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Evaluation of HER2 In Breast Cancer Patients of Saudi Arabia using Conventional Immunohistochemistry and Modern Bright-Field Double IN Situ Hybridization

Shrooq Ali Ahmad Alzahrani[1,*], Jamal Sabir Muhammad Sabir[2], Adeel Gulzar Ahmed Chaudhary[3], Abdelbaset Salem Buhmeida[4]

1.Department of Biology, Faculty of Science, King Abdulaziz University, Jeddah, Kingdom of Saudi Arabia

2.Genomic and Biotechnology Division, Faculty of Science, King Abdulaziz University, P O Box: 80216, Jeddah-21589, Kingdom of Saudi Arabia

3.Department of Laboratory Technology, Faculty of Applied Medical Sciences, King Abdulaziz University, P O Box: 80216, Jeddah-21589, Kingdom of Saudi Arabia

4.Center of Excellence In Genomic Medicine Research, King Abdulaziz University, P O Box: 80216, Jeddah-21589, Kingdom of Saudi Arabia

ABSTRACT

The aim of this study was to evaluate the prevalence of human epidermal growth factor receptor 2 (HER2/neu) gene/protein amplification/expression patterns in breast cancer (BC) of Saudi patients by using conventional immunohistochemistry (IHC) and bright-field double in situ hybridization (BDISH) techniques. King Abdulaziz University Hospital (KAUH), Jeddah, Kingdom of Saudi Arabia. A total of 128 consent patients' (aged 24 to 81 years) samples of breast cancer were subjected to screening for HER2/neu protein expression by immunohistochemistry (IHC) and later for amplification by bright-field dual in situ hybridization (BDISH) method. The clinicohistopathological data of all the patients were collected from the patients' case records. The expression of the HER2/neu protein was evaluated, correlated to HER2/neu gene amplification status in BC and assessed for potential clinical value by correlation measures. IHC data determined that HER2/neu protein expressed in 18-20% (3+) of our BC patients in Saudi cohort. Some samples showed moderate membranous expression of HER2/neu protein and majority of samples showed either negative or 1+ expression. 73% of all the invasive ductal breast carcinoma tissue slides showed non-amplification HER2/neu gene status and 27% showed amplification HER2/neu gene status. Interestingly, there was a reasonable concordance rate (77 %) between IHC and BDISH data in the analyzed cohort. In conclusion, HER2/neu expression/amplification status in breast cancer of Saudi patients is within the range as compared to other ethnic groups. This HER2/neu (over-expression/amplification) status was concordant using either IHC or BDISH and significantly associated with disease aggressiveness and poor outcome.

Keywords: Amplification, BDISH, Breast Cancer, Expression, HER2/neu, IHC.

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INTRODUCTION

In spite of advances in the diagnosis and treatment of human malignancy, cancer remains among the leading causes of morbidity and mortality worldwide, with 7.5 million deaths attributed to cancer in 2008. Breast cancer is now the most frequently diagnosed cancer and the leading global cause of cancer death in women, accounting for 23% of cancer diagnoses (1.38 million women) and 14% of cancer deaths (458,000 women) each year.[1] In other words breast cancer continues to be a major cause of morbidity and mortality throughout the world.[2] In Saudi Arabia, breast cancer is becoming a major health risk and is the most common female malignancy in the country accounting for 26% of all newly diagnosed cancer in females.[3-5] Despite the relatively low incidence of breast cancer in Saudi Arabia compared to other countries, it has been the most common cancer among Saudi females for the past 12 consecutive years.[6] In fact, breast cancer is the single leading cause of cancer death for women within the age group of 20 to 59 years, thus posing a major public health concern.[7]

The prognosis and management of breast cancer is influenced by classic variables such as the histological type, grade and stage, status of estrogen receptor (ER), progesterone receptor (PR) and more recently, human epidermal growth factor receptor 2 (HER2/neu) status.[8] The HER-2 is an important prognostic and predictive factor, because it is commonly amplified and/or overexpressed in approximately 15-34% of invasive breast cancers in humans and in many other types of human malignancies including ovary, lung, stomach and oral.[9,10] Therefore, HER2/neu represents an ideal therapeutic target of breast cancer because of (i) its functional importance in breast cancer growth, (ii) its accessibility as a cell surface receptor, and (iii) its high levels of expression in breast tumors, but low levels in normal tissues.[9,11]

The human epidermal growth factor receptor 2 (HER2) is a receptor found on the surface of the cells. It is encoded by a specific gene called the HER2 /neu gene. It is located on the long arm of chromosome 17q21 and there is usually one copy of gene on each copy of the chromosome 17 pair.[12,13] The receptor is structurally composed of an extracellular ligand-binding domain (632 amino acids), transmembrane domain (22 amino acids), and an intracellular tyrosine kinase catalytic domain (580 amino acids).[10] HER2 can dimerize with various members of the family (HER2, HER3 or HER4). It may dimerize with same member of the family (homodimerization) or with different member of the family (heterodimerization). [14] HER2 plays an important role in all stages of cell growth. Thus, the occurrence of HER2 amplification or overexpression can create a surplus of HER2 signaling. It leads to multiple effects on cancer cells involving cell

proliferation, deoxyribonucleic acid (DNA) synthesis, and tumorigenesis. HER2 is frequently overexpressed in breast, gastric, ovarian and prostate cancers. HER2-positive breast cancers tend to be more aggressive than other types of breast cancer. They are less responsive to hormone treatment, but the treatments like herceptin that specifically target HER2 are very effective.[15,16]

The aforementioned percentage of breast cancer in Saudi Arabia shows that the most dangerous types of cancer are prevalent among women. This study is aimed to evaluate the prevalence of HER2 gene amplification/expression patterns in breast cancer of Saudi patients by using conventional immunohistochemistry (IHC) and bright-field double in situ hybridization (BDISH) techniques. In this study, we also tried to evaluate the concordance rate of HER2 status assay between BDISH and IHC techniques. Correlation of HER2 status with clinicopathological features of invasive ductal carcinoma of breast was also determined.

MATERIALS AND METHOD

Subjects

The current study employed randomly selected 128 consent patients (aged 24 to 81 years) for the breast cancer complications at King Abdulaziz University Hospital (KAUH), Jeddah, Kingdom of Saudi Arabia (KSA) during 2002-2008. The tissue samples of breast cancer were retrieved from consent patients' materials in the archives of KAUH.

Methods

This study was carried out according to King Abdul Aziz University's policy and international ethical guidelines on the care and use of laboratory animals (The National Research Council of The National Academy of Sciences 2011). These guidelines follow the national and international laws and policies (National Institutes of Health Guiding Principles on the Care and Use of Laboratory Animals, USA). The ethical approval was obtained from the Unit of Biomedical Ethics, King Abdulaziz University. All samples were preserved as formalin-fixed paraffin-embedded (FFPE) and subjected to screening for HER2/neu over-expression by IHC and later by BDISH. The clinical and histopathological data of all the patients were collected from the patients' case records. The features of clinicopathological data of patients are given in Table 1.

Table 1: Clinicopathological features of 128 patients of breast cancer

Patients' features	Number of patients (%).
Age	
< 50	38%
≥ 50	62%
Family history	
No	82%

Yes	10%
Unknown	8%
Menopausal status	
Pre-menopausal	65%
Post-menopausal	35%
Tumor size	
< 2 cm	13%
2-5 cm	57%
> 5 cm	30%
Stage of disease	
Low stage- I, II	61%
High stage- III, IV	39%
Grade	
I	21%
II	48%
III	31%
Metastasis	
No	76%
Yes	24%
Lymph node state	
Positive	68%
Negative	32%
Recurrence	
Yes	28%
No	72%
Status at end point	
Alive	77%
Died of disease	23%

Determination of HER2 protein expression by immunohistochemistry (IHC)

Overexpression of HER2/neu protein was detected automatically by IHC. The FDA-approved HER2/neu IHC assay using PATHWAY Her2/neu (4B5) rabbit monoclonal antibody was performed with iView DAB Detection Kit (Ventana Medical Systems, Inc. USA) on BenchMark XT automated IHC/ISH staining system (Ventana). A protocol was established so that the entire assay procedure consisting of deparaffinization with EZ Prep (Ventana, USA) at 75°C, heat pretreated in cell conditioning 1 (CC1) (Ventana) using "conditioner CC1 8 min, mild CC1 30 min" for antigen retrieval at 100°C, and then incubation with the anti-HER2 primary antibody for 16 min at 37°C. The slides were counterstained with hematoxylin II (Ventana) for 4 min and then with bluing reagent (Ventana) for 4 min. At the completion of the run, the slides were removed from the automated slide stainer. After staining step, the slides with residual buffer and liquid coverslip solution on them were rinsed in a mild detergent and then with water only until soap was removed completely from slides. The slides were then immersed into different

concentration of alcohol (70%, 95%, 100%) for 3 min to each. Then one drop of Tissue-Tek Glas mounting medium was applied onto a slide and covered with the glass coverslip.

Determination of HER2/centromere 17 (CEN17) by bright-field double in situ hybridization (BDISH)

HER2/neu and Chromosome 17 probes were detected using two colors chromogenic in situ hybridization (ISH) in formalin-fixed, paraffin-embedded human breast cancer tissue specimens following staining on Ventana BenchMark XT automated slide stainer, by light microscopy. The INFORM HER2/neu Dual ISH DNA probe cocktail contains a HER2/neu probe (labelled with the hapten dinitrophenyl or DNP) and a chromosome 17 centromere probe (labeled with the hapten digoxigenin or DIG) formulated with human placental blocking DNA in a formamide-based buffer. The probes were designed to detect amplification of the HER2/neu gene in solid tumors. A protocol was established so that the entire assay procedure consisting of baking for 30 min, deparaffinization with EZ Prep (Ventana, USA) at 75 °C, cell conditioning 2 (CC2) (Ventana) using "mild CC2 cycle 8 min, standard CC2 cycle 12 min, extended cycle 8 min" followed by protease digestion with ISH protease 3 (Ventana) for 16 min at 37 °C. The genomic DNA in tissue sections and the nick-translated HER2 and CEN17 probes were denatured by heat treatment for 20 min at 80 °C followed by a hybridization step for 6 hours at 44 °C. After that, 3 stringency wash steps were performed at 72 °C with 2× SCC (Ventana). For HER2 gene detection, the slides were incubated with a rabbit anti-DNP antibody for 20 min and then with horseradish peroxidase (HRP)-conjugated anti-rabbit antibody for 16 min at 37°C. The HER2 BDISH signal was detected as metallic silver deposits with silver acetate, hydroquinone, and hydrogen peroxide for 4 min at 37°C. For CEN17 detection, the slides were incubated with a mouse anti-DIG antibody for 20 min and then with an alkaline phosphatase (AP)-conjugated anti-mouse antibody for 24 min at 37°C. The CEN17 BDISH signal was developed as red dot staining with fast red and naphthol phosphate for 8 min. Finally, the slides were counterstained with hematoxylin II for 8 min and with bluing reagent for 4 min. At the completion of the run, slides were removed from the automated slide stainer. The stained slides with residual buffer and liquid coverslip solution on them were rinsed in a mild detergent to remove the coverslip solution, then rinsed with water only until soap was removed completely from the slides then slides were dried at 45°C or 65°C in the oven for 15 min. Slides were finally transferred into xylene bath for approximately 30 seconds, then coverslips were applied by the Tissue-Tek Film Cover slipper.[17]

Scoring system of HER2 gene/protein amplification/ expression status

The scoring method for HER2 protein expression is based on the cell membrane staining pattern. HER2/neu testing results by IHC fall into 3 categories: positive, equivocal, and negative (Table 2). Each of these results trigger different patient management. Breast cancer samples with equivocal IHC were validated using BDISH to determine the HER2 gene amplification status. The interpretation for HER2/neu BDISH testing (HER2/Chr17 ratio) is according to the food and drug administration (FDA) and American society of clinical oncology/college of American pathologists (ASCO/CAP) scoring systems and is given in Table 3. BDISH staining result are visualized via light microscopy in which HER2/neu appears as discrete black signals (silver-enhanced ISH) and Chr17 as red signals (red ISH) in nuclei of cells.

Table 2: Scoring of HER2/neu protein expression validated immunohistochemistry assay

Status	Score	Significance
Positive	3+	Uniform intense membrane staining of > 30% of invasive tumor cells.
Equivocal	2+	Complete membrane staining, nonuniform or weak intensity in at least 10% of the cells, or intense complete membrane staining in 30% or less of tumor cells.
Negative	1+ (- 0)	Weak or incomplete membrane staining in any proportion of tumor cells, or no staining.

Table 3: Scoring of HER2/neu gene amplification by validated by bright-field double in situ hybridization assay

HER2/gene status	Score
Non-amplified	HER2/neu/Chr17 < 1.8
Equivocal	1.8 ≤ HER2/neu/Chr17 ≤ 2.2
Amplified	HER2/neu/Chr17 > 2.2

Statistical analysis

Statistical analysis was performed using the SPSS (Statistical package for the Social Sciences) version 19.0 (IBM Company, NY, USA) software package. Frequency tables were analyzed using the Chi-square test (X^2), with likelihood ratio (LR) or Fischer's exact test to assess the significance of the correlation between the categorical variables. Odds Ratios (OR) and their 95% Confidence Intervals (95%CI) were calculated appropriately, using the exact method. Univariate survival analysis for the outcome measure (disease specific survival, DSS; disease-free survival, DFS) was based on Kaplan-Meier method, with log rank (Mantel-Cox) comparison test. DSS and DFS were calculated, based on the time from diagnosis to death (due to disease) and on the time from diagnosis to the appearance of metastatic disease or recurrence respectively. In all tests, the values $P < 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

Expression patterns of HER2/neu protein in breast cancer

About 128 breast cancer slides with HER2/neu protein were evaluated by IHC technique using the FDA approved Her2/neu gene (clone 4B5). 18-20% of samples showed 3+ve membranous HER2/neu protein expression (Figure 1A). Some samples showed moderate membranous expression of HER2/neu protein (Figure 1B). Majority of samples showed either negative or 1+ expression (Figure 1C).

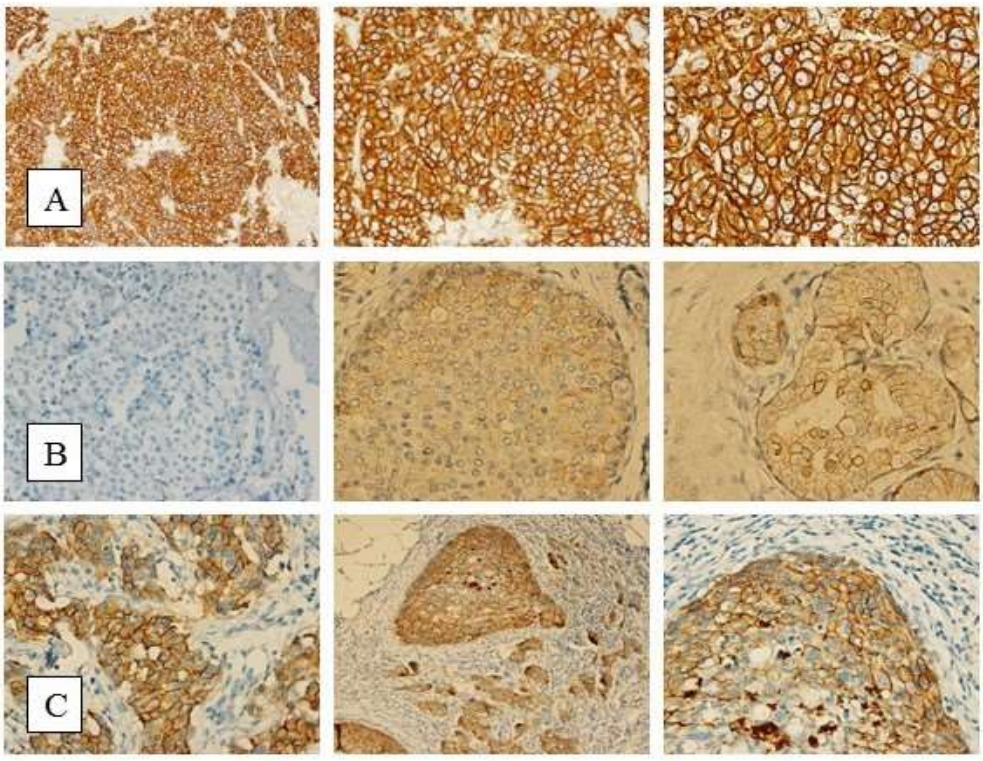

Figure 1: Immunohistochemistry showing the expressions patterns of HER2/neu protein in Saudi breast cancer samples.

(A) Samples showing 3+ membranous expression; **(B)** Samples showing moderate (2+) membranous expression; **(C)** Samples showing either negative 0r 1+ expression. The slides were viewed at 20X and 60 X magnifications.

Amplification pattern of HER2/neu gene in breast cancer

More than 73 breast cancer samples of Saudi females were used for BDISH technique where the HER2 dual ISH 3-in-1 xenograft slides provided by Ventana (Ventana, USA) served as quality control. The HER2/neu amplification status of xenografts is shown in Figure 2A, 2B, 2C. The corresponding frequencies of amplification status of HER2/neu gene evaluated by BDISH technique were: 73% of all the invasive ductal breast carcinoma tissue slides showed non-

amplification HER2 gene status (Figure 3A) and 27% showed amplification HER2/neu gene status (Figure 3B).

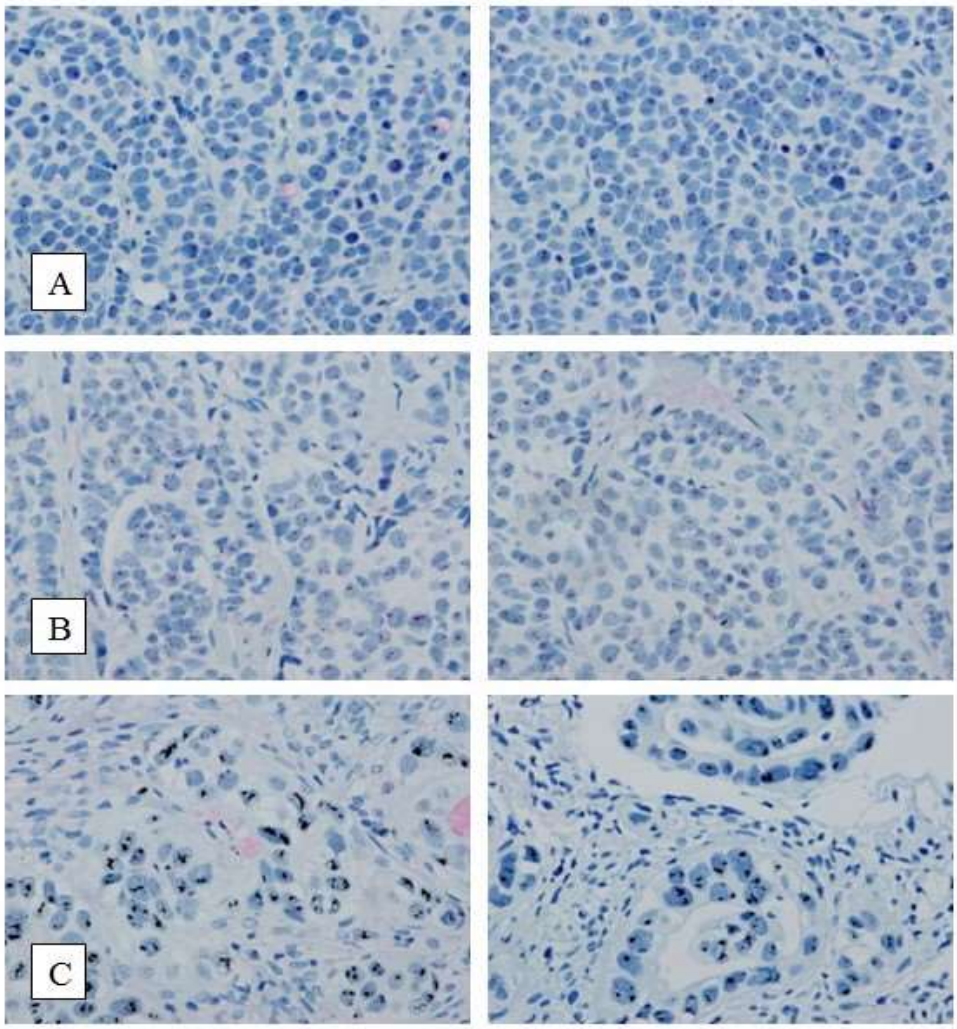

Figure 2: Bright-field double in situ hybridization showing the amplification pattern of HER2/neu gene in Saudi breast cancer samples.

(A) Xenografts showing non-amplified pattern; **(B)** Xenografts showing borderline amplified pattern; **(C)** Xenografts showing amplified pattern. The slides were viewed at 60X magnification.

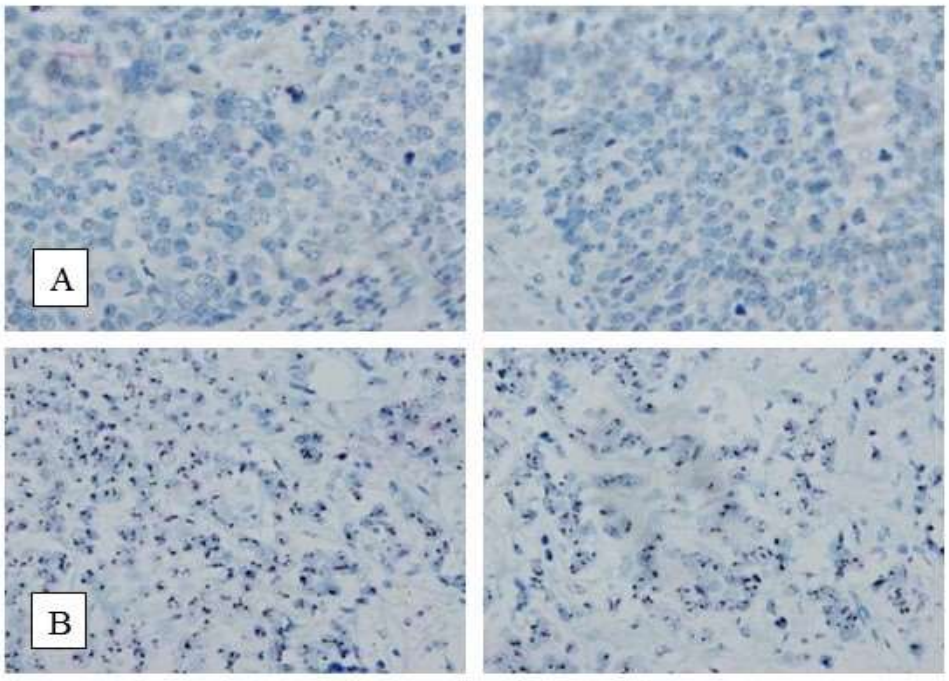

Figure 3: Bright-field double in situ hybridization showing the amplification pattern of HER2/neu gene in invasive ductal carcinomas. (A) Show non-amplified pattern; **(B)** Shows amplified pattern. The slides were viewed at 60X magnification.

Concordance rate between IHC and BDISH for evaluating HER2/neu protein/gene status in breast cancer

The data analysis showed that there is an equitable concordance rate between IHC and BDISH techniques in evaluating HER2/neu status in breast cancer. 73 samples were estimated for concordance rate. 56 out of 73 samples showed concordance, so there is 77 % concordance rate (Table 4).

Table 4: Concordance rate between immunohistochemistry (IHC) and bright-field double in situ hybridization (BDISH) for evaluating HER2/neu protein/gene status in Saudi breast cancer samples

IHC/BDISH of HER2/neu protein/gene status	Expression pattern (0-1)	Expression pattern (2)	Expression pattern (3)	Total
<1.8	36	15	0	51
1.8 - 2.2	0	3	0	3
>2.2	0	2	17	19
Total	36	20	17	**73**

Correlation of IHC HER2/neu protein expression status with clinicopathological features

The correlation of IHC HER2/neu protein expression status with clinicopathological features (cut-off point: 0, 1, 2 and 3) is shown in Table 5. There was significant correlation between

HER2/neu protein expression and age (p<0.01), menopausal status (p<0.03) and borderline significance with family history (p<0.07).

Table 5: Correlation of immunohistochemistry HER2/neu protein status with clinicopathological features (cut-off point: low, moderate and high expression pattern)

Patients' features	HER2/neu protein expression pattern			P- value
	Low	Moderate	High	
Age				
< 50	38	21	17	0.006
>= 50	36	7	3	
Family history				
No	32	11	8	0.03
Yes	2	3	1	
Unknown	0	4	1	
Menopausal status				0.02
Pre-menopausal	34	19	15	
Post-menopausal	28	7	2	
Tumor size				0.55
< 2 cm	7	1	2	
2-5 cm	26	11	8	
> 5 cm	12	9	3	
Stage of disease				0.92
Low stage I, II	38	15	8	
High stage III, IV	23	11	5	
Grade				0.13
I	15	5	4	
II	37	12	5	
III	15	11	9	
Lymph node state				0.54
Positive	34	18	9	
Negative	14	8	7	
Metastasis				0.55
No	39	19	9	
Yes	15	4	2	
Treatment response				0.14
No response	18	3	2	
Objective response	42	22	12	
Recurrence				0.31
Yes	19	5	3	
No	38	20	13	
Status at end point				0.55
Alive	47	20	14	
Died of disease	15	7	2	

Correlation of BDISH HER2/neu gene with clinicopathological features

Correlation of BDISH HER2/neu gene amplification status with clinicopathological features (non-amplified versus amplified) illustrated in Table 6. There was significant correlation

between HER2/neu gene and two clinicopathological features- age ($p<0.04$) and menopausal status ($p<0.01$).

Table 6: Correlation of bright-field double in situ hybridization HER2/neu gene amplification status with clinicopathological features (non-amplified versus amplified)

Patients' features	HER2/neu gene amplification pattern		P- value
	Non-amplified	Amplified	
Age			
< 50	29	15	0.04
>= 50	22	3	
Family history			
No	27	9	0.51
Yes	4	0	
Unknown	4	1	
Menopausal status			0.01
Pre-menopausal	23	14	
Post-menopausal	21	2	
Tumor size			0.76
< 2 cm	5	2	
2-5 cm	25	6	
> 5 cm	16	3	
Stage of disease			0.41
Low stage I, II	28	8	
High stage III, IV	19	3	
Grade			0.40
I	11	3	
II	23	6	
III	14	8	
Lymph node state			0.78
Positive	26	9	
Negative	14	4	
Metastasis			0.76
No	34	8	
Yes	6	1	
Treatment response			0.88
No response	7	2	
Objective response	34	11	
Recurrence			0.38
Yes	11	2	
No	32	12	
Status at end point			0.84
Alive	37	12	
Died of disease	8	3	

Correlation of HER2/neu protein expression status and survival outcome

The Kaplan-Meier analysis showed the relationship between HER2/neu protein expressions profile and disease-free survival (DFS) by using the cut-off point (negative, moderate and strong) expression patterns (Figure 4). At 5 years of survival follow up period, approximately 30% of patients with negative HER2/neu expression showed recurrence of disease as compared to 19% and 18% of both strong and moderate expression patterns respectively (P<0.31). Although the correlation is not statistically significant but still there is a trend that patients with high HER2/neu protein expression pattern have longer disease free survival than those patients with low HER2/neu expression pattern (shorter survival). Univariate (Kaplan-Meier) survival analysis of HER2/neu protein also showed that the patient with high HER2/neu expression pattern has longer disease free survival than those patients with low HER2/neu expression pattern. By using the cut-off point (0, 1, 2, 3) expression patterns at 40 months of survival follow up period, approximately 22% of patients with no (0) HER2/neu expression pattern showed recurrence of disease as compared to 19%, 18% and 17% of (1,2,3) expression pattern, respectively (p<0.48) (Figure 5).

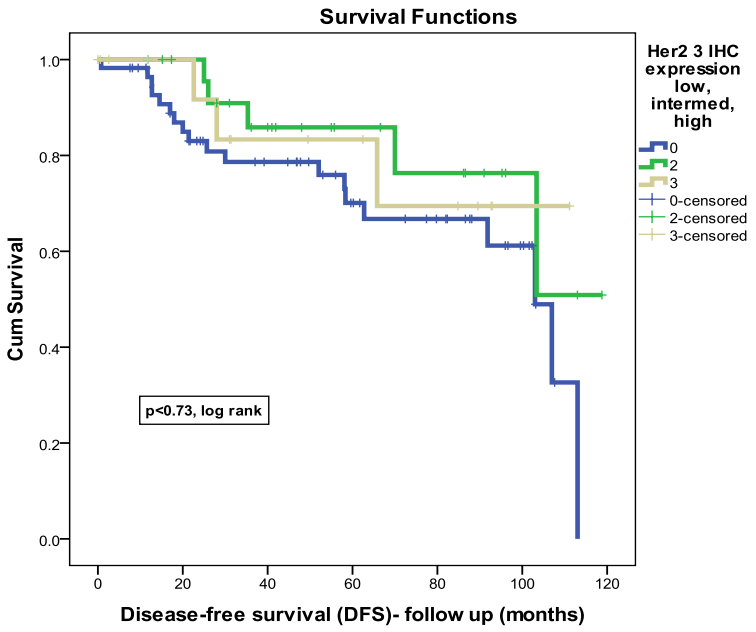

Figure 4: Kaplan-Meier analysis showing the relationship between HER2/neu protein expressions profile and disease-free survival (DFS) by using the cut-off point (negative, moderate and strong) expression patterns. P-value represents log-rank testing of the difference in cumulative survival.

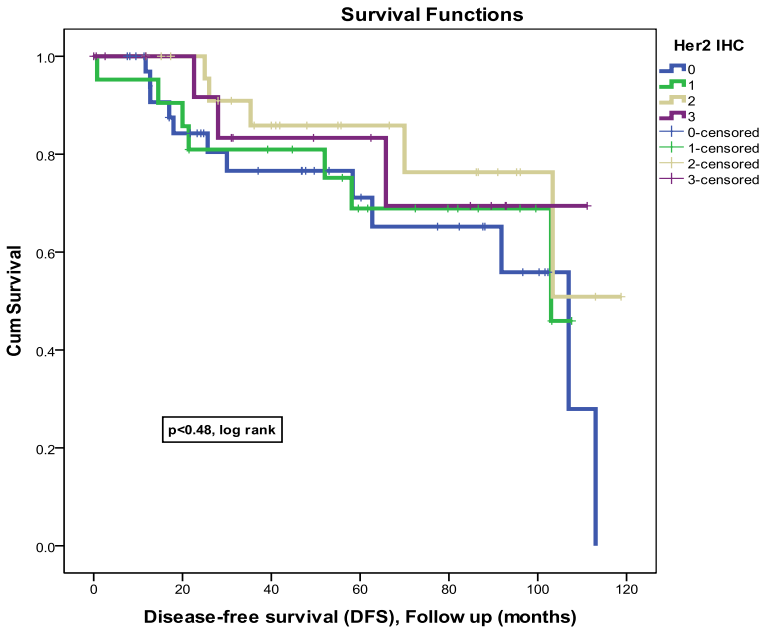

Figure 5: Kaplan-Meier analysis showing the relationship between HER2/neu protein expressions profile and disease-free survival (DFS) by using the cut-off point (0,1,2,3) expression patterns at 40 months of survival follow up period. P-value represents log-rank testing of the difference in cumulative survival.

The relationship between HER2/neu expression profile and disease-specific survival (DSS) by using the cut-off point (0, 1, 2, 3) expression patterns is shown in Figure 6. At 45 month of survival follow up period, approximately 26% of patients with (1) HER2/neu expression are died as compared to 25%, 24%, and 19% of (0, 2 and 3) expression pattern respectively (P<0.98). The Kaplan-Meier curve showed the relationship between HER2/neu expression profile and disease-specific survival (DSS) by using the cut-off point (negative, moderate and strong) expression patterns (Figure 7). At 60 months of survival follow up period, approximately 30% of patients with moderate HER2/neu expression were died of cancer (shorter survival) as compared to 22% and 18% of patients with low and strong expression patterns of their tumor samples respectively (P<0.76).

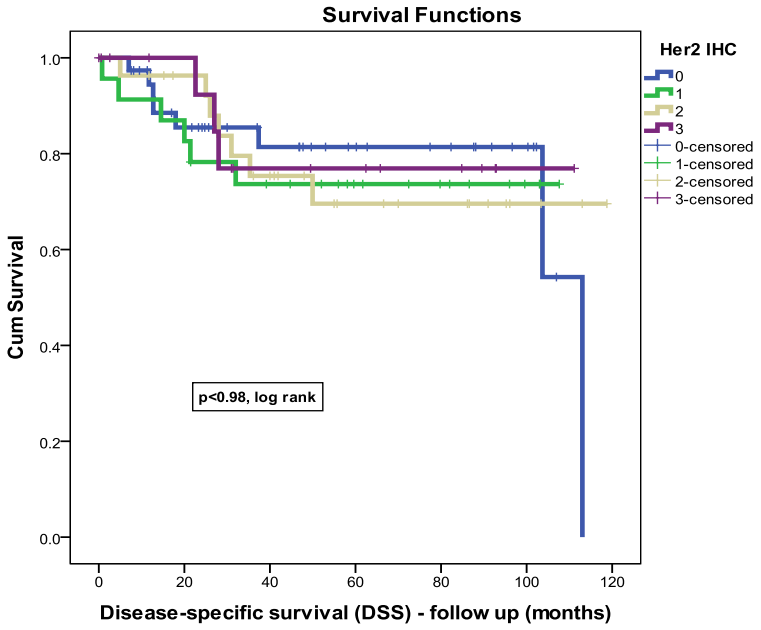

Figure 6: Univariate Kaplan-Meier analysis showing the relationship between HER2/neu protein expressions profile and disease-specific survival (DSS) by using the cut-off point (0,1,2,3) expression patterns. P-value represents log-rank testing of the difference in cumulative survival.

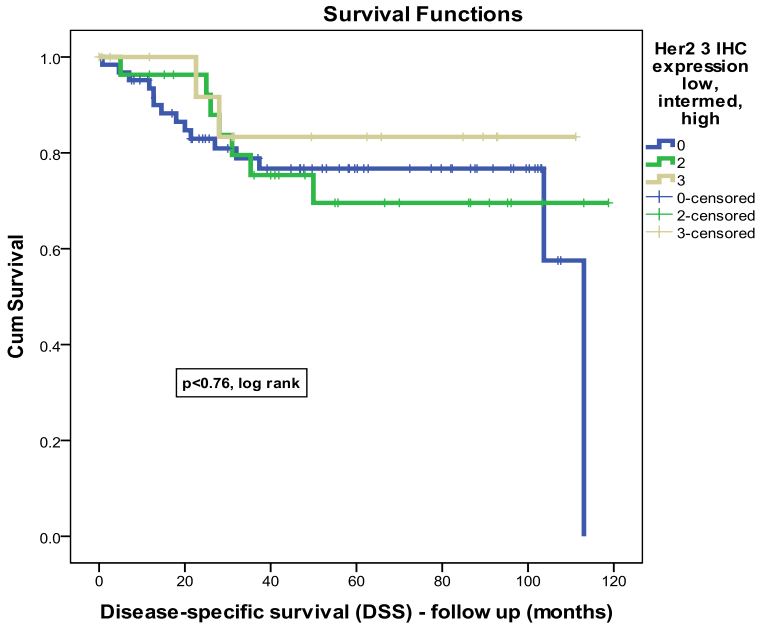

Figure 7: Univariate Kaplan-Meier analysis showing the relationship between HER2/neu protein expressions profile and disease-specific survival (DSS) by using the cut-off point (negative, moderate and strong) expression patterns. P-value represents log-rank testing of the difference in cumulative survival.

Correlation of HER2/neu gene amplification status and survival outcome

The Kaplan-Meier analysis showed the relationship between HER2/neu amplification and disease-free survival (DFS) (Figure 8). At 35 month of survival follow up period, approximately 21% of patients with HER2/neu gene amplification showed recurrence of disease as compared to 19% of patients with non-amplification of HER2/neu gene (P<0.45). Although the correlation is not statistically significant but still there is a trend that patients with amplification HER2/neu gene pattern have shorter disease free survival than those patients with non-amplification HER2/neu gene pattern (longer survival). Univariate (Kaplan-Meier) survival analysis also showed that the patient with amplification HER2/neu gene pattern has shorter disease specific survival than those patients with non-amplification HER2/neu gene pattern. At 40 months of survival follow up period, approximately 30% of patients with HER2/neu gene amplification were dead compared to 21% of patients with non-amplification HER2/neu gene (P<0.65) (Figure 9).

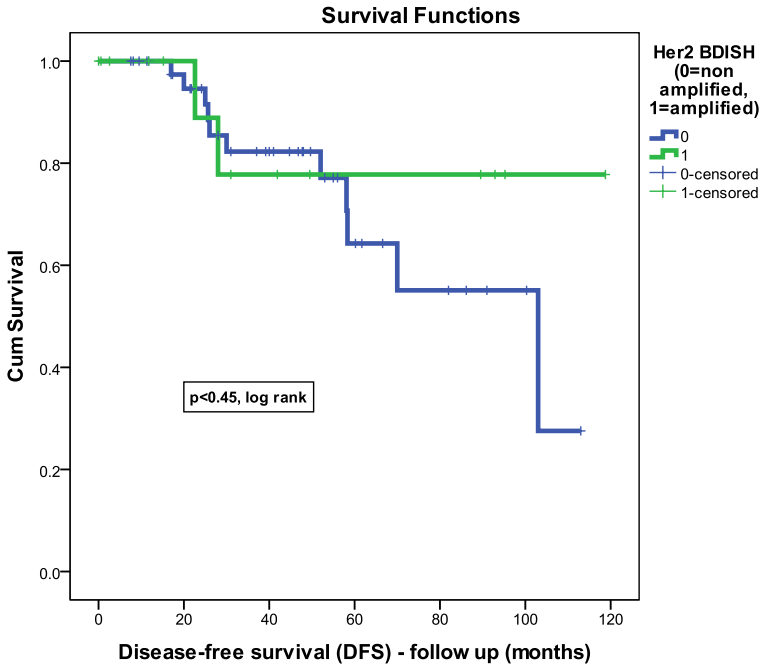

Figure 8: Univariate Kaplan-Meier analysis showing the relationship between HER2/neu gene amplification status and disease-free survival (DFS) by using the cut-off point (non-amplified and amplified). P-value represents log-rank testing of the difference in cumulative survival.

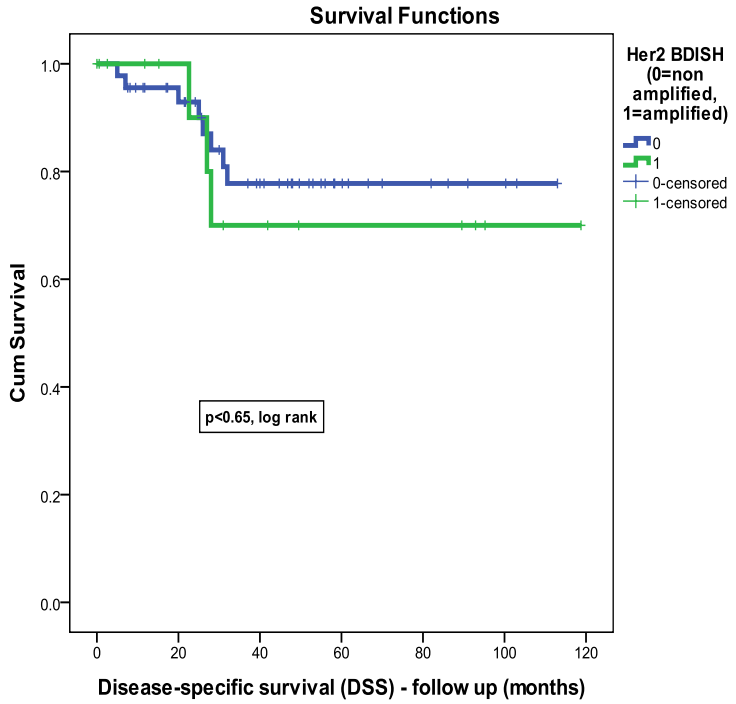

Figure 9: Univariate Kaplan-Meier analysis showing the relationship between HER2/neu gene amplification status and disease-free survival (DFS) by using the cut-off point (non-amplified and amplified) at 40 months of survival follow up period. P-value represents log-rank testing of the difference in cumulative survival.

DISCUSSION

The current study is one among the rare studies where a new technique bright field double in situ hybridization (BDISH) by new machine called Ventana Benchmark XT was used. Considering the overall logistical difficulties, accuracy, time and cost for the double testing of HER2/neu, IHC/BDISH gene analysis may be an efficient and useful approach for HER2/neu screening of breast cancer patients, particularly for laboratories analyzing many breast cancer surgical specimens, where the pathological experience of the staff would guarantee a correct tumor grading. Importantly, this study showed that HER2/neu amplification status in breast cancer of Saudi patients is within the range as compared to other ethnic groups. Moreover, our study revealed a reasonable concordance rate between BDISH and IHC results. To the best of our knowledge, using BDISH technique as an adjunct tool for evaluating HER2/neu gene amplification status in breast cancer is the first study, so far done in Saudi cohort. Therefore, extensive training for both scientific and technical staff is highly recommended to improve the concordance rate between two techniques. However, due to the inherent failures of the IHC assay, BDISH should always be used when the IHC results are inconclusive. The rational

algorithm for HER2/neu testing would be to perform IHC first, followed by BDISH to validate equivocal IHC results. However, internationally there is no consensus about, which technique is the best to start with. Moreover, available data do not clearly demonstrate the superiority of either IHC or ISH as a predictor of benefit from anti-HER2/neu therapy.

CONCLUSION

In conclusion, the larger proportion of patients included in this study was young and at advanced stage. These data emphasize the need to adopt a general population-based screening program, which will reduce the mortality of breast cancer. The availability of patient's medical record obtained from hospitals was the main difficulty in this research, which was a true obstacle to produce realistic results and do the required statistical analysis. Enough efforts should be spent at the level of oncologists, pathologists and surgeons to make the clinicopathological data ready and oriented for research purpose. Therefore, a complete Saudi breast cancer database with complete follow up data should be established to facilitate further research work. However, larger cohort studies are needed to explore the role of different molecular markers as prognosticators of Saudi breast cancer. Scientific collaborations at national and international levels are highly needed for transferring technology, exchanging research ideas and to compare the results of Saudi cohorts with other populations. Establishing the basic platforms of prognostic and predictive genomic models (molecular signatures) to facilitate the approach towards personalized oncology in Saudi Health System is also recommended.

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