

Comparison of IHC, FISH, ER and PR in Breast Cancer in Western Iran

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Received November 21, 2014; Revised December 18, 2014; Accepted December 25, 2014

Abstract To evaluate the concordance and discordance between IHC and FISH results for detection of Her2/neu protein, analyses of ER and PR, and also evaluation of the benefit of adjuvant trastuzumab in patients diagnosed with human epidermal growth factor receptor 2 (HER2) –positive invasive ductal carcinoma enrolled onto the Herceptin adjuvant. IHC analysis of ER, PR and HER2 was performed in 133 patients of breast cancer with invasive ductal carcinoma. 90(67.6%) cases were confirmed by FISH. Statistical analysis was performed with IBM SPSS version 19, Kaplan-Meier method and log-rank test. Age mean of patients was 46.39±10.81 years (range, 24-78 years). Concordance rates between IHC and FISH were 32.4% for IHC 2+ and 81.25% for IHC 3+ (P<0.001). There were 17 and 56 patients of IHC 2+ and 3+ with ER positive and also 23 and 59 patients of IHC 2+ and 3+ had PR positive. The 83 patients had age ≤50 years and 50 patients had >50 years. Of 133 the patients, 48(36.1%) patients were treated with trastuzumab and 85(63.9%) were treated without trastuzumab. The overall survival(OS) for patients treating with trastuzumab were mean of 37.8 months and the OS for patients treating without trastuzumab were mean of 20.8 months (P<0.05). The results are that trastuzumab therapy is effective and improves the survival of HER2-positive breast cancer patients and trastuzumab therapy is effective and tolerated for breast cancer with Her-2 positive. There is a statistically significant relationship between ER positive with PR positive and ER negative with PR negative that these results need to more studies with more patients in the world.

Keywords: ER, FISH, PR, survival, trastuzumab

Cite This Article: Mehrdad Payandeh, Masoud Sadeghi, Edris Sadeghi, and Anita Kabir koohian, "Comparison of IHC, FISH, ER and PR in Breast Cancer in Western Iran." *American Journal of Cancer Prevention*, vol. 2, no. 2 (2014): 37-41. doi: 10.12691/ajcp-2-2-4.

1. Introduction

Breast cancer is the most common cancer (27% of all cancers) and common cause of death (16%) which occurs due to cancers among women, either in developed or developing countries. [1] In Asia the maximum incidence is in 40 – 50 age groups. In Contrast, in western countries the increase in incidence continues as the age increases. [2] Breast tumors are classified histologically according to the location of origin. The ductal tumors develop in breast ducts and represent 80% of tumors. The lobular tumors develop inside the lobes and account for 10 to 15% of cases. Other subtypes represent less than 10% of cases diagnosed per year. Patients with invasive ductal carcinoma present higher lymphatic involvement and worse prognosis than less common types of breast carcinoma [3].

The human epidermal growth factor receptor 2 (HER-2/neu) or (cerbB-2) gene is located on human chromosome 17q21, and is a member of the ErbB family of receptor tyrosine kinases. [4,5] Over-expression of HER-2/neu

gene and its protein are associated breast cancer patient's prognosis and therapy.[4] Trastuzumab (Herceptin®), a recombinant, humanized, monoclonal antibody targeting HER2 is well established as an effective treatment for HER2-positive breast cancer [6,7].

Estrogen receptor(ER) and progesterone receptor (PR) and HER-2 are essential in the estimation process of breast cancer prognosis and play central role in its management and treatment choice worldwide. Hormone receptors and HER-2 expression were evaluated using routine immunohistochemistry (IHC) staining [8].

Hormone receptors including ER and PR status are key molecules in breast cancer. [3,9] The development of drugs to target these hormone receptors, such as tamoxifen, has brought about significant improvement in survival for women with hormone receptor-positive breast cancers. Since information about ER and PR is vital for patient management, quality assurance is important to ensure accurate testing. [10] Protein over-expression detected by IHC or amplification of Her-2 gene analyzed by fluorescence in situ hybridization (FISH) are the two main methods used to detect Her-2 status in clinical practice. [9] IHC is a preferred method for screening and determining

cases which need to be genetically evaluated, [11] and IHC using scoring tools such as the “Hercept scale” and, more recently, the ASCO/CAP scale, was the simplest way to identify positive cases likely to benefit from trastuzumab that is susceptible to interobserver variability and, as with any assay technique, required standardization and validation. [12] Thin tissue section FISH is the routinely used method for the detection of gene status in paraffin-embedded breast cancer samples. [13] However, according to this scale, HER2 amplification of 2+ cases had to be confirmed by FISH [12].

The aim of this study was to compare IHC profiles (ER, PR, HER2) with each other and HER2 FISH in patients with breast cancer, and the effect of trastuzumab on disease free survival(DFS) for first time in west of Iran.

2. Patients and Methods

The study population consists of 133 cases of women with breast cancer at Oncology Clinic, Kermanshah, Iran. All of the patients detected HER2 protein expression by IHC, and Patients were categorized on the basis of IHC values as +2 and +3. Kind of pathology of breast cancer in patients was invasive ductal carcinoma. Patients randomly assigned to receive one year of trastuzumab and one year of observation. Centrally reviewed ER, PR, and HER2 copy numbers were used. The magnitude of trastuzumab benefit was assessed using the Cox proportional hazards model for disease-free survival (DFS) and overall survival (OS).

The specimens for pathology reports were fixed in 10% neutral-buffered formalin (pH=7.4) for 24 hours, then sliced into 4-µm sections. Her-2 protein expression was measured using a commercial available S-P kit. FISH for Her-2 gene amplification was performed in laboratory of Emam Reza Hospital using a commercial available double-color probe. Monoclonal antibodies against ER, PR, HER2, as well as IHC kit were purchased from Pars Azmon Co. Equivalent phosphate-buffered saline (PBS) was used as a negative control for primary antibodies.

2.1. Statistical Analysis

Statistical analysis was performed with IBM SPSS software version 19, and the enumeration data were compared with the Fisher’s exact test. $P \leq 0.05$ was considered to indicate a statistically significant difference (95% CI). Overall survival was calculated using the Kaplan-Meier method and DFS with log-rank test. OS is defined as the time from randomization until death from any cause or endpoint (for 5 years). DFS is defined as the time from treatment with trastuzumab until recurrence of tumor or death (for 3 years).

3. Results

All of the patients were female with age mean of 46.39 ± 10.81 years (range, 24-78 years). Of the 133 patients 37 were classified as IHC 2+ and 96 were classified as IHC 3+. 12 of the IHC 2+ cases and 78 of the IHC 3+ cases were found to be Her2 FISH positive. Also, 25 of the IHC 2+ cases and 18 of the IHC 3+ cases had Her2 FISH negative.

3.1. IHC and FISH

We evaluated the concordance and discordance between IHC and FISH results for detection of Her2/neu protein. The concordance rate is defined as the number of agreed to IHC 2+ and 3+ cases divided by the total number of IHC 2+ and 3+ cases. Also, the discordance rate is defined as the number of discrepant to IHC 2+ and 3+ cases (IHC 2+ or 3+ but Her-2 FISH negative) divided by the total number of IHC 2+ and 3+ cases. Concordance rates were 32.4% for IHC 2+ and 81.25% for IHC 3+. [14] In other hand, discordance rates were 67.6% for IHC 2+ and 18.75% for IHC 3+ (Kappa=0.108, $P < 0.001$) (Table 1).

3.2. ER and PR and Age

There were 17 and 56 patients of IHC 2+ and 3+ with ER positive, while 20 and 40 patients of IHC 2+ and 3+ had ER negative, respectively($P=0.137$). 23 and 59 patients of IHC 2+ and 3+ had PR positive, while 14 and 37 patients of IHC 2+ and 3+ had PR negative, respectively($P > 0.05$) (Table 2).

Table 1. Comparison of the results of IHC and FISH for detection HER2/neu

IHC scoring	Her-2 FISH amplified	Her-2 FISH non-amplified	Concordance by IHC	Discordance by IHC
2+ (n=37)	12	25	(12/37)32.4%	(25/37)67.6%
3+ (n=96)	78	18	(78/96)81.25%	(18/96)18.75%

IHC = Immunohistochemistry; FISH = Fluorescence in situ hybridization, ($P=0.001$).

Table 2. Association analyses of ER and PR with IHC data

IHC scoring	ER		Total	P-value	PR		Total	P-value
	+	-			+	-		
2+ (n=37)	17(23.3)	20(33.3)	37(27.8)	P=0.137	23(28)	14 (27.5)	37(27.8)	P=0.552
3+ (n=96)	56(76.7)	40(66.7)	96(72.2)		59(72)	37(72.5)	96(72.2)	
Total (n=133)	73(100)	60(100)			82(100)	51(100)		

IHC = Immunohistochemistry, ER= Estrogen receptor, PR= Progesterone receptor

Of 133 the patients, 83 patients had age ≤ 50 years that 46 of them were ER positive, 52 were PR positive, 19 had IHC 2+ and 65 had IHC 3+. In the other hand, 50 patients

had >50 years that 27 patients were ER positive, 21 were PR positive, 18 had IHC 2+ and 31 had IHC 3+ ($P > 0.05$) (Table 3).

Table 3. sociation of marker expression with age

Marker expression (n)	Age ≤ 50 years (n=83)	Age >50 years (n=50)	P-value
ER(73)	46(63.01)	27(36.99)	$P > 0.05$
PR(73)	52(71.23)	21(28.77)	$P > 0.05$
HER2 2+(37)	19(51.35)	18(48.65)	
HER2 3+(96)	65(67.70)	31(32.30)	

ER= Estrogen receptor, PR= Progesterone receptor, HER2= human epidermal growth factor receptor 2

Table 4 shows correlation of ER and PR in patients with HER-2(2+) and (3+).Of the 82 patients with PR positive, 65 patients had ER positive and 17 patients were

ER negative. Of the 51 patients with PR negative, 43 patients were ER negative and 8 were ER positive (p<0.001).

Table 4. rrelation of ER and PR in patients with HER2

Marker	ER		Total	P-value
	+	-		
PR				
+	65(89)	17(28.3)	82(61.7)	P<0.001
-	8(11)	43(71.7)	51(38.3)	
Total	73(100)	60(100)		

ER= Estrogen receptor, PR= Progesterone receptor.

3.3. OS and DFS

Of 133 the patients, 48(36.1%) patients were treated with trastuzumab and 85(63.9%) were treated without trastuzumab. Figure 1 shows percent of OS, During a 60 months (2010-2014) from date of diagnosis Hre2 by IHC in all of the patients with mean of 41 months. One way of summarizing survival data is to report the percent of patients still alive at a fixed point in time. We might initially restrict our analysis to patients for who we have complete information on the first two years of follow-up. In summary, 87 patients completed a two-year follow-up,

of whom 22 died and 65 were still alive. Therefore, 46 women were lost to follow-up before completing a two-year period and should therefore be excluded from the analysis.Fig.2 shows percent of DFS, During a 36 months from date of treatment with trastuzumab. Patients were excluded from the analysis were lost to follow-up before completing a one-year period of treatment. Figure 2A shows the DFS for patients treating with trastuzumab with mean of 27.8 months, and Figure 2B shows the DFS for patients treating without trastuzumab with mean of 20.8 months (P=0.006, 95% CI of ratio= 0.031 to 0.561, Hazard ratio=0.133).

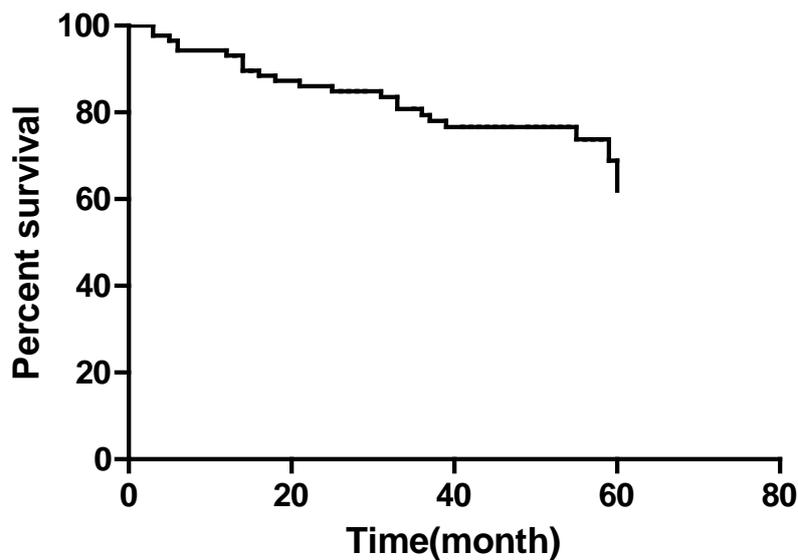


Figure 1. Overall survival from date of diagnosis Hre2 by immunohistochemistry (IHC 2+ and IHC 3+) for all of the patients (n=133)

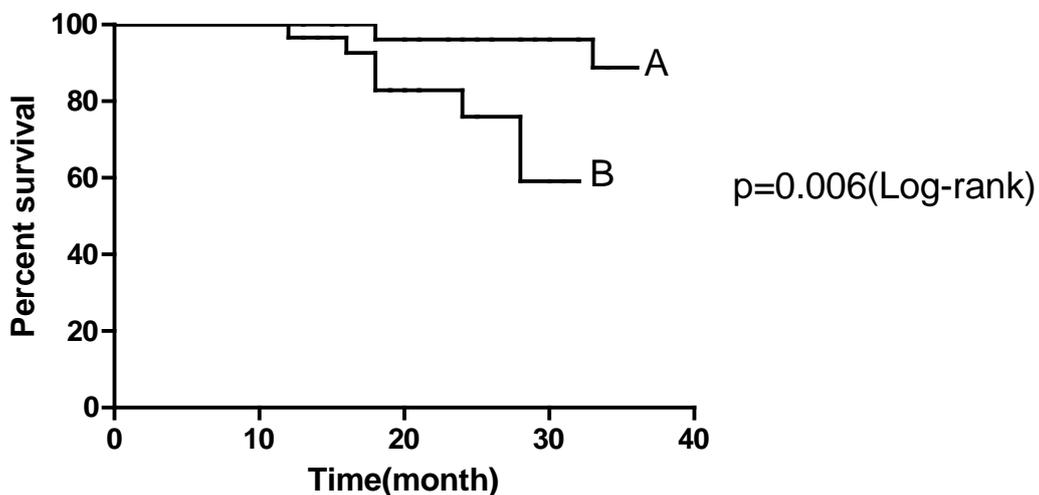


Figure 2. Disease free survival from date of diagnosis Hre2 by immunohistochemistry (IHC 3+): (A) for patients treating with trastuzumab(B) patients treating without trastuzumab

Furthermore, 29 patients with IHC 2+ that were treated with trastuzumab for 3 years from date of treatment, one patient dead. But 29 patients without trastuzumab, 8 patient dead.

4. Discussion

HER2, a proto-oncogene, also known as c-erbB-2 or HER2/neu, located on chromosome 17q21, is considered to be closely associated with the occurrence and development of breast cancer. [15] Therefore, the need for accurate detection of the Her2 alteration has now become even more important, because therapeutic decisions for patients with breast cancer are increasingly dependent on this information, [11,16] We evaluated results of IHC and FISH among 133 patients with HER2 positive (Table 1). There was a concordance rate between IHC 3+ and FISH, but was a discordance rate between IHC 2+ and FISH. This results were statistically significant ($P < 0.01$). A few of studies [11,17-19] show a concordance rate between IHC 3+ and discordance rate between IHC 2+ with FISH. But, other studies [14,20] show a concordance rate between IHC 2+ and FISH. Therefore, agreement between IHC and FISH are still controversial, in our concordance in the cases of IHC 3+ is similar with theirs, but there are some differences in the cases of IHC 2+. This may due to the different sensitivity and specificity of the antibodies and probe used in this study, [14] or because of subjective interpretative variation in reporting, variable pre-analytical conditions, imperfect IHC methodology [19].

ER and PR play important roles in the growth and differentiation of breast cancers while their expression levels are decisive factors guiding the endocrine treatment of breast cancers, and important prognostic markers. [15] Our study showed that in all of the patients, the positive rates of ER and PR were 54.8% and 61.6%, but results weren't statistically significant ($P > 0.05$).

Several studies [15,21,22,23] revealed the relationship of expression between subtypes (ER, PR and HER2) with age. ER and PR-high expression were observed in females who were 50 or younger, [15,24] and in our study, too. Her2- high expression was in female with >50 years, [15] but we showed that it was in female ≤ 50 years such as Shomaf M et al. [25] There were no significant differences between the subtypes regarding age (Table 3).

Of 73 patients with ER positive, 65(89%) had PR positive and of 60 patients with ER negative, 43(71.7%) had PR negative that results were statistically significant (Table 4). Also, a study [16] confirmed our study.

Trastuzumab is a humanized monoclonal antibody directed against the HER2/neu oncoprotein and has the ability to inhibit tumor growth in breast cancer patients overexpressing HER2 [26].

Mean of OS for 48 patients treating with trastuzumab and 85 patients without it were 27.8 and 20.8 months (Figure 2).

The five-year OS rate was 74.71% for patients with IHC 2+ and IHC 3+ that is favorable in comparison with previously reported series [27].

The three-year DFS between the two subgroups was significant (Figure 2). The DFS rate for patients treating with trastuzumab and without trastuzumab was 93.3% and 76.6%, respectively. Therefore, trastuzumab therapy

improves the survival of HER2-positive breast cancer patients.

5. Conclusions

We therefore advise clinicians are required FISH analysis as a predictive in breast cancer patients with IHC score 2+. Also trastuzumab therapy is effective and improves the survival of HER2-positive breast cancer patients. There is a statistically significant relationship between ER positive with PR positive and ER negative with PR negative that these results need to more studies with more patients in the world.

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