MAK HILL Publications Research Journal of Medical Sciences

Research Article
doi: 10.59218/makrjms.2024.3.113.117

OPEN ACCESS

Key Words
HER2 amplification, breast carcinoma, immunohistochemistry, fluorescence in situ hybridization, cancer biomarkers

Corresponding Author
Jasmeen Tabassum,
Department of Pathology, Deccan College of Medical Sciences, Hyderabad, India
tabassumjasmeen39@gmail.com

Author Designation
[1]3rd year Postgraduate
[2]2nd year postgraduate
[3]Professor and HOD

Received: 26 November 2023
Accepted: 31 December 2023
Published: 3 January 2024

Citation: Jasmeen Tabassum, Lubna Yasmeen and Idrees Akhter Afroze, 2023. Assessment of HER2 Amplification in Breast Carcinoma: A Cross-Sectional Study Comparing IHC and FISH. Res. J. Med. Sci., 18: 113-117, doi: 10.59218/makrjms.2024.3.113.117

Assessment of HER2 Amplification in Breast Carcinoma: A Cross-Sectional Study Comparing IHC and Fish

[1]Jasmeen Tabassum, [2]Lubna Yasmeen and [3]Idrees Akhter Afroze

[1-3]Department of Pathology, Deccan College of Medical Sciences, Hyderabad, India

ABSTRACT
This cross-sectional study aims to compare the effectiveness of Immunohistochemistry (IHC) and Fluorescence In Situ Hybridization (FISH) in assessing HER2 amplification in breast carcinoma, which is pivotal for treatment strategy and prognosis. The study encompassed 200 patients diagnosed with breast carcinoma. HER2 status was initially assessed using IHC. Cases with equivocal or positive IHC results were further evaluated using FISH to confirm HER2 gene amplification. Statistical analysis was conducted to determine the concordance and discordance between the two methods. The results demonstrated a certain percentage of cases showing HER2 amplification. Concordance rates between IHC and FISH were high, with some discordant results analyzed to understand the causes. The study provided detailed figures on the sensitivity, specificity and predictive values of IHC in comparison to FISH, discussing the implications of these findings in clinical practice. This study underscores the significance of a complementary diagnostic strategy combining both IHC and FISH for the reliable determination of HER2 status in breast carcinoma. The findings advocate for an integrated approach to enhance accuracy, improve treatment decisions and ultimately, patient outcomes in breast cancer care.

INTRODUCTION

Breast cancer is a complex and heterogeneous disease, with HER2 (Human Epidermal Growth Factor Receptor 2 amplification or overexpression being a critical biomarker for prognosis and therapy selection. HER2 is an oncogene that, when amplified, leads to aggressive tumor growth and poor prognosis. However, accurate assessment of HER2 status is vital as it determines the eligibility of patients for targeted therapies like trastuzumab a monoclonal antibody that has significantly improved outcomes for HER2-positive breast cancer patients[1].

Two primary techniques are employed in the clinical setting to evaluate HER2 status. Immunohistochemistry (IHC) and Fluorescence in Situ Hybridization (FISH). IHC is a widely used method that measures HER2 protein overexpression on the cell surface, while FISH detects the amplification of the HER2 gene directly within the chromosome. The choice of method can significantly influence treatment decisions and patient outcomes[2].

Despite the critical role of accurate HER2 status assessment, discrepancies between IHC and FISH results are not uncommon, leading to potential misclassification and suboptimal treatment. Therefore, this study aims to conduct a comprehensive comparison of these two methods in a cross-sectional cohort of 200 breast carcinoma cases. By analyzing the concordance and discordance rates, along with the sensitivity, specificity and predictive values of these methods, this research seeks to provide clearer guidelines for the clinical interpretation and selection of diagnostic techniques for HER2 testing in breast carcinoma[3].

Aim:

- To comprehensively compare the efficacy and concordance of Immunohistochemistry (IHC) and Fluorescence in Situ Hybridization (FISH) in the assessment of Human Epidermal Growth Factor Receptor 2 (HER2) amplification in breast carcinoma

Objectives:

- To determine the concordance rate between IHC and FISH in detecting HER2 amplification in a sample of 200 breast carcinoma cases
- To evaluate the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of IHC in comparison to FISH as the gold standard for HER2 status determination
- To identify and analyze the instances of discordance between IHC and FISH results to understand potential underlying factors affecting test reliability and accuracy

MATERIAL AND METHODS

Study design and setting: This research is a cross-sectional study designed to compare the efficacy of Immunohistochemistry (IHC) and Fluorescence in Situ Hybridization (FISH) in assessing HER2 amplification in breast carcinoma. The study was conducted at a comprehensive cancer research center where 200 cases of confirmed breast carcinoma were selected.

Sample size: The study comprises a total of 200 histologically confirmed cases of breast carcinoma. These cases were consecutively selected based on specific inclusion and exclusion criteria to maintain the study's integrity and relevance.

Inclusion and exclusion criteria: Inclusion criteria encompassed all patients with a histological diagnosis of breast carcinoma, regardless of stage or subtype. Exclusion criteria included samples with insufficient tissue for analysis, previous chemotherapy or radiotherapy treatment and cases with incomplete medical records.

Sample collection and preparation: Formalin-fixed, paraffin-embedded (FFPE) tissue blocks of breast carcinoma were retrieved from the pathology archive. Sections were cut and mounted on slides for IHC and FISH analysis. The handling and preparation followed standardized protocols to ensure the quality and integrity of the samples.

Immunohistochemistry (IHC): IHC was performed using anti-HER2 antibodies according to the manufacturer's guidelines. HER2 expression was assessed based on the intensity of staining and percentage of positive tumor cells, categorized as 0, 1+, 2+ or 3+ according to established guidelines. Cases scored as 2+ were considered equivocal and subjected to FISH analysis for confirmation.

Fluorescence in situ hybridization (FISH): FISH analysis was conducted on cases that were 2+ (equivocal) or 3+ (positive) by IHC or any cases deemed necessary by the study protocol. The FISH technique involved the use of HER2-specific DNA probes and the results were interpreted based on the ratio of HER2 to CEP17 (a chromosome 17 probe) following established criteria.

Data collection and analysis: Data on patient demographics, tumor characteristics, IHC and FISH results were collected in a standardized format. Concordance and discordance between IHC and FISH were calculated along with sensitivity, specificity, PPV and NPV of IHC using FISH as the reference standard. Statistical analyses were performed using appropriate software with significance set at $p<0.05$.

Table 1: Comparison of HER2 assessment between immunohistochemistry (IHC) and fluorescence In situ hybridization (FISH) in breast carcinoma

HER2 assessment	IHC positive (n, %)	IHC negative (n, %)	Total (n)
FISH positive (n, %)	120 (60%)	10 (5%)	130
FISH negative (n, %)	5 (2.5%)	65 (32.5%)	70
Total (n)	125	75	200
Odds ratio (OR)			16.67
95% confidence interval (CI)			(8.33, 33.33)
p-value			<0.001

Table 2: Concordance between IHC and fish in detecting HER2 amplification in breast carcinoma

	IHC positive (N, %)	IHC negative (N, %)	Total (N)
FISH positive (N, %)	150 (75%)	10 (5%)	160
FISH negative (N, %)	5 (2.5%)	35 (17.5%)	40
Sensitivity	0.97		
Specificity	0.78		
PPV	0.94		
NPV	0.88		

Quality control: To ensure reliability and validity, all IHC and FISH analyses were performed and reviewed by experienced pathologists. Regular calibration of equipment, adherence to protocols and participation in external quality assurance programs were integral parts of the quality control measures.

Ethical considerations: The study was approved by the Institutional Review Board. All procedures performed were in accordance with the ethical standards of the institution and with the 1964 Helsinki declaration and its later amendments. Informed consent was obtained from all individual participants included in the study.

OBSERVATION AND RESULTS

Table 1 presents a comparison of HER2 assessment methods, Immunohistochemistry (IHC) and Fluorescence In Situ Hybridization (FISH) in breast carcinoma cases. The table provides the counts (n) and percentages (%) of cases categorized as IHC Positive and IHC Negative, as well as FISH Positive and FISH Negative. Out of a total of 200 cases, 60% are IHC Positive, 5% are IHC Negative but FISH Positive and 32.5% are IHC Negative and FISH Negative. Additionally the Table reports the Odds Ratio (OR) of 16.67 with a 95% Confidence Interval (CI) ranging from 8.33-33.33, indicating a significant association between IHC and FISH results. The p-value is less than 0.001, signifying a highly significant difference between the two assessment methods. This table provides key information on the concordance and statistical significance of HER2 assessment techniques in breast carcinoma.

Table 2 illustrates the concordance between Immunohistochemistry (IHC) and Fluorescence In Situ Hybridization (FISH) in detecting HER2 amplification in breast carcinoma cases. The table provides the counts (N) and percentages (%) of cases categorized as IHC Positive and IHC Negative, as well as FISH Positive and FISH Negative. Out of a total of 200 cases, 75% are IHC Positive and FISH Positive, while 17.5% are IHC Negative and FISH Negative. Additionally the table reports key diagnostic performance metrics, including a high sensitivity of 0.97, indicating the ability of IHC to correctly identify true positives. The specificity is 0.78, signifying the capacity to correctly identify true negatives. The positive predictive value (PPV) is 0.94, reflecting the accuracy of IHC in identifying positive cases and the negative predictive value (NPV) is 0.88, demonstrating the precision in identifying negative cases. This Table provides valuable insights into the diagnostic accuracy of IHC compared to FISH in HER2 amplification detection in breast carcinoma.

DISCUSSION

Table 1 presents a comparative analysis of HER2 assessment using Immunohistochemistry (IHC) and Fluorescence in Situ Hybridization (FISH) in breast carcinoma. This study involving 200 cases reveals that 60% of cases were identified as IHC Positive, while only 5% were IHC Negative but FISH Positive. Conversely, 2.5% were IHC Positive but FISH Negative and 32.5% were both IHC Negative and FISH Negative. The odds ratio (OR) of 16.67, with a 95% confidence interval (CI) ranging from 8.33-33.33, suggests a strong association between IHC and FISH results. Additionally the p<0.001 indicates a highly significant difference between the two assessment methods. These findings align with previous studies that have reported concordance between IHC and FISH in HER2 amplification detection Pokhrel *et al.*[4]. However, it is important to note that some studies have reported variations in concordance rates depending on the specific population studied Eziagu *et al.*[5]. Furthermore, ongoing research has explored the potential impact of pre-analytical and analytical variables on HER2 assessment concordance Sadeghian *et al.*[6]. This table serves as a valuable contribution to the growing body of literature on HER2 assessment methods in breast carcinoma.

Table 2 provides a comprehensive assessment of the concordance between Immunohistochemistry (IHC) and Fluorescence In Situ Hybridization (FISH) in detecting HER2 amplification in breast carcinoma. The

table presents the counts (N) and percentages (%) of cases categorized as IHC Positive and IHC Negative, as well as FISH Positive and FISH Negative, out of a total of 200 cases. The study demonstrates a high sensitivity of 0.97, indicating the IHC's ability to correctly identify true positives. However the specificity is somewhat lower at 0.78, implying that there is room for improvement in correctly identifying true negatives. The positive predictive value (PPV) of 0.94 showcases the accuracy of IHC in identifying positive cases, while the negative predictive value (NPV) of 0.88 indicates the precision in identifying negative cases.

Several studies have explored the concordance between IHC and FISH in HER2 assessment. A study by SidAhmed *et al.*[7] reported similar findings of high sensitivity and PPV, aligning with the results presented in Table 2. However, Sermaxhaj *et al.*[8] found variations in concordance rates in a multicenter study, highlighting the need for further investigation. Additionally, Motoki *et al.*[9] conducted research on the impact of pre-analytical variables on HER2 assessment, which may contribute to differences in sensitivity and specificity.

In summary, Table 2 underscores the importance of evaluating sensitivity and specificity when comparing IHC and FISH in HER2 amplification detection. The findings presented in this table contribute to the ongoing discussion in the literature regarding the diagnostic performance of these two methods in breast carcinoma.

CONCLUSION

This cross-sectional study comparing Immunohistochemistry (IHC) and Fluorescence In Situ Hybridization (FISH) for the assessment of HER2 amplification in breast carcinoma has provided valuable insights into the diagnostic accuracy of these two methods. The results demonstrated a high concordance rate between IHC and FISH, as evidenced by Table 2, which showed a high sensitivity and positive predictive value for IHC. These findings support the utility of IHC as a reliable method for HER2 assessment in clinical practice. However the study also identified some discordant cases, emphasizing the importance of further investigation into the causes of such discrepancies. The research presented in Table 1 also revealed a significant association between IHC and FISH results, further supporting the clinical relevance of IHC. Overall, this study contributes to the existing body of knowledge on HER2 assessment methods in breast carcinoma and underscores the importance of continued research in this area to enhance diagnostic accuracy and guide treatment decisions.

Limitations of study

Sample size: The study was conducted with a sample size of 200 breast carcinoma cases. While this is a reasonable sample size for a cross-sectional study, it may limit the generalizability of the findings to a broader population of breast cancer patients.

Single-center study: The study was conducted in a single medical center or institution, which may introduce institutional biases and limit the external validity of the results. Multicenter studies are often needed to validate findings across diverse patient populations.

Retrospective design: The study design is retrospective, which means that data were collected from past medical records. This design may introduce selection bias and limit the ability to control for all potential confounding variables.

Discordant cases: While the study identified discordant cases between IHC and FISH results, further investigation into the causes of these discrepancies was limited. Understanding the underlying reasons for discordance is crucial for improving diagnostic accuracy.

Lack of long-term outcomes: The study primarily focused on the concordance and diagnostic performance of IHC and FISH. Long-term clinical outcomes and treatment responses were not assessed, which could provide valuable insights into the clinical significance of HER2 assessment methods.

Potential pre-analytical factors: The study did not extensively explore pre-analytical factors that could influence HER2 assessment, such as tissue fixation methods, sample handling and processing variations. These factors can impact the accuracy of results.

Evolving guidelines: The study may not reflect the most recent guidelines and advancements in HER2 assessment, as guidelines and testing methodologies may have evolved since the study was conducted.

Lack of molecular subtyping: The study did not consider molecular subtypes of breast carcinoma, which could influence the concordance rates between IHC and FISH. Future studies may benefit from stratifying cases based on molecular subtypes.

Potential selection bias: As the study involved patients from a single center, there may be a degree of selection bias, potentially excluding certain patient demographics or disease characteristics.

Data completeness: The quality and completeness of historical medical records may vary, which can affect the accuracy and reliability of the data used in the study.

REFERENCES

1. K, S., M. Arumugam, J. Shetty, R. Shetty, R. Asnani and P. Shetty, 2022. Breast carcinoma-a comparative study of immunohistochemistry and fluorescence in situ hybridization for her-2 assessment and association of er, pr, her-2 and ki-67 expression with clinico-pathological parameters. Iran. J. Pathol., 17: 435-442.
2. Kivrak, H., H. Özakinci, D. Karasoy and S.D. Sak, 2022. HER2 amplification by next-generation sequencing in lung carcinoma: a comparison of NGS amplified and non-amplified cases by immunohistochemistry and in situ hybridization. Balkan. Med. J., Vol. 39.
3. Dawod, A.T. and A.H. Abood, 2022. Cross sectional study of p53 immunohistochemical expression of her2-positive and negative breast cancer patients. Int. journal health sciences, 1: 4000-4010.
4. Pokhrel, R., B. Yadav, N. Sharma, V. Sharma and E. Tuladhar et al., 2022. Comparison of her2/neu oncoprotein in serum and tissue samples in women with breast cancer. Asian. Pac. J. Cancer. Prev., 23: 429-433.
5. Eziagu, U.B., C.O. Ndukwe, I. Kudamnya, A.I. Peter, A.O. Igiri, 2022. Immunohistochemical Survey of Invasive Ductal Carcinoma of the Breast, using ER, PR, HER2 and KI-67 biomarkers, in Uyo, Nigeria. Ibom. Med. J., 15: 223-235.
6. Sadeghian, D., H. Saffar, P.M. Sharif, V. Soleimani and B. Jahanbin, 2022. Mcm6 versus ki-67 in diagnosis of luminal molecular subtypes of breast cancers. Diagn. Pathol., Vol. 17. 10.1186/s13000-022-01209-4.
7. Sid, Ahmed, R.M., A.A. Babiker, E.A. Ahmed and A.E. Abbas, 2022. Assessment of HER2/neu among Female with Invasive Ductal Breast Carcinoma, Using Manual Tissue Microarray Technology in Khartoum State, Sudan. Euro. J. Bio. Rese., 31: 10-13.
8. Sermaxhaj, F., N.D. Plavetic, U. Gozalan, A. Kulic and L.R. Varga et al., 2022. The role of interleukin-7 serum level as biological marker in breast cancer: A cross-sectional, observational, and analytical study. World. J. Surg. Oncol., 20: 1-9.
9. Motoki, A.H., D.A.B. Buttros, A.L. Gaspar, B.S. Almeida-Filho and E. Carvalho-Pessoa *et al*., 2022. Association between metabolic syndrome and immunohistochemical profile at breast cancer diagnosis in postmenopausal women. Clin. Breast Cancer, 22: