Original article

Evaluation of HER2 Gene Amplification by FISH in Immuno-histochemically Equivocal Breast Cancer Cases

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ABSTRACT

Background: Breast cancer is a global health concern, especially in Europe and America, with higher incidence and mortality rates. HER2-positive breast cancer accounts for 20-25% of diagnoses, known for its aggressive behavior. To assess HER2 status, various methods are available, including PCR, IHC, FISH, and CISH. Immunohistochemistry (IHC) and Fluorescence in Situ Hybridization (FISH) are the primary techniques for HER2 evaluation. IHC and FISH are the predominant methods employed for evaluating HER2 status. IHC gauges the expression of the HER2 protein on the cell surface, while FISH identifies HER2 gene amplification at the DNA level. The objective of this study is to know the prevalence of FISH positive cases in IHC , HER2 equivocal cases of breast cancer.

Patients and methods: This descriptive cross sectional study was carried out in Al Hada, Armed Forces Hospital in Taif region for two years. The study includes individuals aged 18 or older classified as HER2-equivocal (IHC 2+) invasive breast cancer per ASCO/CAP guidelines within three months, who provide informed consent for tissue and clinical data use. Exclusions include confirmed HER2-positive or HER2-negative status, insufficient tumor tissue, prior neoadjuvant or HER2-targeted therapy, metastatic disease, severe comorbidities, life expectancy under six months, or inability to consent.. IHC testing was conducted on collected breast tissue with standard protocols. FISH testing was performed to assess HER2 gene amplification at the DNA level on equivocal cases of tissue samples. The results obtained from the FISH testing were analyzed. The degree of concordance between the two methodologies was thoroughly assessed.

RESULTS: The study analyzed 100 patients aged between 22 and 80 years, with 89% being over the age of 41. The majority of the tumors were identified as ductal carcinoma. Among the patients with HER2-positive equivocal immunostaining, 78% of those results showed no HER2/neu gene amplification on FISH analysis.

Conclusion: These findings emphasize the combination of IHC and FISH, to accurately assess HER2 status and guide appropriate treatment decisions.

Keywords:

Immunohistochemistry (IHC), Fluorescence In-Situ Hybridization (FISH), HER2/neu gene & Breast Cancer.

INTRODUCTION

Breast cancer is a pervasive global health issue, ranking among the most commonly diagnosed malignancies worldwide. When examining global cancer data, it becomes evident that both Europe and America face significantly higher incidence and mortality rates relatedto breast cancer. Within China, breast cancer incidence has surged to 20 cases per 100,000 individuals, while in Pakistan it has reached to a percentage of 69.1 per 100,000 indicating an upward trend.[1,2]

HER2-positive breast cancer constitutes a substantial subset of breast cancer cases, representing approximately 20-25% of diagnoses. The HER2 gene, belonging to the epidermal growth factor receptor family, encodes a receptor tyrosine kinase that catalyzes cell division and increased cell motility.[2] The genetic alteration is linked to more aggressive tumor behavior and a less favorable prognosis, underscoring its significance as a therapeutic target with trastuzumab[2,3]

To assess HER2 status accurately, various methodologies are available, including polymerase chain reaction (PCR), immunohistochemistry (IHC), fluorescence in situ hybridization (FISH), and chromogenic in situ hybridization (CISH).[1,6]

IHC and FISH are the predominant methods employed for evaluating HER2 status. IHC gauges the expression of the HER2 protein on the cell surface, while FISH identifies HER2 gene amplification at the DNA level. IHC with FISH is used in many laboratories for equivocal

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cases of HER 2 according to ASCO/CAP recommendations[4,5]. This research endeavors to conduct a prospective study within our local setting, Al Hada, Armed Forces Hospital in Taif region for two years, encompassing 100 cases of breast carcinoma of all types that are equivocal with HER2.

The main rationale of this study is to evaluate the accuracy of diagnostic methods in HER2 equivocal breast cancer cases. Determining HER2 status is essential for guiding treatment, but differences between Immunohistochemistry (I HC) and Fluorescence In-Situ Hybridization (FISH) results create challenges in these borderline cases. This study helps to compare these methods to improve reliability in diagnosing HER2 status and ensure patients receive the most appropriate therapies.

PATIENTS AND METHODS

This is a cross sectional study conducted at Al Hada Armed Forces Hospital in the Taif region (2 years) from February 2022 to January 2024 after taking informed consent.

The study focused on breast cancer patients with HER2-equivocal (2+) amplification status as determined by initial diagnostic tests.A total of 100 patients with breast cancer were enrolled. The sample size was estimated using prevalence of breast cancer was 24%[7] at 7% margin of error and 95% confidence level using following formula:

$$n = \frac{z^2 - \frac{a}{2}\, p(1-p)}{d^2}$$

Comprehensive clinical data, including patient histories, diagnostic reports, and medical records, will be collected at the time of recruitment. Breast tissue specimen will be obtained through both CNB and excision. These samples will be subjected to immunohistochemistry (IHC) testing following standard protocols to determine HER2 protein expression levels (Figure 1). The study was conducted utilizing one of the FDA-approved assay kits, stained with HercepTest using DakoCytomation standard protocols. Immunostaining was categorized using the following scale using ASCO/CAP guidelines: 0 indicated an absence of staini ng; Negative (0/+) represented a faint and incomplete membranous pattern; Equivocal (2+) denoted a weak -moderate complete membranous pattern (>%10 tumor cells) or intense complete membranous staining in less than 30%, and Positive (3+) indicated a strong membranous pattern in more than 30% of tumor cell 2+ are denoted as equivocal cases.[8-10]

In parallel, fluorescence in situ hybridization (FISH) testing is performed prospectively on the same tissue samples to assess HER2 gene amplification at the DNA level. According to ASCO/CAP guidelines for FISH.[11]

Positive: HER2/CEP17 ratio ≥2.0 or HER2 copy number ≥6.0 signals/nucleus

Negative: HER2/CEP17 ratio <2.0 and HER2 copy number <4.0 signals/nucleus

Equivocal:

- Ratio ≥2.0, but copy number <4.0
- Ratio <2.0, with copy number 4.0–6.0

FISH testing was applied on IHC equivocal cases only and results were analyzed.

The inclusion criteria are those who have a recent diagnosis of invasive breast cancer (within the last three months) based on core biopsy or surgical pathology. Only patients classified as HER2-equivocal (IHC 2+) during preliminary testing, according to ASCO/CAP guidelines, will be eligible. Participants must provide written informed consent for study participation, including the collection of tissue samples and the use of clinical data for researchpurposes. The study is open to individuals of any gender aged 18 years or older.

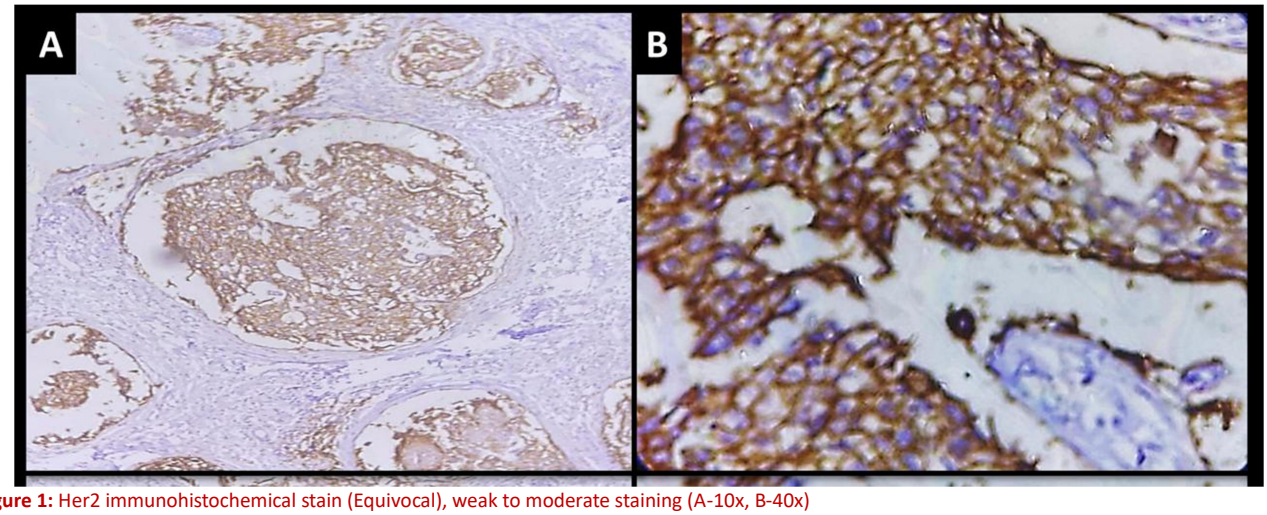

Figure 1: Her2 immunohistochemical stain (Equivocal), weak to moderate staining (A-10x, B-40x)

Exclusion criteria include patients with a confirmed HER2-positive (IHC 3+ or FISH-amplified) or HER2-negative (IHC 0/1+ or FISH non-amplified) status. Additionally, patients with insufficient or non-representative tumor tissue samples, those who have received neoadjuvant chemotherapy, targeted HER2 therapy, or endocrine therapy before enrollment, and those presenting with distant metastatic disease at diagnosis will be excluded. Patients with severe comorbid conditions or a life expectancy of less than six months, as well as individuals unable to provide informed consent due to cognitive impairment or other conditions, are also not eligible for the study.

Data was analyzed by SPSS version 20. The qualitative variables like age, grading of tumor, carcinoma was shown by frequency & percentages. The quantitative variables are expressed as median ± interquartile range. Association of different types of Breast carcinoma with FISH test results was estimated by chi square test. A p-value of <0.05 was taken as statistically significant.

RESULTS

Analysis of the 100 cases revealed that the patients' ages ranged from 22 to 80 years, with a median age of 62 years. Notably, 89% of the cases involved women over the age of 41(Table 1).

Among 100 breast cancer patients with HER2-equivocal (2+) IHC results, 78% did not show HER2 gene amplification on FISH, highlighting the need for FISH to confirm HER2 status in equivocal cases (Table 2). Clinico-pathological factors are compared with FISH results (Table 3, 4, 5).

DISCUSSION

Breast cancer represents a significant global health concern, with early and accurate determination of HER2 status being crucial for appropriate treatment decisions. The equivocal classification of HER2 status, often observed in a subset of breast cancer patients, poses a diagnostic challenge, and various methods, including Immunohistochemistry and Fluorescence In Situ Hybridization, are employed to resolve this ambiguity.[12-16] This discussion focuses on the comparative analysis of IHC and FISH for HER2 equivocal cases in breast cancer, drawing insights from recent research findings.

The current management approach for breast carcinoma patients relies on the tumor's pathology and the status of prognostic markers such as ER, PR, and HER-2/neu. Tumors with HER-2/neu amplification or overexpression are recognized for their aggressive nature, often presenting as higher grade tumors with a rapid proliferation rate and a tendency for lymph node involvement.[12,15]

Table1: Frequency of patients in different age groups

Age	Cases (n)	Percentage
21-40	11	11%
41-80	89	89%
Total	100	100%

Table 2: Concordance on HER-2 Status between IHC & FISH

Total cases (n)	IHC status	HER-2 Status by FISH			
		Amplified (n)	%	Non-amplified	%
100	Equivocal/ 2+	22	22%	78	78%

Table 3: comparison of FISH results with different age groups

Age	FISH Positive (n)	FISH Negative (n)
21-40	5	6
41-80	17	72
Total	22	78

Table 4: Comparison of FISH results with grades of breast carcinoma

Grading of carcinoma	FISH Positive	FISH Negative	Total
Ductal			
Well differentiated	2	10	12
Lobular differentiated	8	22	30
Poorly differentiated	11	36	47
Lobular			
Well differentiated	0	0	0
Lobular differentiated	0	1	1
Poorly differentiated	1	2	3
Others	0	7	7
Total	22	78	100

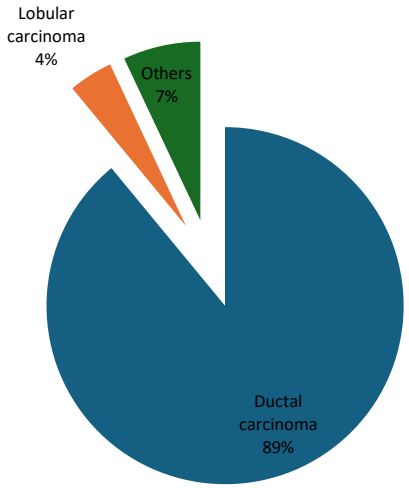

Figure 2: Frequency types of breast carcinoma

Out of the 100 patients in our study, 89% were diagnosed with Invasive Ductal Carcinoma, 4% with Lobular Carcinoma, and the remaining 7% with other types of carcinomas (Figure 2).

Among different pathological subgroups, the proportion of equivocal or 2+ tumors that may be found positive via FISH analysis should be considered.

Correlation studies investigating the connection between grade, stage, tumor size, nodal involvement, and hormone receptor status with HER-2/neu amplification status have validated this consistency.

Recent studies have emphasized the importance of assessing the concordance between IHC and FISH results in HER2 equivocal cases. Some studies have reported low concordance rates, For instance, a study by Dolan et al.[16] demonstrated substantial concordance between IHC and FISH in cases of HER2 equivocal amplification. These findings align with the study by Schalper et al.[17] and which also reported a level of agreement between IHC and FISH results (Figure 3).

The low concordance rate between immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH) for HER2-equivocal breast cancer cases raises significant implications for the accurate diagnosis and management of HER2-positive breast cancer. In HER2-equivocal cases, where IHC results (2+) fail to definitively categorize HER2 status, reliance solely on IHC may result in misclassification, either overestimating or underestimating HER2 amplification. The findings from this study demonstrate that a substantial proportion of IHC-equivocal cases fail to show HER2 gene amplification when analyzed using FISH, indicating the inherent limitations of IHC in borderline cases.[16]

One possible explanation for this low concordance is the subjective interpretation of IHC results. HER2 IHC testing relies on protein expression levels that may not correlate directly with gene amplification, particularly in equivocal cases. FISH, on the other hand, provides a more objective assessment of HER2 status by quantifying HER2 gene amplification at the DNA level. This discrepancy underscores the importance of confirmatory FISH testing to ensure accurate classification of HER2 status in equivocal cases.[16,17]

Furthermore, the observed discordance has clinical implications, as misclassification of HER2 status can affect treatment decisions and patient outcomes. HER2-positive breast cancer patients are candidates for HER2-targeted therapies, such as trastuzumab, which have been shown to improve survival. Incorrectly categorizing a HER2-equivocal patient as HER2-negative may deny them access to these life-saving treatments, while false-positive results may expose patients to unnecessary treatment-associated toxicities and costs.

Another factor contributing to the low concordance may be the inherent tumor heterogeneity observed in HER2-equivocal cases. Tumors with mixed HER2 expression can lead to inconsistent results across different diagnostic modalities. This emphasizes the need for rigorous and standardized testing protocols and highlights the importance of integrating molecular assays like FISH or more advanced techniques such as next-generation sequencing (NGS) to complement IHC results.[18]

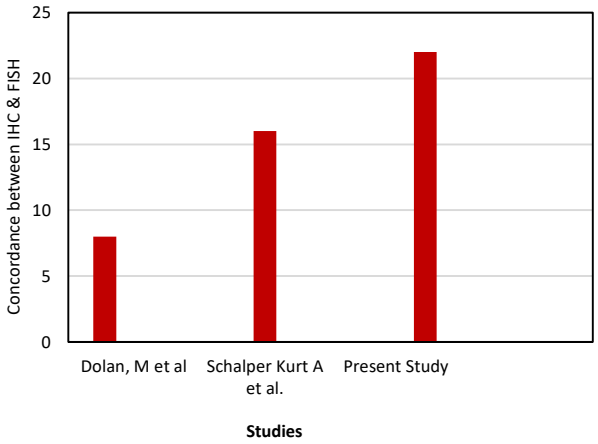

Figure 3: Concordance rates between IHC and FISH

The findings from this study advocate for the routine use of FISH or other confirmatory molecular diagnostic tools in HER2-equivocal cases. Incorporating reflex FISH testing into diagnostic algorithms for all HER2 IHC 2+ cases can significantly reduce diagnostic uncertainties and ensure patients receive appropriate treatment. Additionally, continuous efforts to refine IHC protocols, improve inter-observer agreement, and establish clear guidelines for HER2 testing will be essential to enhance diagnostic accuracy.[18,19]

Considering the cost-effectiveness and practicality of diagnostic methods is essential in healthcare settings. Recent research by Smith et al.20highlighted that IHC is more cost-effective and readily applicable in routine clinical practice, making it a preferred method for some institutions. However, FISH is still essential for confirming HER2 amplification in some cases.

IHC and FISH are important tools for HER2 status testing in breast cancer. However, discordant results can occur, so standardized protocols and quality control are essential. Additionally, integrated algorithms that combine data from both methods could improve accuracy.

Given recent research, institutions should carefully consider concordance, cost-effectiveness, and practicality when choosing between IHC and FISH for equivocal cases. Collaborative efforts are needed to establish standardized HER2 testing guidelines that account for the strengths and limitations of both methods.

It is essential to acknowledge the limitations of our study. The sample size, while representative, may not capture the full spectrum of breast cancer cases. Furthermore, our study did not investigate the impact of different laboratory techniques or interpretation criteria

on the results. Future research could explore these factors in greater detail.

CONCLUSION

The low concordance between Immunohistochemistry (IHC) and Fluorescence In-Situ Hybridization (FISH) in HER2 equivocal cases underscores the challenges in accurately determining HER2 status using IHC alone. This discordance suggests the value of using both IHC and FISH, along with additional molecular techniques, to enhance diagnostic accuracy and ensure appropriate treatment decisions. Further research is needed to improve diagnostic criteria and patient management for HER2-equivocal breast cancer.

Author Contributions

HA: Conception and design, analysis and interpretation of data, drafting the article, critical revision for important intellectual content, final approval.
MQ: Conception and design, analysis and interpretation of data.
RB: Analysis and interpretation of data, drafting the article.
SP: Acquisition of data, conception and design, analysis and interpretation.
N: Analysis and interpretation of data, proofreading.
R: Conception and design, analysis and interpretation of data.

REFERENCE

1. Sui W, Ou M, Chen J, Wan Y, Peng H, Qi M. Comparison of immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH) assessment for Her-2 status in breast cancer. World journal of surgical oncology. 2009 Dec;7:1-6.
2. Hanif M, Sabeen B, Maqbool A, Ahmed A, Nadeem F, Habib S. Breast cancer: Incidence (Thirteen year data analysis) and one year clinicopathological data of patients in a tertiary care cancer hospital. Int J Biol Biotechnol. 2015 Apr;12(3):373-9.
3. Murthy SS, Sandhya D G, Ahmed F, Goud K I, Dayal M, Suseela K. 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 Microbiol 2011;54:532-8
4. Bradley R, Braybrooke J, Gray R, Hills R, Liu Z, Peto R, Davies L. Trastuzumab for early-stage, HER2-positive breast cancer: a meta-analysis of 13 864 women in seven randomised trials. The Lancet Oncology. 2021 Aug 1;22(8):1139-50.
5. Yu D, Zhou C, Xu J, Gao Y, Zheng J, Li X, et al. Comparative accuracy of human epidermal growth factor receptor 2 (HER2) status determination in matched HER2 equivocal and non-equivocal breast cancer patients: a 2020 meta-analysis. Front Oncol. 2021;11:1071.
6. Lim TH, Lim AS, Tien SL, Tan PH. Impact of the updated 2018 American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) guidelines on human epidermal growth factor receptor 2 (HER2) gene testing in invasive breast cancers: a single center study. Ann. Diagn. Pathol.. 2022 Jun 1;58:151935.
7. Tufail M, Wu C. Exploring the burden of cancer in Pakistan: an analysis of 2019 data. J Epidemiol Glob Health. 2023;13(2):333-43.
8. Brackstone M, Baldassarre FG, Perera FE, Cil T, Chavez Mac Gregor M, Dayes IS. Management of the axilla in early-stage breast cancer: Ontario Health (Cancer Care Ontario) and ASCO guideline. J Clin Oncol. 2021 Sep 20;39(27):3056-82.
9. Denduluri N, Somerfield MR, Chavez-MacGregor M, Comander AH, Dayao Z, Eisen A, Freedman RA, et al. Selection of optimal adjuvant chemotherapy and targeted therapy for early breast cancer: ASCO guideline update. J. Clin. Oncol.. 2021 Feb 20;39(6):685-93.
10. Henry NL, Somerfield MR, Dayao Z, Elias A, Kalinsky K, McShane LM, et al. Biomarkers for systemic therapy in metastatic breast cancer: ASCO guideline update. J. Clin. Oncol.. 2022 Sep 20;40(27):3205-21.
11. Zare S, Rong J, Daehne S, Roma A, Hasteh F, Dell'Aquila M, et al. Implementation of the 2018 American Society of Clinical Oncology/College of American Pathologists Guidelines on HER2/neu Assessment by FISH in breast cancers: predicted impact in a single institutional cohort. Mod Pathol. 2019 Nov 1;32(11):1566-73.
12. Agersborg S, Mixon C, Nguyen T, Aithal S, Sudarsanam S, Blocker F, et al. Immunohistochemistry and alternative FISH testing in breast cancer with HER2 equivocal amplification. Breast Cancer Research and Treatment. 2018 Jul;170:321-8.
13. Nagarjun BR, Parikh B, Patel MN, Trivedi PJ, Patel DM. Indian data on HER2 fluorescence in situ hybridization in invasive breast cancer with immunohistochemically equivocal results as per 2018 ASCO/CAP guidelines. South Asian J Cancer. 2022 Aug 23;11(04):281-6.
14. Rosenberg CL. Polsomy 17 and Her-2 amplification: true, and unrelated. J Clin Oncol 2008; 26: 4856-8.
15. Lin L, Sirohi D, Coleman JF, Gulbahce HE. American society of clinical oncology/college of American pathologists 2018 focused update of breast cancer HER2 FISH testing guidelines results from a national reference laboratory. Am J Clinic Pathol. 2019 Sep 9;152(4):479-85.
16. Dolan M, Snover D. Comparison of immunohistochemical and fluorescence in situ hybridization assessment of HER-2 status in routine practice. American Journal of Clinical Pathology. 2005 May 1;123(5):766-70.
17. Schalper KA, Kumar S, Hui P, Rimm DL, Gershkovich P. A retrospective population-based comparison of HER2 immunohistochemistry and fluorescence in situ hybridization in breast carcinomas: impact of 2007 American Society of Clinical Oncology/College of American Pathologists criteria. Arch Pathol Lab Med. 2014 Feb 1;138(2):213-9.
18. Atallah N, Makhlouf S, Li XM, Zhang Y, Mongan NP, Rakha E. Prediction of response to anti-HER2 therapy using a multigene assay. Mod. Pathol. 2025 Jan 16:100713.
19. Aznab M, Izadi B, Amirian F, Khazaei S, Madani SH, Ramezani M. Comparison of immunohistochemical methods (IHC) and fluorescent in situ hybridization (FISH) in the Detection of HER 2/Neu gene in Kurdish patients with breast cancer in Western Iran. Int. J. Hematol. Oncol. Stem Cell Res.2022 Oct 10;16(4):217.
20. Smith I, Robertson J, Kilburn L, Wilcox M, Evans A, Holcombe C, et al. Long-term outcome and prognostic value of Ki67 after perioperative endocrine therapy in postmenopausal women with hormone-sensitive early breast cancer (POETIC): an open-label, multicentre, parallel-group, randomised, phase 3 trial. The Lancet Oncology. 2020 Nov 1;21(11):1443-54.