Evaluation of HER2/Neu gene

in equivocal cases assessed by IHC in invasive breast cancer

Evaluación del gen HER2 / Neu en casos equívocos evaluados por IHC en cáncer de mama invasivo

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Abstract

Introduction: "HER2 is a proto-oncogene" establish on "chromosome 17q" coding tyrosine kinase receptor situated on the external membrane of the breast epithelial cells. "HER2" acts on epidermal growing element to regulator numerous cellular tasks, like propagation and differentiation of cell, cell persistence, "apoptosis, angiogenesis, migration, and metastasis", the aim of study is showed the frequency of "Her-2/neu gene" extension in Ca. breast identified as "score 2+ by IHC" and to companion with (age, ER status and the pathological grade and stage). **Method:** This study was conducted over three years period from 2017-2019 and included 77 cases of invasive ductal carcinoma with equivocal Her 2 IHC results (2+). Specimen included 43 mastectomies and 34 breast core needle biopsies referred to Duhok central laboratory and Vajin private laboratory. ER status (negative versus positive) and ki67 were already done by immunohistochemistry technique for all the cases. Relation of Her2 gene status was studied in relation to different parameters including age, estrogen receptor (ER) status and ki67, for mastectomy cases, also tumor grade and pathological stage were available. **Results:** Cross sectional study of 77 patients with mean age (49 ± 11) years old, (26%) of patients with amplified Her2 status, (84%) of patients with positive estrogen receptor, (48%) of patients with grade II and (41.6%), with high Ki 67 (more than 20), (31.2%) of patients with T2 tumor size, (13%) of patients with N1, N2 lymph node include, finally (63.3%) of patients are below age 50 years. There is a significant association between Her2 status and grade of tumor. **Conclusion:** FISH is best method for evaluation of "Her-2/neu gene status" particularly for confusing patients evaluated by IHC that are no attended by correct gene strengthening in the patients with breast Ca. Amplified Her2 position related with grade III of tumor.

Keywords: HER2/Neu gene, equivocal cases, IHC, invasive breast cancer.

Resumen

Introducción: "HER2 es un protooncogén" establecido en el "cromosoma 17q" que codifica el receptor de tirosina quinasa situado en la membrana externa de las células epiteliales mamarias. "HER2" actúa sobre el elemento de crecimiento epidérmico para regular numerosas tareas celulares, como propagación y diferenciación celular, persistencia celular, "apoptosis, angiogénesis, migración y metástasis", el objetivo del estudio es mostrar la frecuencia de "Her-2 / neu extensión del gen "en Ca. mama identificada como "puntuación 2+ por IHC" y con la que acompaña (edad, estado de ER y grado y estadio patológico). Método: Este estudio se realizó durante un período de tres años entre 2017 y 2019 e incluyó 77 casos de carcinoma ductal invasivo con resultados equívocos de Her 2 IHC (2+). La muestra incluyó 43 mastectomías y 34 biopsias con aguja gruesa de mama derivadas al laboratorio central de Duhok y al laboratorio privado de Vajin. El estado de ER (negativo versus positivo) y ki67 ya se realizaron mediante técnica de inmunohistoquímica para todos los casos. Se estudió la relación del estado del gen Her2 en relación con diferentes parámetros, incluida la edad, el estado del receptor de estrógeno (RE) y ki67, para los casos de mastectomía, también se dispuso del grado del tumor y el estadio patológico. Resultados: Estudio transversal de 77 pacientes con edad media (49 ± 11) años, (26%) de pacientes con estado de Her2 amplificado, (84%) de pacientes con receptor de estrógeno positivo, (48%) de pacientes con grado II y (41,6%), con alto Ki 67 (más de 20), (31,2%) de los pacientes con tamaño de tumor T2, (13%) de los pacientes con N1, N2 incluyen ganglios linfáticos, finalmente (63,3%) de los pacientes están por debajo 50 años de edad. Existe una asociación significativa entre el estado de Her2 y el grado del tumor. Conclusión: FISH es el mejor método para la evaluación del "estado del gen Her-2 / neu", en particular para pacientes confusos evaluados por IHC que no son atendidos por el fortalecimiento correcto del gen en pacientes con Ca de mama. Posición de Her2 amplificada relacionada con el grado III del tumor.

Palabras clave: gen HER2 / Neu, casos equívocos, IHC, cáncer de mama invasivo.

Introduction

"HER2 is a proto-oncogene" establish on "chromosome 17q" coding tyrosine kinase receptor situated on the external membrane of the breast epithelial cells. "HER2" acts on epidermal growing element to regulator numerous cellular tasks, like propagation and differentiation of cell, cell persistence, "apoptosis, angiogenesis, migration, and metastasis", "Her-2/neu gene amplification" happens in (20 to 30%) of breast Ca.[1,2], it associate with quick tumor development, increased danger of reappearance after operation, deprived reaction to conservative chemotherapy and poorer prognosis with decreased general survival rate[3,4]. Testing of Her-2/neu status is important subsequently the support of "trastuzumab" for the management of Ca. breast with Her-2 positive, "trastuzumab" link to the extracellular area of "Her-2/neu" and prevent propagation of malignant cells that over express Her-2/neu also encourages antibody apposite to malignant cells[5]. "Lapatinib, Her-1/Her-2" double receptor preventer introduced for treatment patients with stating gene amplification. Inappropriately, the management is costly and transports severe opposing effects like cardiotoxicity as well as Herceptin. Together drugs being effected only in cancers with gene magnification[1,6]. Correct valuation of Her-2/neu status in specific malignant cells is obligatory before presentation of particular treatment plans and has thus occupied on excessive in usual work up of Carcinoma of breast [7–8]. The two utmost broadly used skills for evaluation of "Her-2/neu are IHC and FISH", together apply on formalin fixed paraffin entrenched tissues. "FISH" have good sensitivity besides specificity in identifying "Her- 2" magnification but needs special equipment and knowledge to achieve and understand the consequences[1,9]. "IHC for Her-2/neu protein" is broadly used but untrue positive and untrue negative consequences are current. Relative studies state that great association between IHC investigation and FISH in patients with "IHC scores 0, 1+ and 3+". Disagreeing outcomes are significant in quantity of patients with vague 2+ immunostaining[7,10,11]. The aim of study is showed the frequency of "Her-2/neu gene" extension in breast cancer identified as "score 2+ by IHC" and to associate with (age, ER status and the pathological grade and stage).

Material and Method

This study was conducted over three years period from 2017-2019 and included a total of 77 cases of invasive ductal carcinoma with equivocal Her 2 IHC results (2+). Specimen included 43 mastectomies and 34 breast core needle biopsies referred to Duhok central laboratory and Vajin private laboratory. ER status (negative versus positive) and ki67 were already done by immunohistochemistry technique for all the cases. Relation of Her2 gene status was studied in relation to different parameters including age, estrogen receptor (ER) status and ki67, for mastectomy cases, also tumor grade and pathological stage were available. Molecular identification of Her2/Neu gene using Flourescent in situ hybridization was performed using Zyto*Light* SPEC ERBB2/CEN 17 Dual Color Probe that is designed to detect the mentioned gene in solid

malignancies like breast, stomach and bladder cancers. "The SPEC ERBB2/CEN 17 Double Color Probe is a combination of an orange fluorochrome direct branded CEN 17 probe exact for the alpha satellite centromere region of chromosome 17 and a green fluorochrome direct-labeled SPEC ERBB2 probe specific for the chromosomal region 17q12-q21.1 harboring the ERBB2 gene". In a standard interphase nucleus, (2) orange and (2) green signals are predictable. In a cell with magnification of the "ERBB2 gene locus", numerous duplicates of the green signal or green signal clusters will detected. **Statistical analysis**: SPSS 25.0 was used to perform statistical analysis of the study. The work out data were formulated as counts and percentages. Chi-square and Fisher exact tests were used to describe the association of these data. Level equal to 0.05 or below is the lower level of accepted statistically significant difference.

Fig (1): Fluorescence in situ hybridization (FISH) image showing Negative (A) and Positive (B) results for HER2/Neu gene amplification with over all ratio >2.
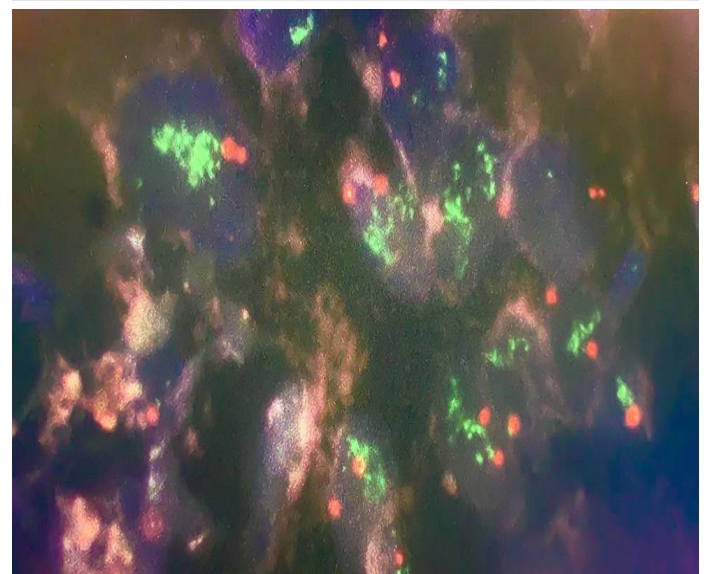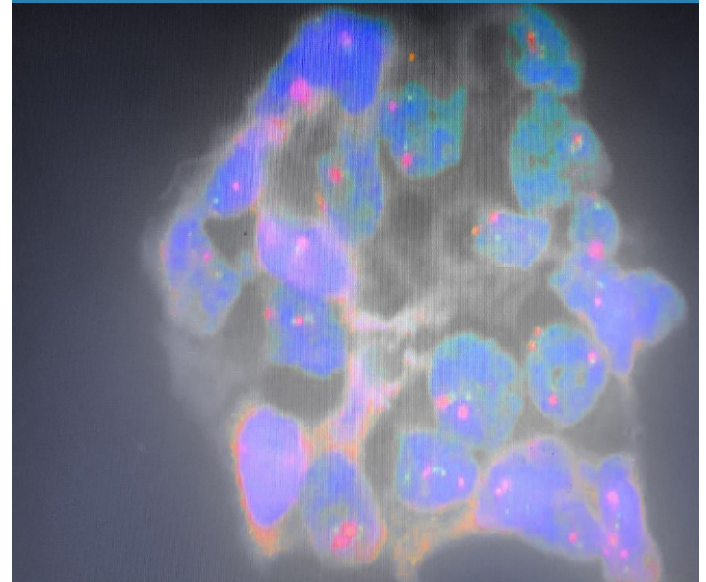
(A)   (B)

Results

Cross sectional study of 77 patients with mean age (49 ± 11) years old, (26%) of patients with amplified Her2 status, (84%) of patients with positive estrogen receptor, (48%) of patients with grade II and (41.6%) with grade III, (50.6%) of patients with high KI 67 (more than 20), (31.2%) of patients with T2 tumor size, (13%) of patients with N1, N2 lymph node include, finally (63.3%) of patients are below age 50 years. As show in table 1.

Table 1: distribution of variables.			
variables		frequency	percentage
Her2 status	amplified	20	26.0
	non amplified	57	74.0
Estrogen receptor	positive	65	84.4
	negative	12	15.6
Grade	I	8	10.4
	II	37	48.1
	III	32	41.6
KI67	below 20	38	49.4
	20 and above	39	50.6
tumor size	no	35	45.5
	T1	11	14.3
	T2	24	31.2
	T3	5	6.5
	T4	2	2.6
lymph node	N0	22	28.6
	N1	10	13.0
	N2	10	13.0
	no	35	45.5
Age	below 50 years old	49	63.6
	50 and above	28	36.4

According to fig 1; Her2 status consist of two types in current study, (25.97%) of patients with amplified Her2 status while (74%) of patients with no amplified Her2 status.

Fig (2): Her2 status distribution.
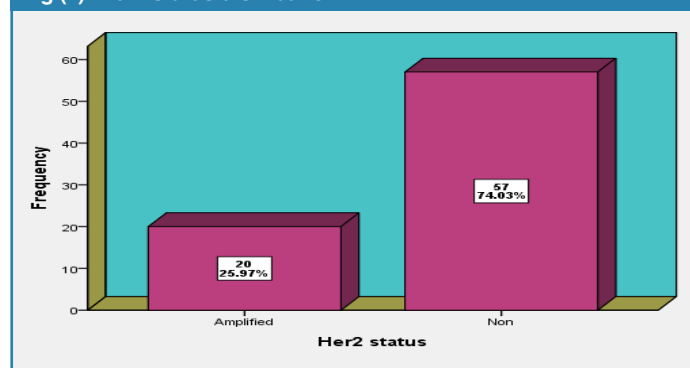
According to table (2); there is significant association between Her2 status and grade of tumor, (65%) of amplified Her2 status patients are in grade III, and (25%) of amplified Her2 status patients are in grade II. There is no significant association between Her2 status and (Estrogen receptor, Ki67, age, tumor size and lymph node involvement).

Table 2: association between Her2 status and (Grade, Estrogen receptor, KI67, age, tumor size and lymph node involvement).

variables		Her2 status Amplified	Her2 status NON Amplified	P-value
Estrogen receptor	negative	2	10	
	%	10.0%	17.5%	0.34
	positive	18	47	
	%	90.0%	82.5%	
	total	20	57	
	%	100.0%	100.0%	
	below 20	8	30	
	%	40.0%	52.6%	
KI67	below 20	12	27	0.24
	%	60.0%	47.4%	
	total	20	57	
	%	100.0%	100.0%	
	< 50 years old	12	37	
Age	%	60.0%	64.9%	
	50 and above	8	20	0.45
	%	40.0%	35.1%	
	total	20	57	
	%	100.0%	100.0%	
	I	2	6	
	%	10.0%	10.5%	
Grade	II	5	32	**0.038**
	%	25.0%	56.1%	
	III	13	19	
	%	65.0%	33.3%	
	total	20	57	
	%	100.0%	100.0%	
	no	9	26	
	%	45.0%	45.6%	
	T1	3	8	
	%	15.0%	14.0%	0.84
Tumor size	T2	5	19	
	%	25.0%	33.3%	
	T3	2	3	
	%	10.0%	5.3%	
	T4	1	1	
	%	5.0%	1.8%	
	total	20	57	
	%	100.0%	100.0%	
	N0	4	18	
	%	20.0%	31.6%	
	N1	2	8	
	%	10.0%	14.0%	0.27
lymph node	N2	5	5	
	%	25.0%	8.8%	
	no	9	26	
	%	45.0%	45.6%	
	total	20	57	
	%	100.0%	100.0%	

P-value ≤ (0.05) significant.

Discussion

The evaluation of Her-2/neu status become usual practice in aggressive carcinoma of breast. Patients with "HER2 amplification" have advantage from effective treatments with trastuzumab[12,13]. Numerous methods are obtainable for such documentation. IHC suggested as an early assessment for "HER2 status", followed by FISH if consequences are unsatisfying[12]. While the whole association between "FISH and IHC" is high (94–98% of breast cancer), and an absolute outcome by IHC will be mostly consistent with FISH, equivocal patients vary significantly[14–17]. Studies show that (20–28%) of IHC equivocal patients have gene augmentation by FISH[12, 13, 14, 18]. "The 2013 ASCO–CAP" standards for defining HER2 amplification by FISH increase in equivocal patients significantly from the earlier 2007 standards[19]. Other FISH investigations recommended for equivocal patients, but the dependability of these testing and the clinical significance of the consequences are not fine recognized. In current study is about these "equivocal IHC Her 2 (2+)" patients followed by "FISH HER2 analysis" which demonstrates 25.9% of cases to be positive for HER2 gene amplification using FISH test which is similar to other studies.[20,21]. It is been stated that IHC equivocal results which do not show gene amplification are possibly due to preanalytical factors like tissue fixation and processing (which can affect the epitope retrieval process) and protein expression due to polysomy[12]. Regarding grading there is significant association between grade III invasive ductal carcinoma and Her2/Neu gene amplification. No association between age and HER2 gene amplification is detected as in studies that show the same results[22-24].

Conclusion

FISH is best method for evaluation of "Her-2/neu gene status" particularly for confusing patients evaluated by IHC that are no attended by correct gene strengthening in the patients with breast Ca. Amplified Her2 position related with grade III of tumor.

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