer Res Treat* 1992;22:207-19.
11. Nafissi MN. Breast cancer survival analysis based on immunohistochemistry subtypes (ER/PR/HER2): A retrospective cohort study. *Arch Iran Med* 2016;19:680.
12. Turner NH, Di Leo A. HER2 discordance between primary and metastatic breast cancer: Assessing the clinical impact. *Cancer Treat Rev* 2013;39:947-57.
13. Chan A, Morey A, Brown B, Hastrich D, Willsher P, Ingram D. A retrospective study investigating the rate of HER2 discordance between primary breast carcinoma and locoregional or metastatic disease. *BMC Cancer* 2012;12:555.
14. Aurilio G, Disalvatore D, Pruneri G, Bagnardi V, Viale G, Curigliano G, *et al.* A meta-analysis of oestrogen receptor, progesterone receptor and human epidermal growth factor receptor 2 discordance between primary breast cancer and metastases. *Eur J Cancer* 2014;50:277-89.
15. Falck A-K, Fernö M, Bendahl P-O, Rydén L. Does analysis of biomarkers in tumor cells in lymph node metastases give additional prognostic information in primary breast cancer? *World J Surg* 2010;34:1434-41.

Source of Support: Nil. **Conflict of Interest:** None declared.