

ORIGINAL ARTICLE

A comparison study of HER2 protein overexpression and its gene status in breast cancer

Suria Hayati MD PAUZI¹, Hanis Nabihah SAARI¹, Muhammad Ridzuan ROSLAN¹, Sharifah Nor Shairah SYED KHAIR AZMAN JAMALULIL¹, Irene Scully TAUAN¹, Fatin Adiola MOHD RUSLI¹, Azimatun Noor AIZUDDIN²

¹Department of Pathology, Faculty of Medicine, Universiti Kebangsaan Malaysia Medical Centre, Jalan Yaacob Latif, Bandar Tun Razak, Cheras, 56000, Kuala Lumpur, Malaysia and ²Department of Community Health, Faculty of Medicine, Universiti Kebangsaan Malaysia Medical Centre, Jalan Yaacob Latif, Bandar Tun Razak, Cheras, 56000, Kuala Lumpur, Malaysia

Abstract

Introduction: Evaluation of HER2 status in breast cancer using immunohistochemistry (IHC) and in-situ-hybridisation (ISH) study is important to establish prognosis and to select patient for targeted therapy. **Objective:** The study aims to determine the concordance between HER2 protein IHC score and its gene status by dual-colour dual-hapten in-situ-hybridization (DDISH) study. **Materials and Methods:** Retrospective study was performed on 767 referred breast cancer cases over a period of five years. The HER2 IHC score (the initial and repeat test score) and the results of HER2 gene status by DDISH were retrieved from the histopathological reports. The agreement between initial IHC score with repeat test score was measured using Cohen Kappa. Chi square test analyzed the association between HER2 IHC score with its gene status by DDISH. **Results:** The concordance of HER2 IHC score between the initial and repeat test were 52.7% and 89.4% for IHC score 2+ and 3+ respectively. There was moderate agreement of HER2 IHC score between the initial and repeat test score ($\kappa = 0.526$, $p < 0.001$). A significant association noted between HER2 IHC score with its gene status by DDISH ($p < 0.001$). Only 56 out of 207 cases (27.1%) with 2+ IHC score showed HER2 gene amplification while the majority of cases with 3+ IHC score were gene-amplified (446 out of 451, 98.9%). **Conclusion:** ISH study should be done in all IHC-equivocal cases (2+) to select patient for targeted therapy. Gene amplification must also be confirmed in IHC-positive cases (3+) to prevent from giving non-effective treatment with possible adverse effects to patient with non-amplified HER2 gene.

Keywords: breast cancer, HER2 gene, HER2 immunohistochemistry, DDISH

INTRODUCTION

Breast cancer is one of a major public health problem throughout the world and it is the most common cancer among woman.¹ It is the second leading cause of cancer fatality in women after lung cancer.² It has also been noted that young age at diagnosis correlates with a worse clinical outcome compared to their older counterparts.³

HER2 (Human epidermal growth factor receptor-2) gene is a proto-oncogene and is situated on chromosome 17q21⁴. This gene encodes for HER family proteins of type 1 transmembrane growth factor receptors. It promotes intracellular signaling pathways in

reaction to extracellular signals that comprise of extracellular ligand-binding domain, a transmembrane domain and an intracellular tyrosine kinase domain.⁵ Signaling through this receptor will significantly increase cellular proliferation and prevent apoptosis. Point mutation within the transmembrane domain will cause translation of mutated protein that finally will stimulates receptor dimerization and enhanced tyrosine kinase activity that is recognized as overexpression of HER2 gene.⁵ HER2 gene amplification is consistently discovered in breast, ovarian and gastric cancers with a percentage 10-30% of these tumors show

HER2 gene amplification⁶. Among invasive breast cancer, HER2 protein overexpression has been reported in approximately 25-30% of the cases. A shorter disease-free survival and therapeutic resistant are also observed among patients with HER2 amplification.⁴

Assessment of HER2 status is important to establish prognosis as well as to establish patient's eligibility for HER2-targeted therapy. Currently, immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH) are widely used methods for determination of HER2 status in breast carcinoma. Other alternative methods to detect HER2 gene status in a tumour include quantitative PCR and the newly developed chromogenic dual in situ hybridization (DISH).⁷

HER2 immunohistochemistry (IHC) test is performed regularly in all breast cancer cases. As in any laboratory test, the IHC test is subjected to several pre-analytical, analytical and post analytical variables which may affect the final interpretation of the result. The confirmation of HER2 gene status performed in our centre is by dual-colour dual-hapten in-situ hybridization study (DDSIH) which is interpreted using the light microscope. The DDISH test is not widely available unlike the immunohistochemistry (IHC) test which is routinely performed by all major hospital in Malaysia. There are only few referral hospital laboratories which performed the DDISH test, one of which is our centre which also received breast cancer cases from other hospitals for confirmation of HER2 gene amplification status. At the current moment, the DDISH study is routinely performed in all breast cancer cases with positive HER2 IHC (3+) and equivocal score (2+) to confirm the HER2 gene amplification status.

The purpose of this study is to evaluate variability and concordance of HER2 IHC scoring between different hospital laboratories and to also determine the concordance between the HER2 protein expression by IHC with its HER2 gene amplification status by DDISH study.

MATERIALS AND METHODS

This is a retrospective and descriptive study of 767 referred breast cancer cases that were sent to our institution for confirmation of HER2 gene status from January 2013 to December 2017. Our institution received referral for all breast cancer cases from the East Coast of Malaysia which was scored as IHC 2+ and 3+ for confirmation of gene status. Formalin-fixed tissue blocks were

received from these hospitals, following which a repeat HER2 IHC test was regularly performed as well as the DDISH study for confirmation of the gene status. We do not routinely receive HER2 IHC-stained slides from these hospitals nor have the information regarding the IHC protocol for each hospital. For this study, the data was retrieved from the histopathological report and no repeat assessment of the stained slides were performed.

The data was analysed using Statistical Packaged for the Social Science, (SPSS) software version 23. Cohen Kappa coefficient test was used to measure the agreement between the initial HER2 protein score with the repeat test done on the same tissue sections from the tumour specimen. Chi Square test was performed to look at the association between HER2 IHC score with the gene amplification status by DDISH.

RESULTS

A total of 767 referred breast cancer cases were included in this study. All of these patients are female, the majority of which are diagnosed at the age between 41-50 years old (250 cases) followed by those 51-60 years of age (226 cases). Most of them are Malays while other races comprise between 1-8% of the overall referred breast cancer cases (Table 1).

Concordance between the initial and the repeat HER2 IHC score

The cases are composed of 321 breast cancer cases scored as equivocal (IHC score 2+) and 443 IHC-positive (score 3+) cases. Although we routinely received only HER2 IHC score 2+ (equivocal) and 3+ (positive) for HER2 gene confirmation, we found that three cases scored as 1+ (negative) were also sent for DDISH study. No specific reason was stated on why the study was requested. (Table 2).

During the evaluation of inter-laboratory concordance, it was noted that the highest number of discordant results are observed in cases with equivocal IHC score (2+). Among the 321 cases with the initial IHC score of 2+, a repeat IHC study show a concordance of 52.7% with only 169 cases achieved a similar score. 29 other cases show a negative result (score 1+) and 55 cases scored as positive (3+) (Table 2).

The cases that were scored as 3+ in the initial test achieved a better concordance rate of 89.4% whereby the majority of the cases (396 out of 443 cases) showed similar positive score on the repeat IHC study. Interestingly, 9 cases with the

TABLE 1: Age and race distribution of the study population

Demographic	n (%)
Age	
21-30	24 (3.1 %)
31-40	102 (13.3 %)
41-50	250 (32.6 %)
51-60	226 (29.5 %)
61-70	118 (15.4 %)
71-80	39 (5.1 %)
80-90	8 (1.0 %)
Race	
Malay	680 (89%)
Chinese	64 (8%)
Indian	15 (2%)
Others	8 (1%)

initial score of 3+ showed a negative IHC result (1+) on the repeat IHC study while 38 other cases were re-scored as equivocal (2+) (Table 2).

A 100% concordance rate was also observed among the three negative (1+) cases with non-amplified HER2 gene was later confirmed by DDISH study. Kappa coefficient test showed an overall moderate agreement with kappa value of 0.526 (p value <0.001). (Table 2).

Concordance between IHC and DDISH study

As part of this study, we had also evaluated our own institution's concordance between IHC score (from the repeat IHC test done at our laboratory) and the gene amplification status by DDISH. In general, 66% of all cases exhibit gene amplification by DDISH. Most of the 109 IHC-negative cases (score 0 or 1+) (96.3%) showed a non-amplified HER2 gene while gene amplification was seen in the remaining 4 cases (3.7%) (Table 3).

As expected, HER2 gene amplification was proved in 98.9% (446 out of 451 cases) of IHC-positive (3+) cases with only 5 cases showed a non-amplified gene on DDISH. The majority

of the 207 IHC-equivocal (2+) cases (72.9%) showed a non-amplified HER2 gene while in 56 cases (27.1%) the gene was confirmed to be amplified. A further statistical analysis using chi-square test showed a significant association between the IHC score and the gene amplification status by DDISH (p<0.001) (Table 3).

DISCUSSION

In the present study, we demonstrate an overall inter-laboratory concordance rate in HER2 IHC scoring of 74.1%. A study done on 394 breast cancer cases in 2002 has compared IHC score between two laboratories performing HER2 IHC on the same tumour tissue sections using similar primary antibodies however with different immunostaining protocol and different scoring system. This study has reported a concordance rate of 84.5% and had concluded that standardization of IHC method is important for the IHC result to be duplicable.⁸ Unfortunately, in our study we do not have the information on the IHC protocol employed by each hospital.

TABLE 2: Concordance between initial IHC score and score of the repeat test

HER2 IHC score from the referred hospital	Number of cases according to score from repeat HER2 IHC study					Concordance (%)
	0	1+	2+	3+	Total	
1+	-	3	-	-	3	100 %
2+	29	68	169	55	321	52.7 %
3+	-	9	38	396	443	89.4 %

Kappa Coefficient 0.526, p<0.001.

TABLE 3: Concordance between IHC and DDISH

IHC score (repeat test)	n	Gene amplification status by DDISH	
		Amplified (%)	Non-amplified (%)
0 or 1+ (Negative)	109	4 (3.7%)	105 (96.3%)
2+ (Equivocal)	207	56 (27.1%)	151 (72.9%)
3+ (Positive)	451	446 (98.9%)	5 (1.1%)

Significant association between IHC score and the gene amplification status by DDISH (p<0.001)

A ring study done in Japan between one coordinating institution and 10 participating laboratories, in which 20 breast cancer tissue samples were immunostained by the respective laboratories had found a good agreement ($\kappa = 0.718$) in their HER2 IHC scoring.⁹ In our study, we reached a moderate agreement ($\kappa = 0.526$) between the score from the referring hospitals and the repeat score at our centre.

Both immunohistochemistry (IHC) and in-situ hybridization (ISH) techniques have their own advantages and disadvantages. IHC is cheaper and is more convenient with lesser time needed to perform the study. However, IHC stain may be affected by several factors and inevitably, this might lead to interlaboratory discrepancies for HER2 protein IHC score analysis. Some of the factors include tissue fixation and type of antibodies used.¹⁰ In one study, a higher percentage of positive HER2 IHC was seen with antibody A0485 (Dako) (52.2%) as compared with HercepTest (33.3%).¹¹ The authors suggested that the higher sensitivity observed with antibody A0485 was attributed to the polyclonal nature of the antibody.¹¹ The different monoclonal and polyclonal antibodies used to detect HER2 protein identify different intra or extracellular epitope domain on the HER2 receptor protein. False negative staining may be obtained with the shedding of the extracellular domain if the antibody used is specifically directed to that domain.^{11,12} Partial degradation of HER2 protein as a consequence of tumour necrosis or chemotherapy may also affect the result of IHC stain. In this situation, HER2 protein may only be detected by the more sensitive polyclonal antibody.¹¹

Usage of different IHC scoring system by different institutions may affect the final IHC reporting¹³ however in our study, all hospitals are using the standard ASCO/CAP 2013 (American Society of Clinical Oncology/ College of American Pathologist) guideline. Inter-observer variability can also be one of the factors affecting

IHC result interpretation nevertheless this factor cannot be evaluated in our study as the data obtained from the histopathological report was based on the score of repeat IHC staining on the tissue section from same tumour tissue and not by reevaluating the previously-stained tissue section from the referred hospitals. Nonetheless, the inter-observer agreement may significantly affect the IHC scoring which may vary with stain intensity. Thomas *et al* had observed the agreement in IHC scoring was high for stain intensities interpreted as 0 (no stain) or 3+ (intense, complete staining). However, for stain intensities interpreted as 1+ and 2+, the inter-observer agreement was generally poor and this further support the important role of ISH study in evaluating cases with score 1+ and 2+.¹⁴

On the other hand, ISH (in-situ-hybridization) study is more expensive though it is less subjective. The result is also more duplicable as DNA is more stable and less influenced by the fixation¹³. Due to its specificity and sensitivity, FISH (in situ hybridization) has been considered as gold standard currently with several comparative studies had observed a high concordance rate of IHC score 3+ or 1+/0 in this technique in breast carcinomas.^{10,15,16} Nonetheless, FISH study is more time consuming as each cell will need to be assessed in FISH.¹³ The inability to archive FISH slides has also limit its usage in most pathology laboratories.¹⁷ Despite several alternative tests had been developed to replace FISH, the most potential test that could pass 95% correlation would be chromogenic dual in situ hybridisation (DISH).⁷ Two studies had demonstrated more than 95% correlation between FISH and DISH results in accordance to the recommendation by ASCO/CAP guideline.^{18,19} The concordance rate between IHC and ISH varies in different studies. This disagreement is expected as IHC and ISH evaluate distinct target – concentration of protein in IHC versus gene copies in ISH.¹³

An overall 66% of IHC-equivocal (2+) and

positive (3+) cases in the present study exhibit gene amplification by DDISH. On the other hand, almost all (105 out of 109) of IHC-negative cases was confirmed to have a non-amplified HE2 gene with a concordance of 96.3%. Other studies show similar findings with a concordance rate of between 95 – 100% were observed in HER2 negative (1+) cases and its non-amplified gene status by FISH.²⁰⁻²² Nevertheless, 4 out of 109 HER2-negative cases in our series showed gene amplification by DDISH. This discrepant findings of negative IHC-staining in gene-amplified case may be attributed to possible differences in the duration of fixation as delayed or insufficient tissue fixation may result in degradation of epitope¹¹. Different method of antigen retrieval may also affect the final staining.¹¹ However, in this current study we do not have the information on the technique of antigen retrieval, cold ischaemic time nor the duration of fixation from each hospital.

Studies from India and Iran had reported the detection of gene amplification by FISH between 25% to 32.4 in equivocal cases (2+ IHC score) with non-amplification was seen in 67.6 to 73%.^{20,23,24} This is comparable to our findings of gene amplification obtained in only 27.1% of 2+ cases. However, in 2018 Lateef et al has reported higher percentage of amplified gene status (69.2%) in equivocal IHC score albeit a much smaller number of cases (26 cases).²⁰

Several studies from had reported concordance of 84-100% between HER2 IHC score of 3+ and amplified gene status by ISH^{20,22,25} which is almost similar to our series of 98.9% concordance rate. HER2 protein overexpression without gene amplification may result from antigen over-retrieval, protein over-expression secondary to elevated transcriptional activity or polysomy 17¹¹. Another possible reason is a misinterpretation of weak to moderate staining as intense staining or if the interpretation of staining is performed in non-suitable crushed tumour areas.²⁰

IHC and ISH have different sensitivity and specificity. The disagreement between HER2 protein status by IHC and HER2 gene amplification status by ISH was expected especially in equivocal cases (2+). Nonetheless, ISH is more duplicable and consistent compared to IHC due to the stability of DNA structure which is less affected by fixation of tissue as opposed to protein. Furthermore, ISH interpretation which is based on counting distinct signals representing the chromosomal area of the amplified gene is more objective¹³ compared to the degree of staining intensity in IHC.

CONCLUSION

In summary, although HER2 status can be indirectly assessed by the less expensive IHC studies, disagreement between IHC and ISH is expected especially in IHC equivocal (2+) cases. Therefore, we recommend ISH study to be done in all equivocal cases to avoid missing patients that could have benefited from the targeted therapy. On the other hand, even though high concordance is noted among positive IHC cases (3+), administering therapy just based on the IHC results may not be suggested as HER2 gene can be non-amplified though IHC positive. Furthermore, ISH is less costly as compared to the targeted therapy not to mention the possible adverse drug effects despite non-effective therapy in non-amplified cases. Laboratories should also continuously optimize the IHC protocol to ensure reliable HER2 IHC results and to participate in HER2 EQA programme to evaluate their performance.

Statement conflict of Interest: The authors declared that there was no conflict of interest

REFERENCES

1. Ferlay J, Hery C, Autier P, Sankaranarayanan R. Breast cancer epidemiology. In: Li CI, ed. Vol 1. LLC, 233 Spring Street, New York, NY 10013, USA: Springer; 2010:1-19.
2. DeSantis C, Ma J, Bryan L, Jemal A. Breast cancer statistics, 2013. *CA Cancer J Clin.* 2014;64(1):52-62.
3. Assi HA, Khoury KE, Dbouk H, Khalil LE, Mouhieddine TH, El Saghir NS. Epidemiology and prognosis of breast cancer in young women. *J Thorac Dis.* 2013;5(Suppl 1):S2.
4. Yano T, Ohtsu A, Boku N, Hashizume K, Nakanishi M, Ochiai A. Comparison of HER2 gene amplification assessed by fluorescence in situ hybridization and HER2 protein expression assessed by immunohistochemistry in gastric cancer. *Oncol Rep.* 2006;15(1):65-71.
5. Moasser M. The oncogene HER2: its signaling and transforming functions and its role in human cancer pathogenesis. *Oncogene.* 2007;26(45):6469-6487.
6. Hynes NE, Stern DF. The biology of erbB-2/neu/HER-2 and its role in cancer. *Biochim Biophys Acta.* 1994; 1198(2-3):165-184.
7. Layfield LJ, Wallander ML, Tripp SR, Redpath S, Banks PM. Comparison of Dual-ISH (DISH) With Fluorescence In Situ Hybridization (FISH) and Correlation With Immunohistochemical Findings for HER2/Neu Status in Breast Carcinoma. *Appl Immunohistochem Mol Morphol.* 2017;25(4):231-236.
8. Gancberg D, Järvinen T, Leo Ad *et al.* Evaluation of HER-2/NEU protein expression in breast cancer by immunohistochemistry: an interlaboratory study

- assessing the reproducibility of HER-2/NEU testing. *Breast Cancer Res Treat.* 2002;74(2):113-120.
9. Umemura S, Osamura RY, Akiyama F *et al.* What causes discrepancies in HER2 testing for breast cancer? A Japanese ring study in conjunction with the global standard. *Am J Clin Pathol.* 2008;130(6):883-891.
 10. Zhang H, Ren G, Wang X *et al.* HER-2 gene amplification by fluorescence in situ hybridization (FISH) compared with immunohistochemistry (IHC) in breast cancer: a study of 528 equivocal cases. *Breast Cancer Res Treat.* 2012;134(2):743-749.
 11. Kovacs A, Stenman G. HER2-testing in 538 consecutive breast cancer cases using FISH and immunohistochemistry. *Pathol Res Pract.* 2010;206(1):39-42.
 12. Lebeau A, Deimling D, Kaltz C *et al.* Her-2/neu analysis in archival tissue samples of human breast cancer: comparison of immunohistochemistry and fluorescence in situ hybridization. *J Clin Oncol.* 2001;19(2):354-363.
 13. Wang S, Saboorian MH, Frenkel E, Hynan L, Gokaslan ST, Ashfaq R. Laboratory assessment of the status of Her-2/neu protein and oncogene in breast cancer specimens: comparison of immunohistochemistry assay with fluorescence in situ hybridisation assays. *J Clin Pathol.* 2000;53(5):374-381.
 14. Thomas TA, Hayes MM, Spinelli JJ *et al.* HER-2/neu in Breast Cancer: Interobserver Variability and Performance of Immunohistochemistry with 4 Antibodies Compared with Fluorescent In Situ Hybridization. *Mod Pathol.* 2001;14(11):1079-1086.
 15. Mrozkowiak A, Olszewski WP, Piascik A, Olszewski WT. HER2 status in breast cancer determined by IHC and FISH: comparison of the results. *Pol J Pathol.* 2004;55(4):165-171.
 16. Kuo S-J, Wang BB-T, Chang C-S *et al.* Comparison of Immunohistochemical and Fluorescence In Situ Hybridization Assessment for HER-2/neu Status in Taiwanese Breast Cancer Patients. *Taiwan J Obstet Gynecol.* 2007;46(2):146-151.
 17. Mostafa NA, Eissa SS, Belal DM, Shoman SH. Assessment of Her-2/neu gene amplification status in breast carcinoma with equivocal 2+ Her-2/neu immunostaining. *J Egypt Natl Canc Inst.* 2011;23(1):41-46.
 18. Brüggmann A, Lelkaitis G, Nielsen S, Jensen KG, Jensen V. Testing HER2 in breast cancer: a comparative study on BRISH, FISH, and IHC. *Appl Immunohisto M M.* 2011;19(3):203-211.
 19. Bartlett JMS, Campbell FM, Ibrahim M *et al.* A UK NEQAS ICC and ISH multicentre study using the Kreatech Poseidon HER2 FISH probe: intersite variation can be rigorously controlled using FISH. *Histopathology.* 2010;56(3):297-304.
 20. Lateef F, Jamal S, Nasir S. Her-2/neu Oncogene Amplification by Fluorescence In Situ Hybridization and Protein Overexpression on Immunohistochemistry in Breast Cancer. *J Coll Physicians Surg Pak.* 2018;28(8):581-585.
 21. Varga Z, Noske A, Ramach C, Padberg B, Moch H. Assessment of Her-2/neu status in breast cancer: Overall positivity rate and accuracy by fluorescence In Situ hybridization and Immunohistochemistry in a single institution over 12 years: a quality control study. *BMC Cancer.* 2013;13:615.
 22. Singhai R, Patil V, Patil A. Immunohistochemical (IHC) HER-2/neu and fluorescent-in situ hybridization (FISH) gene amplification of breast cancer in Indian women. *Asian Pac J Cancer.* 2011;12(1):179-183.
 23. Murthy SS, Sandhya DG, Ahmed F *et al.* Assessment of HER2/Neu status by fluorescence in situ hybridization in immunohistochemistry-equivocal cases of invasive ductal carcinoma and aberrant signal patterns: a study at a tertiary cancer center. *Indian J Pathol Micro.* 2011;54(3):532-538.
 24. T. García-Caballero I, MD Menéndez, A. Vázquez-Boquete I, R. Gallego I, J. Forteza, M. Fraga. HER-2 status determination in breast carcinomas. A practical approach. *Histol Histopathol.* 2006;21(3):227-236.
 25. Panjwani P, Epari S, Karpate A *et al.* Assessment of HER-2/neu status in breast cancer using fluorescence in situ hybridization & immunohistochemistry: Experience of a tertiary cancer referral centre in India. *Indian J Med Res.* 2010;132:287-294.