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HER2/neu estimation by ISH (FISH and CISH) technique in equivocal cases (+2) by IHC in breast cancer

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Abstract
Background: The HER-2/neu oncogene, a member of the epidermal growth factor receptor family, plays a significant role in the development of human breast cancer due to its common amplification. Its detection is paramount for determining the suitability of patients for trastuzumab (Herceptin) therapy and informing treatment decisions involving anthracycline-based and hormonal regimens. The aim of the study is to HER2/neu estimation by ISH (FISH and CISH) technique in equivocal cases (+2) by IHC in breast cancer.
Method: Cross-sectional analysis of 52 female breast cancer patients under FISH and 150 under CISH. CISH from Department of pathology/medical city teaching complex/oncology teaching hospital and FISH from AL SHARIQA specialized lab/diagnostic centre from January 2019 to May 2023. Both groups of females were asked their age (years) and FISH or CISH results (positive or negative).
Results: Breast cancer patients average 53 ± 11 years. Table 1 shows 38.5% of females aged 50–59 and 30.8% aged 40–49. Only 21.2% of women had HER 2 Neu-positive FISH. 78.8% had HER 2 Neu-negative FISH. FISH for HER 2 Neu is 100% positive in 30-39-year-old women. 30% of women aged 50–59 had HER 2 Neu-positive FISH. 6.3% and 7.7% of women aged 40–49 and ≥ 60 had positive FISH for HER 2 Neu. Breast cancer patients average 50.9 ± 12.2. Table 3 shows 30.7% of women aged 40–49 and 28% aged 50–59. Only 27.3% of women have HER 2 Neu-positive CISH. 72.7% have HER 2 Neu-negative CISH. Age does not affect HER 2 Neu CISH.
Conclusion: FISH and CISH showed various age-HER-2/neu oncogene amplification relationships. FISH showed an age-group-HER-2/neu amplification connection, whereas CISH did not. These results emphasise the relevance of the detection technique in interpreting HER-2/neu status in breast cancer and recommend additional study to improve patient classification and therapy decision.

Keywords: HER2/neu, estimation, ISH, FISH, CISH, technique, IHC, breast cancer

Introduction
The HER-2/neu oncogene, a member of the epidermal growth factor receptor family, plays a significant role in the development of human breast cancer due to its common amplification. Its detection is paramount for determining the suitability of patients for trastuzumab (Herceptin) therapy and informing treatment decisions involving anthracycline-based and hormonal regimens. Moreover, accurate HER-2/neu status assessment can provide crucial prognostic information [1]. However, misleading or inconclusive results from HER-2/neu tests could lead to inappropriate treatment plans [2], emphasizing the necessity for a reliable assay for the determination of HER-2/neu oncogene status. Historically, molecular methods such as the Southern, Northern, and Western blot techniques have been employed for HER-2/neu detection. Yet, these methods proved impractical for surgical pathology due to their laborious nature [3]. Consequently, immunohistochemical methods, which preserve morphologic features and can be conducted on archival specimens, became widely adopted. Cases with strong positive (3 +) scoring warrant inclusion of trastuzumab in treatment regimens, while completely or essentially negative (0 and 1 +) scoring are contraindications. Equivocal (2 +) IHC indicates that the cancer should undergo additional testing with FISH to determine trastuzumab eligibility. Despite the broad utility, these methods had shortcomings. Variation in results was common because of the different commercially available antibodies used [4]. For instance, in one retrospective study involving 187 breast cancers with documented HER-2/neu amplification, sensitivities ranged from 6% to 82%, depending on the antibody used [4].

The introduction of the FDA-approved HercepTest (DAKO, Carpinteria, CA) promised standardization, but the assay had many limitations [4, 5]. Issues ranged from unreliability in negative results due to a lack of positive internal controls for checking fixation, processing, or assay problems, to uncertainties in positive results due to potential scoring misinterpretations or technical factors [6-8]. Even the presence of interobserver variability in scoring led to significant differences in HER-2/neu reporting [5]. Despite being the only commercially available test kit with FDA approval, the HercepTest was not shown to be superior to other methods, and was found to be more expensive [5]. Fluorescence in situ hybridization (FISH), with its high sensitivity (96.5%) and specificity (100%), is considered the "gold standard" for detecting HER-2/neu amplification [6]. Despite its merits, the method necessitates a fluorescence microscope and specialized training for interpretation, making it costly and technically demanding (figure1). Furthermore, fluorescence fades quickly, making it an impermanent record. Chromogenic in situ hybridization (CISH), which offers a more cost-effective and permanent solution than FISH, appears to be a promising alternative. Like FISH, CISH uses similar tissue preparation and probe hybridization procedures, but it employs a peroxidase reaction for probe detection that can be seen using light microscopy [5] (figure2). This eliminates the need for fluorescence and allows for easy visualization of tissue alongside the amplification product. Nevertheless, as a relatively new technique, the correlation between CISH and FISH results has yet to be fully established in clinical settings [5]. The aim of the study is to HER2/neu estimation by ISH (FISH and CISH) technique in equivocal cases (+2) by IHC in breast cancer.

Method

Cross sectional study of 2 groups of patients, group (1); 52 female's patients with breast Ca. under FISH technique while group (2); 150 female's patients have breast Ca. under CISH technique. The data CISH collected from Department of pathology / medical city teaching complex / oncology teaching hospital and FISH collected from AL SHARIQA specialized lab/AL SHARIQA diagnostic center from period January 2019 to May 2023. All females in both groups asked about their age (years), and what the outcome of FISH technique or CISH technique either positive or negative. CISH done by ventana bench mark XT, FISH done by dako omnis hybridization. SPSS 22 was used for statistical analysis, and frequency and percentage were utilized for categorical data, while mean, median, and standard deviation were used for continuous data. Chi-square is used to analyses the connection between categorical data; a P-value of less than or equal to 0.05 is considered significant.

Results

Fish

Patients with breast cancer the mean age of patients 53 ± 11 years old. As shown in table 1; 38.5% of females at age group 50-59 years and 30.8% of them at age group 40-49 years old. Just only 21.2% of females have positive FISH for HER 2 Neu. And 78.8% have negative FISH for HER 2 Neu.

Table 1: Distribution of patients according to the variables of study

Variables		Frequency	Percentage
Age groups (years)	30-39	3	5.8
	40-49	16	30.8
	50-59	20	38.5
	≥60	13	25.0
Fish for HER 2 Neu	Negative	41	78.8
	Positive	11	21.2

As shown in table 2; there is significant association between FISH for HER 2 Neu and age group, 100% of females at age group 30-39 years are positive FISH for HER 2 Neu. While 30% of females at age group 50-59 years are positive FISH for HER 2 Neu. While just 6.3% and 7.7% of females at age group 40-49 years and ≥ 60 years have positive FISH for HER 2 Neu.

Table 2: Association between FISH for HER 2 Neu and age group

Variables			FISH for HER 2 Neu	Total	P-value
		Negative	Positive		
Age groups (years)	30-39	0	3	3	
		0.0%	100.0%	100.0%	
	40-49	15	1	16	
		93.8%	6.3%	100.0%	0.001
	50-59	14	6	20	
		70.0%	30.0%	100.0%	
	≥60	12	1	13	
		92.3%	7.7%	100.0%	

P-value ≤ 0.05 (significant).

CISH

Patients with breast cancer the mean age of patients 50.9 ± 12.2 years old. As shown in table 3; 30.7% of females at age group 40-49 years and 28% of them at age group 50-59 years old. Just only 27.3% of females have positive CISH for HER 2 Neu. And 72.7% have negative CISH for HER 2 Neu.

Table 3: Distribution of patients according to the variables of study

Variables		Frequency	Percentage
Age groups (years)	20-29	2	1.3
	30-39	24	16.0

	40-49	46	30.7
	50-59	42	28.0
	≥60	36	24.0
CISH for HER 2 Neu	Negative	109	72.7
	Positive	41	27.3

As shown in table 4; there is no significant association between CISH for HER 2 Neu and age group.

Table 4: Association between CISH for HER 2 Neu and age group

Variables			CISH for HER 2 Neu	Total	P-value
		Negative	Positive		
Age groups (years)	20-29	2	0	2	
		100.0%	0.0%	100.0%	
	30-39	17	7	24	
		70.8%	29.2%	100.0%	0.38
	40-49	30	16	46	
		65.2%	34.8%	100.0%	
	50-59	30	12	42	
		71.4%	28.6%	100.0%	
	≥60	30	6	36	
		83.3%	16.7%	100.0%	

P-value ≤ 0.05 (significant).

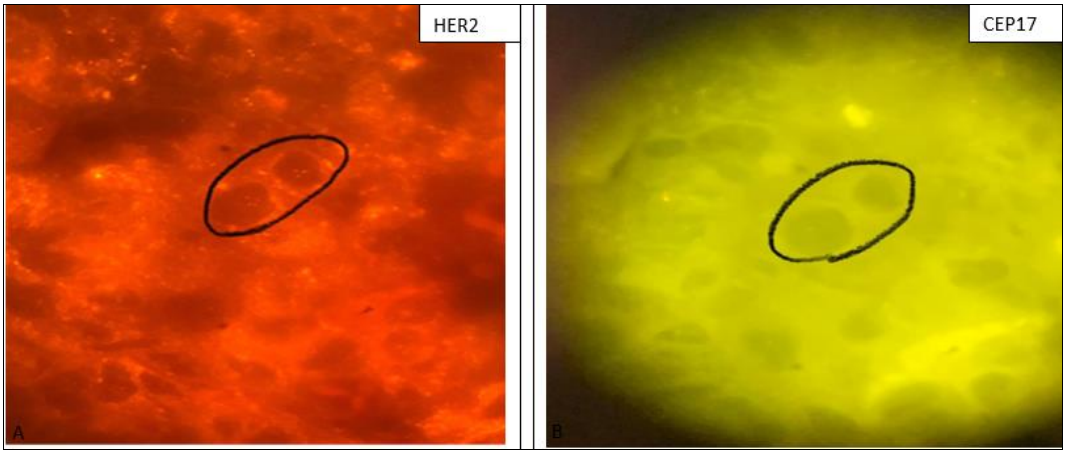

Fig 1: Fish: A. HER2 gene Amplified. Show numerous copies of the gene. B. HER2 Not amplified. Show CEP17 signals only

Fish: Fluorescence in situ hybridization, CEP17: Chromosome 17, HER2: Human epidermal growth receptor2

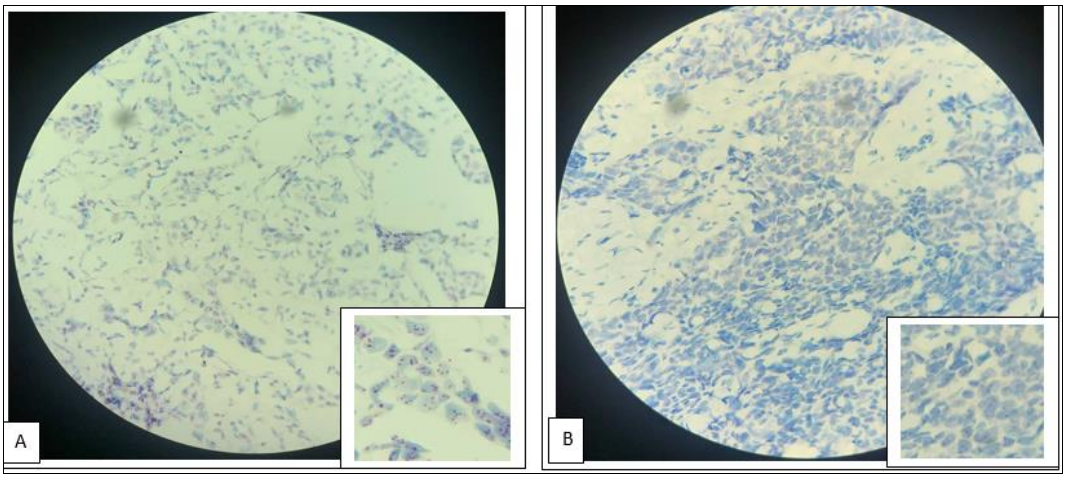

Fig 2: CISH: A. HER2 Amplified. This dual-CISH shows an increased number of HER2 genes (red probes) and a normal number of CEP17 signals (blue probes). B. HER2 Not Amplified.

Cish: Chromogenic in Situ Hybridization, CEP17: Chromosome 17, HER2: Human epidermal growth receptor 2

Discussion

In the study focusing on breast cancer patients, the demographic and clinical data reveal some insightful patterns. The mean age of the patients is 53 ± 11 years, highlighting that the condition predominantly affects middle-aged to older women. The distribution of age within

the study population illustrates that the age group of 50-59 years includes 38.5% of the females, followed by 30.8% in the 40-49 years' age bracket. This observation might suggest a higher vulnerability or likelihood of developing breast cancer during these age periods [9]. Various studies have linked the risk of breast cancer to age, with the likelihood increasing as women approach and pass through menopause, mainly due to hormonal changes and other genetic and lifestyle factors [10,11]. A key finding from this study is the HER-2/neu oncogene status among the patients. Only 21.2% of females were found to have positive FISH for HER-2/neu, while a significant majority, 78.8%, tested negative. This pattern reflects the broader statistics observed in breast cancer cases, where approximately 15-20% of all breast cancers are found to be HER-2/neu positive [12]. HER-2/neu-positive breast cancers are known to be more aggressive and are associated with a higher recurrence rate and poorer prognosis compared to HER-2/neu-negative cases [13]. The use of FISH in determining HER-2/neu status has been established as a highly sensitive and specific method [14]. Therefore, these results not only provide a snapshot of the HER-2/neu status in the study population but also lend support to the existing literature regarding the prevalence of HER-2/neu amplification among breast cancer patients**.** In the current study, there appears to be a notable association between age groups and the presence of HER-2/neu oncogene amplification as determined by FISH. Strikingly, all the females in the 30-39 age group tested positive for HER-2/neu. This percentage gradually decreased in older age groups, with 30% of females aged 50-59 years being HER-2/neu positive, and only a fraction of females in the 40-49 years and ≥ 60 years' age groups (6.3% and 7.7% respectively) showing HER-2/neu amplification. These findings resonate with those of several prior studies. For instance, a study by Lateef F *et al*. [15] demonstrated that younger women were more likely to be diagnosed with HER-2/neu-positive breast cancers. This could be attributed to the observation that aggressive breast cancer subtypes, which often exhibit HER-2/neu overexpression, are more common in younger women Valentin MD *et al*. [16]. Additionally, Yuan P *et al.* [17] suggest that the higher incidence of HER-2/neu overexpression in younger patients may be related to underlying genetic and hormonal differences that alter with age. Contrarily, a lower incidence of HER-2/neu positivity in older patients, as observed in our study, has also been highlighted in past literature. Al Tamimi *et al*. [18] noted a declining rate of HER-2/neu amplification with increasing age, particularly in women aged 60 and over. This pattern could potentially be linked to differing tumor biology in older women, or to post-menopausal hormonal changes that influence breast cancer subtypes (Rosner *et al*). [19]. The 100% HER-2/neu positivity rate seen in the 30-39 years' age group in our study is a compelling observation, further supporting the suggested correlation between younger age and increased HER-2/neu amplification. This phenomenon is mirrored in a study by Slamon *et al*. [20], which reported higher rates of HER-2/neu oncogene amplification among premenopausal women. Nonetheless, the complexity and multifactorial nature of this relationship necessitate further exploration. It is critical to continue investigating these age-related patterns in HER-2/neu amplification, as they hold potential implications for patient care and treatment strategies. Our study reveals that the mean age of breast cancer patients is 50.9 ± 12.2 years. In terms of age distribution, 30.7% of the females fall into the age group of 40-49 years, while 28% are in the 50-59 years group. Upon assessing HER-2/neu oncogene amplification via chromogenic in situ hybridization (CISH), only 27.3% of the females were found positive, with the majority (72.7%) testing negative. Interestingly, unlike the findings related to FISH, there was no discernible association between CISH results for HER-2/neu and age group. These results are consistent with findings from previous studies. For example, Gong *et al*. [21] reported no significant correlation between age and HER-2/neu overexpression when using CISH, further establishing the validity of our observations. Moreover, the reported prevalence of HER-2/neu positivity aligns with the literature. In a study conducted by Wolff *et al*. [22], the rate of HER-2/neu positive breast cancers, as determined by CISH, ranged from 15% to 20%. This is slightly lower than our finding (27.3%), but within a comparable range. One potential reason for the observed lack of correlation between age and HER-2/neu positivity via CISH could be the differential sensitivity and specificity of CISH compared to FISH. In contrast to FISH, CISH allows visualization of tissue morphology along with gene amplification Tse *et al*. [23]. This could result in a more precise determination of HER-2/neu status, which may not be as impacted by age-associated factors.

Conclusion

In conclusion, our study highlighted the differential relationship between age and HER-2/neu oncogene amplification based on whether fluorescence in situ hybridization (FISH) or chromogenic in situ hybridization (CISH) was used. While a significant association was found between age group and HER-2/neu amplification using FISH, this association was absent with CISH. These findings emphasize the importance of the detection method in interpreting HER-2/neu status in breast cancer, and warrant further investigation to refine patient stratification and treatment selection.

Conflict of Interest

Not available

Financial Support

Not available

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