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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 14 (10): 584-589, 2011
ISSN 1028-8880 / DOI: 10.3923/pjbs.2011.584.589

Expression of Epidermal Growth Factor Receptor Tyrosine Kinase Family in Fine Needle Aspiration and Permanent Specimens of Invasive Lobular and Ductal Breast Cancers

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Abstract: Recently, the role of HER-2/Neu gene amplification has been enthusiastically investigated in breast cancer. Determining the HER-2/Neu status could be achieved by evaluating either histologic samples or cytologic specimens obtained by Fine Needle Aspiration (FNA). This study aimed at determining the concordance of HER-2/Neu expression in FNA and histologic sections. FNA samples, as well as their corresponding histologic sections of 90 cases with breast cancer were evaluated in Tabriz Sina Teaching Center in a 13-month period of time. The immunohistochemistry was employed for determining the HER-2/Neu amplification for both methods. The concordance rate and agreement were determined between the two methods. Ninety specimens of women with a mean age of 50.93±10.64 (29-84) years were assessed. There were 84 cases with invasive ductal carcinoma and 6 cases with invasive lobular carcinoma. Lymph nodes were involved in 50 cases and there were vascular and neural involvement in 40 and 35 cases, respectively. Her-2/Neu was not detected in 27 cases (30%) with weak and strong amplifications in 47 (52.2%) and 16 (17.8%) cases of FNA specimens, respectively. Her-2/Neu was not detected in 29 cases (32.2%) with weak and strong amplifications in 42 (46.7%) and 19 (21.1%) cases of histologic specimens, respectively. The concordance rate was 70% between the two methods. The agreement was statistically significant between the two methods, as well (kappa = 0.51, $p<0.001$). HER-2/neu gene amplification can be reliably estimated by immunohistochemistry on breast cancer FNAs and a good correlation has been found between this and results on histological sections.

Key words: Invasive breast cancer, immunohistochemistry, epidermal growth factor receptor tyrosine kinase family, lobular carcinoma, ductal carcinoma

INTRODUCTION

The incidence of breast cancer is variable in different countries; however, its gradual increase is a common feature and a potential concern (Beckmann, 2002; Hashemi and Karami-Tehrani, 2006). The annual mortality rate of this malignancy is estimated to be about 400,000 cases all over the world. It is the most common cancer among Iranian women with more than 7000 new diagnosed in each year (Abbasi *et al*., 2009a). Malignancy of the breast is one of the commonest causes of death in women aged between 40-44 years, especially in advanced stages and after metastasis (Owiredu *et al*., 2009; Cong and Tsokos, 2010; Kumar *et al*., 2010; Khorshid, 2011). Although, there was an escalating trend in prevalence of breast cancer in the late decade, the mortality rate has been decreased concomitantly. Improvements in diagnostic and therapeutical approaches have been claimed in this regard (Rosai and Ackerman, 2004). Positive family history always has been considered as a major risk factor in breast cancer. So, genetics could be blamed as a potent underlying determiner in these patients (Martin and Weber, 2000). Tumor markers are frequently used for screening and monitoring in oncology (Mohammadzadeh *et al*., 2010; Frempong *et al*., 2008; Suman and Kaiser, 2006; Kumar and Jamil, 2006; Zia *et al*., 2007; Khurshid, 2001; Abbasi *et al*., 2009b). Human epidermal growth factor receptor-2 (HER-2) and tumor antigen Neu (ErbB2) which are known as HER-2/Neu together are members of epidermal growth factor receptor tyrosine kinase family (Olsson and Bladstrom, 2002; Weidner *et al*., 1991; Wood and Skandalakis, 2010; Nakopoulou *et al*., 2002). Overexpression of HER-2/Neu has been proposed as an indicator of a more invasive

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breast cancer and a predictor of poor out come (De Waal and Leenders, 2005; Tsutsui *et al*., 2003; Zhu *et al*., 2005). Fine Needle Aspiration (FNA) in patients with suspected breast malignancies is a very important diagnostic approach, because it is a minimally invasive but rather sensitive method. There is very limited number of studies regarding the expression rate of HER-2/Neu in FNA specimens in breast cancer with heterogeneous and inconclusive results (Nizzoli *et al*., 2003; Bozzetti *et al*., 2002). This study aimed at evaluating the expression rate of HER-2/Neu in FNA and permanent specimens of invasive breast cancer.

MATERIALS AND METHODS

Setting and design: In a 13-month period of time (March 2010-April 2011), 90 samples of invasive breast cancer including both cytological (FNA) and permanent specimens were evaluated in an analytic-descriptive setting. HER-2/Neu expression was assessed in these specimens by an immunohistochemical method. The concordance and agreement rates of HER-2/Neu expression were determined between the FNA and permanent specimens. This study was performed in Imam Reza Teaching Center, Tabriz, Iran. This study is approved by the Ethics Committee of Tabriz University of Medical Sciences.

Sample size: The sample size was determined by considering a 97% concordance rate between results of the two methods (Lee *et al*., 2008), $\alpha = 0.05$ and $d = 0.8$. Based on these values and by using ratio estimation formula, the sample size was calculated 89 cases. Finally, 90 patients with suspected breast lump undergoing excisional biopsy were enrolled in the study.

Procedures and grading: FNA was performed in all cases. The permanent samples were fixed in 10% formalin and embedded in liquid paraffin. Four micron slices were cut and hematoxylin-eosin staining was performed. Expression of HER-2/Neu was determined by using a standard immunohistochemical kit (Hercept Test, DAKO®, Denmark). FNA and permanent slides were evaluated by two skilled pathologists in a blind manner. If there were conflicts in reports, the final decision was made after negotiation. The histological type of tumor was made according the World Health Organization (WHO) criteria (Tavassoli and Devilee, 2003). The histological grade was reported based on the Nottingham grading system (Elston and Ellis, 2002). The status of HER-2/Neu expression was determined based on the extension of membranous staining: negative (score 0 or +1), weak positive (score +2) and positive (score +3) (Rosai and Ackerman, 2004). It should be emphasized the permanent findings were considered as the final (gold standard) results.

Variables: The studied variables were age, diagnosis, marginal involvement, grade (in Invasive ductal carcinoma only), lymph node involvement and its number, vascular invasion, neural invasion and expression of HER-2/Neu (in FNA and permanent specimens separately).

Statistical analysis: The data were analyzed by SPSS software, version 15.0 (IBM, Chicago). These data were expressed as Mean±Standard Deviation (range) or frequency (percentage). The kappa coefficient was calculated for expression of agreement by crosstabs method. Values>0.5 indicate high agreements between the results of immunohistochemical assessment on the FNA and permanent specimens. The logistic regression analysis was used for determining the causes of non-agreement. Compressions with a p-value$\leq$0.05 were considered statistically significant.

RESULTS

Ninety women with invasive breast cancer were studied. Characteristics of the patients, as well as the studied variables are summarized in Table 1. The age of patients ranged between 29 and 84. The invasive ductal carcinoma to the invasive lobular carcinoma ratio was 14 to 1. The margins were confined to muscles in majority of cases, followed by skin and nipple. All the invasive ductal carcinoma cases were grade II or III (Table 1).

Concordance of HER-2/Neu expression rates between the FNA and permanent specimens are summarized in Table 2.

Accordingly, Considering the status of HER-2/Neu expression, in the FNA samples there were 27 negative cases (30%), 47 weak positive cases (52.2%) and 16 positive cases (17.8%). The corresponding rates were 29 (32.2%), 42 (46.7%) and 19 (21.1%) cases in the permanent samples, respectively (Table 2).

As shown in Table 2, there was a significant agreement between the results of two methods (kappa coefficient = 0.52, p-value<0.001); i.e., the results of two methods were similar in majority of cases including negative cases (70.4%), weak positive cases (70.2%) and positive cases (68.8%) (Table 2).

Percentages of similar and different results regarding the expression rate of Her-2/Neu in FNA and permanent specimens are depicted in Fig. 1. Accordingly, there were 63 (70%) matched and 27 (30%) different results between

Table 1: Evaluated variable in the studied population

Variable	Amount
Age (year)	50.93±10.64
Type of tumor	
Invasive ductal carcinoma	84 (93.3)
Invasive lobular carcinoma	6 (6.7)
Margins	
Muscles	11 (12.2)
Skin	2 (2.2)
Muscles, nipple and skin	2 (2.2)
Muscles and nipple	1 (1.1)
Grade (Invasive ductal carcinoma)	
II	81 (96.4)
III	3 (3.6)
Lymph node involvement	50 (55.6)
Number of involved lymph nodes	6.82±2.46
Vascular invasion	40 (44.4)
Neural invasion	35 (38.9)

Data are shown as Mean±Standard Deviation or frequency (percentage)

Table 2: Concordance of the results in fine needle aspiration and permanent specimens with regard to HER-2/Neu expression

	Permanent specimens			
Method	Negative	Weak positive	Positive	Total
FNA specimens				
Negative	19 (70.4)	7 (25.9)	1 (3.7)	27 (30)
Weak positive	7 (14.9)	33 (70.2)	7 (14.9)	47 (52.2)
Positive	3 (18.8)	2 (12.5)	11 (68.8)	16 (17.8)
Total	29 (32.2)	42 (46.7)	19 (21.1)	90 (100)

Data are shown as frequency (percentage). FNA: Fine needle aspiration

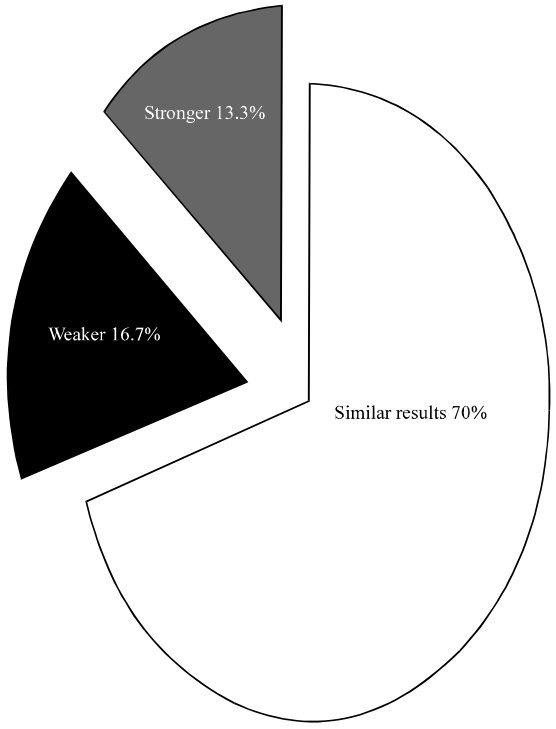

Fig. 1: Percentages of similar and different (weaker or stronger) results regarding the expression rate of HER-2/Neu in fine needle aspiration and permanent specimens

Table 3: p-values of different variables in contribution of unmatched results between the fine needle aspiration and permanent specimens of invasive breast cancer

Variable	Matched (n = 63)	Unmatched (n = 27)	p-value
Age of patients (year)	50.89±11.41	51.04±8.76	0.11
Lymph node involvement	34 (54.0)	16 (59.3)	0.66
Marginal status			
Muscle	11 (17.5)	3 (11.1)	0.13
Skin	3 (4.8)	1 (3.7)	
Nipple	2 (3.2)	1 (3.7)	
Grade of tumor			
II	59 (93.7)	22 (81.5)	0.76
III	3 (4.8)	0 (0)	
Vascular involvement	27 (42.9)	13 (48.1)	0.99
Neural involvement	24 (38.1)	11 (40.7)	0.92

Data are shown as Mean±Standard Deviation or frequency (percentage). $p \leq 0.05$ is considered statistically significant

the two evaluations. Only in different results, there were 15 cases (16.7%) with weaker expression and 12 cases (13.3%) with stronger expression of HER-2/Neu in FNA specimens comparing with the permanent findings (Fig. 1).

Probable contributors to unmatched results between the FNA and permanent specimens were evaluated by logistic regression method. Studied variables between the two matched and unmatched groups, as well as the corresponding p-values are summarized in Table 3. Accordingly, none of the studied variables including age of patients ($p = 0.11$), lymph node involvement ($p = 0.66$), marginal status including muscle, nipple or skin ($p = 0.13$), grade of tumor including II or III ($p = 0.76$), vascular involvement ($p = 0.99$) or neural involvement ($p = 0.92$) were associated with this heterogeneity of results.

DISCUSSION

The expression rate of HER-2/Neu was 70% (52.2% weak positive, 17.8% positive) in FNA specimens and 67.8% (46.7% weak positive and 21.1% positive) in permanent specimens. The expression rate of this marker has been reported 10-30% in previous reports on breast cancer specimens (Gullick *et al*., 1991; Hartmann *et al*., 1994; Rilke *et al*., 1991). As seen, the expression rate is higher in our cases comparing with the reported rates in the literature. This is mainly due to invasive nature of cancer in our population. It is thought that there is a strong direct association between the expression rate of HER-2/Neu and aggressiveness of breast cancer (Beatty *et al*., 2004). So, the higher rate of HER2/Neu expression in our study comparing to that in similar reports on invasive and noninvasive cancers was expectable. To the best of our knowledge, there is no other similar report in this group of patients. The method of evaluation and considering the week positive cases in our setting may further justify this heterogeneity between the reports (Bozzetti *et al*., 2002). The concordance rate

was 73% in present study relating to the expression of HER-2/Neu in FNA and permanent samples. There was also a significantly high agreement between the two results (kappa coefficient = 0.52, p-value<0.001). So the FNA could be proposed as a minimally-invasive method for acquiring specimens for determining the expression of this marker in breast cancer comparing with the more invasive biopsies. Wu *et al.* (2000) and Stomper *et al.* (2000) also recommended FNA specimens for evaluating the expression rate of HER-2 in breast cancer. These reports are in line with ours. The concordance rate between the FNA and permanent specimens of breast cancer relating to the expression rate of HER-2/Neu was 75% in a report by Nizzoli *et al.* (2003) in 24 cases. Although, the result of this study is very similar to ours, they did not confine the samples to invasive cases. Likewise, the sample size is more in our series and this may lead to a more powerful design. Bozzetti *et al.* (2002) evaluated the results of immunohistochemical evaluation of HER-2/Neu expression in FNA and permanent samples of breast cancer in 66 cases. The overall expression rate was 27% and the concordance rate between the two methods was 92%. The expression rate is clearly higher in our study comparing with that in the mentioned report (67.8-70% vs. 27%). The cause of this difference was discussed earlier. Although, the concordance rate was higher in the mentioned study comparing with ours, again it should be reminded that the cases in our series were invasive breast cancers and this may justify the possible and albeit minimal differences. The concordance rates between the two methods in breast cancer varies between 56 and 92% in different settings (Lee *et al.*, 2008; Beatty *et al.*, 2004; D'Alfonso *et al.*, 2010; Klorin and Keren, 2003; Troncone *et al.*, 1996; Jorda *et al.*, 1994; Corkill and Katz, 1994). Our reports lie in this range, too. This heterogeneity and wide range of expression rates is due to different sample sizes, methods of fixation, methods of examination, grades of tumor and skill of the examiners. Replacement of permanent specimens with the FNA samples in evaluating the expression rate of HER-2/Neu as a diagnostic or prognostic marker could be justified as follows:

- Planning a better therapeutic process especially in patients in need of chemotherapy prior to operation and in patients with small and non-surgical lesions
- Reproducibility of FNA samples comparing with the permanent specimens (Nizzoli *et al.*, 2003)

In the present study, the results of FNA were underestimated (false negative) in 16.7% and overestimated (false positive) in 13.3% considering the results of permanent specimens as the gold standard. Nizzoli *et al.* (2003) claimed that high rate of false positive results in FNA samples is a major limitation of this method in patients with breast cancer. Our findings are in contrast with this claim. Small samples size and lower experience in interpreting the findings may limit the results of the mentioned study comparing with ours. So it could be concluded that the false positive or negative results are not very common in evaluating the HER-2/Neu expression in FNA specimens of invasive breast cancer.

CONCLUSION

Based on the findings of present study, there was a significant agreement between the results of immunohistochemical investigation of HER-2/Neu expression in FNA and permanent specimens of invasive breast cancer. So the cytologic samples could be used instead of the permanent specimens in this regard. This is the first study on the invasive ductal and lobular carcinomas of breast and so, further studies may be helpful in elucidating the findings. Employment of newer methods in evaluating the expression rate of HER-2/Neu such as Fluorescent In-situ Hybridization (FISH) is recommended in future studies in this regard.

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