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RESEARCH PAPER

Comparative study of Her-2, p53, Ki-67 expression and clinicopathological characteristics of breast cancer in a cohort of northern China female patients

Li Ding, Zijin Zhang, Yan Xu, and Yongqiang Zhang

Department of Oncology, Beijing Hospital, National Center of Gerontology, Beijing, China

ABSTRACT

The objective was to study the relationship among Her-2, Ki-67, p53 expression and the clinicopathologic characteristics of breast cancer in the patients of northern China. Expression of Her-2, Ki-67, p53 and clinical characteristics of 260 breast cancer patients were retrospectively studied. Her-2 overexpression led to higher incidence rates of infiltrating ductal carcinoma and axillary lymph node metastasis, bigger diameters of the primary tumors, later pTNM staging, and a lower incidence rate of ductal carcinoma *in situ* ($p < 0.05$). High expression of ER and PR led to fewer patients classified histologically in higher grade ($p = 0.001$), while high expression of Ki-67 and p53 caused more patients classified histologically in higher grade ($p = 0.001$). In patients histologically classified in grade 1 and 2, the expression of Ki-67 and p53 was significantly ($p = 0.001$) higher, and the expression of ER and PR was significantly lower, in Her-2 positive patients than Her-2 negative patients. Breast cancer with Her-2 overexpression was more likely to recur and metastasize than Her-2 negative breast cancer. Higher coincidence of high expression of p53 and Ki-67 with Her-2 overexpression and more progressed tumors suggested that in addition to p53, Ki-67 might also be a prognostic biomarker of breast cancer.

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Introduction

Breast cancer is globally the leading cause of death in women and ranks second in cancer-related mortality.[1] Incidence rates of breast cancer in most regions of the world, especially in developing nations, are increasing.[2,3] In China, breast cancer–related death is the fourth among all other cancers in women, and the incidence rate of breast cancer in urban areas is higher than in rural areas. There are clear differences in clinicopathological characteristics of breast cancer between central China and Western countries.[4]

The human epidermal growth factor receptor 2 gene (Her-2/neu) is a member of HER family and encodes a receptor of molecular mass 185 kDa. These receptors are single transmembrane proteins consisting of an intracellular tyrosine binding domain with various tyrosine phosphorylation sites[5,6] and an extracellular domain for ligand binding and a cytoplasmic tail.[7] After dimerization, Her-2 induces various cellular functions such as cell growth, differentiation and survival through a different cascade. Through MAPK and PI3K signaling pathways, Her-2 prevents apoptosis and promotes cell proliferation.[8,9] Her-2 overexpression leads to an aggressive form of breast cancer,[10] which comprises 25% of all breast cancer cases.[11] The Her-2 positive breast cancer patients have a lower survival rate than patients without Her-2 overexpression. Her-2 has been used as a predictive and prognostic biomarker of breast cancer.[12-14]

P53, encoded by gene Tp53, is a transcription factor that as a tumor suppressor regulates the cell cycle. Mutation in the p53 genes causes the formation of proteins that are more stable than the wild type protein; the mutant proteins accumulate and can be analyzed by immunohistochemistry.[15,16] The human p53 is a nuclear phosphoprotein composed of 393 amino acids. It represses or activates gene expression through binding at many sites of chromatin.[17,18] Its level is low due to a shorter half-life in unarrested cells, but under stress conditions such as DNA damage, it is stabilized by posttranslational modifications.[19] P53 controls cellular functions of cell cycle control, DNA repair, apoptosis, angiogenesis, and cellular stress response through targeting genes such as Mdm2, WAFI/CIPI,

CONTACT Yongqiang Zhang 13701314630@163.com Department of Oncology, Beijing Hospital, National Center of Gerontology, No. 1 DaHua Road, Dong Dan, Beijing, 100730, China.

WIPI, BAX, PIG3, FASL, CSR, P21, etc.[20] P53 is regulated at different levels.[21] The human p53 gene encodes many isoforms of p53 protein through alternative splicing, alternative promoter usage and alternative initiation of translation.[22] Many mutations in the Tp53 gene have been found in breast cancers[17,23] and compose approximately 20–40% of all cases depending upon tumor size and stage of the disease. Mutation in the Tp53 gene seems to be an early event in breast tumorigenesis.[24]

Progesterone receptors (PR) are encoded by the gene PGR and consist of 933 amino acids. Two progesterone receptors, PRA and PRB, are transcribed by the same gene using alternative promoters. PRA and PRB are identical except that the N-terminal of PRB contains an extra 164 amino acids. PRA represses the activity of PRB, while PRB is a major activator of transcription factors.[25] During tumorigenesis, the ratio of PRA to PRB alters, resulting in more PRA than PRB. PR expression is a marker for normal estrogen receptor (ER) functions. Breast cancer patients with PR and ER show better prognosis and response to endocrine therapy than patients that lack these receptors.[26]

Ki-67 is a nuclear protein of molecular mass 359 kDa and is commonly used for the detection and quantification of proliferating cells. An increase in its expression is associated with cell growth.[27] It is commonly used as a diagnostic marker in various cancers including breast cancers.[28,29]

It was found that Her-2 and Tp53 genes were both present on chromosome 17 and there was a strong association between Her-2 overexpression and p53 mutations in breast cancer.[30,31] ER and PR were negatively correlated with Her-2 overexpression.[32,33] The association between Her-2 and Ki-67 in breast cancer patients[5,6,31,34,35] makes Ki-67 an emerging biomarker for breast cancer.[36] This study retrospectively compared 160 cases of Her-2 overexpressing breast cancer patients with 100 cases of Her-2 negative patients in terms of clinicopathological characteristics and the expression of p53 and Ki-67, and further uncovered the connection among Her-2, p53 and Ki-67.

Results

Effect of Her-2 overexpression on clinic pathological characteristics

The demographic, clinic and pathological characteristics were compared between Her-2 positive and negative patients (Table 1). There were no significant differences in age ($p = 1.000$), onset time of breast cancer related to menopause ($p = 0.130$), tumor location ($p = 0.430$), first symptoms including the formation of lumps in breasts, nipple discharge, formation of axillary lumps and calcification ($p = 0.550$), and vessel carcinoma embolus ($p = 0.126$). The incidence rate of infiltrating ductal carcinoma was significantly higher (88.68% vs. 76.53%, $p = 0.002$) while the incidence rate of ductal carcinoma *in situ* was significantly lower (11.32% vs. 23.47%, $p = 0.002$) in Her-2 positive breast cancer patients than in Her-2 negative patients. The diameters of the primary tumors were significantly larger in Her-2 positive patients than in Her-2 negative patients ($p = 0.036$), with more T2 and T3 tumors in the Her-2 positive group than the Her-2 negative group. There was a significantly higher incidence rate of axillary lymph node metastasis in Her-2 positive patients than in Her-2 negative patients, with

Table 1. Comparison of demographic, clinic and pathological characteristics of Her-2 positive and negative patients.

Variables	HER-2 negative n = 99	HER-2 positive n = 159	*P*
Age(year), mean±sd.	50.06 ± 13 .77	49.23 ± 10 .45	1.000
Onset time, n (%)			0.134
Pre-menopause	51(51.52)	97(61.01)	
Post-menopause	48(48.48)	62(38.99)	
Tumor location, n (%)			0.430
Left	43(43.43)	81(50.94)	
Right	55(55.56)	77(48.43)	
Bilateral	1(1.01)	1(0.63)	
First symptoms			0.550
Lump	92(92.93)	152(95.60)	
Nipple discharge	4(4.04)	4(2.52)	
Axillary lump	2(2.02)	3(1.89)	
Calcification	1(1.01)	0(0.00)	
Vessel carcinoma embolus, n (%)			0.126
No	90(90.91)	134(84.28)	
Yes	9(9.09)	25(15.72)	
Pathological type, n (%)			0.002
IDC	75(76.53)	141(88.68)	
Other	23(23.47)	18(11.32)	
Histological grade, n (%)			0.001
1	14(14.14)	6(3.77)	
2	57(57.58)	81(50.94)	
3	28(28.28)	72(45.28)	
Diameter of lumps, n (%)			0.036
T1	58(58.59)	67(42.14)	
T2	37(37.37)	82(51.57)	
T3	4(4.04)	10(6.29)	
axillary lymph node metastasis, n (%)			0.024
N0	66(66.67)	78(49.06)	
N1	20(20.20)	45(28.30)	
N2	4(4.04)	19(11.95)	
N3	9(9.09)	17(10.69)	
pTNM staging, n (%)			0.033
0–I	42(42.42)	44(27.67)	
II	45(45.45)	83(52.20)	
III	12(12.12)	32(20.13)	

more N1 (28.30% vs. 20.20%, p = 0.024) and N2 (11.95% vs. 4.04%, p = 0.024) cases in Her-2 positive patients than those who were Her-2 negative. There were significantly more patients classified histologically as higher grade in the Her-2 positive group than in the Her-2 negative group (grade 3, 45.28% vs. 28.28%, p = 0.001). There was a significant difference in pTNM staging (p = 0.033); more Her-2 positive patients than Her-2 negative patients were in stage II (52.20% vs. 45.45%) and stage III (20.13% vs. 12.12%).

Effect of ER, PR, Ki-67 and p53 on clinicopathological characteristics

The effects of ER expression (Table 3), PR expression (Table 4), Ki-67 expression (Table 2) and p53 expression (Table 5) on clinicopathological characteristics were studied through comparisons in breast cancer patients. ER (Table 3, p = 0.187), PR (Table 4, p = 0.589), Ki-67(Table 2, p = 0.076) and p53 (Table 5, p = 0.523) expression did not influence the incidence rate of infiltrating ductal carcinoma in breast cancer patients. There were significantly fewer patients classified histologically as higher grade in the ER positive group than in the ER negative group (grade 3, 27.95% vs. 56.70%, p = 0.001) (Table 3) and in the PR positive group than in the PR negative group (grade 3, 29.33% vs. 51.85%, p = 0.001) (Table 4). Furthermore, there were significantly more patients classified histologically as higher grade in the Ki-67 high expression group than in the low expression group (grade 3, 45.08% vs. 18.75%, p = 0.001) (Table 2) and in the p53 positive group than in the p53 negative group (grade 3, 47.41% vs. 31.69%, p = 0.015) (Table 5). There was no significant difference in pTNM staging between the ER positive and ER negative groups (Table 3), the PR positive and PR negative groups (Table 4), the Ki-67 high expression and low expression groups (Table 2), or the p53 positive and p53 negative groups (Table 5).

Table 2. Comparison of pathological type, histological grade and pTNM staging between Ki-67 low expression and high expression breast cancer patients*.

Variables	KI-67 low expression n = 64 (%)	KI-67 high expression n = 193 (%)	*P*
Pathological type, n (%)			0.076
IDC	49(76.56)	166(86.01)	
Other	15(23.44)	27(13.99)	
Histological grade, n (%)			0.001
1	10(15.63)	10(5.18)	
2	42(65.63)	96(49.74)	
3	12(18.75)	87(45.08)	
pTNM staging, n (%)			0.202
0–I	27(42.19)	59(30.57)	
II	29(45.31)	99(51.30)	
III	8(12.50)	35(18.13)	

*No Ki-67data available for one case of patient.

Table 3. Comparison of pathological type, histological grade and pTNM staging between ER negative and positive breast cancer patients.

Variables	ER negative n=97	ER positive n=161	*P*
Pathological type, n (%)			0.1867
IDC	85(87.63)	131(81.37)	
Other	12(12.37)	30(18.63)	
Histological grade, n (%)			< 0.001
1	4(4.12)	16(9.94)	
2	38(39.18)	100(62.11)	
3	55(56.70)	45(27.95)	
pTNM staging, n (%)			0.0923
0-I	25(25.77)	61(37.89)	
II	56(57.73)	72(44.72)	
III	16(16.49)	28(17.39)	

Difference in the expression of KI-67, ER, PR and P53 between HER2 positive and negative breast cancer patients

The difference of the expression of Ki-67, ER, PR and p53 between Her-2 positive and negative breast cancer patients was also studied (Table 6).

In histological grade 1 and 2 breast cancer patients, the expression of Ki-67 was significantly higher in Her-2 positive patients than Her-2 negative patients (p = 0.001), with higher coincidence rates of Her-2 positive/Ki-67 high expression than Her-2 positive/Ki-67 low expression (86.21% vs. 13.79%) and Her-2 negative/Ki-67 low expression than Her-2 negative/

Table 4. Comparison of pathological type, histological grade and pTNM staging between PR negative and positive breast cancer patients.

Variables	PR negative n = 108 (%)	PR positive n = 150 (%)	*P*
Pathological type, n (%)			0.589
IDC	92(85.19)	124(82.67)	
Other	16(14.81)	26(17.33)	
Histological grade, n (%)			0.001
1	6(5.56)	14(9.33)	
2	46(42.59)	92(61.33)	
3	56(51.85)	44(29.33)	
pTNM staging, n (%)			0.091
0-I	29(26.85)	57(38.00)	
II	62(57.41)	66(44.00)	
III	17(15.74)	27(18.00)	

Table 5. Comparison of pathological type, histological grade and pTNM staging between p53 negative and positive breast cancer patients.

Variables	P53 negative n = 142 (%)	P53 positive n = 116 (%)	*P*
Pathological type, n (%)			0.523
IDC	117(82.39)	99(85.34)	
Other	25(17.61)	17(14.66)	
Histological grade, n (%)			0.015
1	15(10.56)	5(4.31)	
2	82(57.75)	56(48.28)	
3	45(31.69)	55(47.41)	
pTNM staging, n (%)			0.115
0-I	49(34.51)	37(31.90)	
II	75(52.82)	53(45.69)	
III	18(12.68)	26(22.41)	

Ki-67 high expression (56.34% vs. 43.66%). The expression of p53 was also significantly higher in Her-2 positive patients than Her-2 negative patients (p = 0.001), with higher coincidence rates of Her-2 positive/p53 positive than Her-2 positive/p53 negative (52.87% vs. 47.13%) and Her-2 negative/p53 negative than Her-2 negative/p53 positive (78.87% vs. 21.13%). Additionally, ER expression was significantly lower in Her-2 positive patients than Her-2 negative patients (p = 0.001) with a higher coincidence rate of Her-2 negative/ER positive than Her-2 negative/ER negative (91.55% vs. 8.45%), and PR expression was significantly lower in Her-2 positive patients than Her-2 negative patients (p = 0.001) with a higher coincidence rate of Her-2 negative/PR positive than Her-2 negative/PR negative (84.51% vs. 15.49%) (Table 6). There was no difference in the expression of ER, PR or Ki-67 between Her-2 positive patients and Her-2 negative patients of histological grade III (Table 6).

In all the patients studied, the expression of Ki-67 was significantly higher in Her-2 positive patients than Her-2 negative patients (p = 0.001) with higher coincidence rates of Her-2 positive/Ki-67 high expression than Her-2 positive/Ki-67 low expression (86.16% vs. 13.84%) and Her-2 negative/Ki-67 low expression than Her-2 negative/Ki-67 high expression (57.14% vs. 42.86%). The expression of p53 was also significantly higher in Her-2 positive patients than Her-2 negative patients (p = 0.001) with higher coincidence rates of Her-2 positive/p53 positive than Her-2 positive/p53 negative (54.72% vs. 45.28%) and Her-2 negative/p53 negative than Her-2 negative/p53 positive (70.71% vs. 29.29%). Finally, ER expression was significantly lower in Her-2 positive patients than Her-2 negative patients (p = 0.001) with a higher coincidence rate of Her-2 negative/ER positive than Her-2 negative/ER negative (79.80 % vs. 20.20%), and PR expression was significantly lower in Her-2 positive patients than Her-2 negative patients (p = 0.001) with a higher coincidence rate of Her-2 negative/PR positive than Her-2 negative/PR negative (74.75 % vs. 25.25%) (Table 6).

Discussion

This study retrospectively analyzed 260 breast cancer patients of northern China for the expression of Her-2, PR, ER, p53, Ki-67 and clinicopathological characteristics. It was found that the expression of Her-2, PR, ER, p53 and Ki-67 influenced the clinicopathological characteristics of breast cancer. The effect of the expression of Her-2, PR, ER, Ki-67 and p53 on clinicopathological characteristics suggests that these genes may be used as predictive and/or prognosis biomarkers for breast cancer. The coincidence of Her-2

Table 6. Comparison of the expression of Ki-67, ER, PR and p53 between Her-2 positive and negative breast cancer patients.

	Histological grade 1 and 2			Histological grade 3			Total subjects		
Variables	HER-2 -tive n = 71 (%)	HER-2 +tive n = 87 (%)	*P*	HER-2-tive n = 28 (%)	HER-2 +tive n = 72 (%)	*P*	HER-2-tive n = 99 (%)	HER-2+tive n = 159 (%)	*P*
KI-67*			<0.001			0.593			<0.001
Low	40(56.34)	12(13.79)		2(7.41)	10(13.89)		42(42.86)	22(13.84)	
High	31(43.66)	75(86.21)		25(92.59)	62(86.11)		56(57.14)	137(86.16)	
ER			<0.001			0.531			<0.001
Negative	6(8.45)	36(41.38)		14(50.00)	41(56.94)		20(20.20)	77(48.43)	
Positive	65(91.55)	51(58.62)		14(50.00)	31(43.06)		79(79.80)	82(51.57)	
PR			<0.001			0.451			<0.001
Negative	11(15.49)	41(47.13)		14(50.00)	42(58.33)		25(25.25)	83(52.20)	
Positive	60(84.51)	46(52.87)		14(50.00)	30(41.67)		74(74.75)	76(47.80)	
P-53			<0.001			0.531			<0.001
Negative	56(78.87)	41(47.13)		14(50.00)	31(43.06)		70(70.71)	72(45.28)	
Positive	15(21.13)	46(52.87)		14(50.00)	41(56.94)		29(29.29)	87(54.72)	

*No Ki-67data available for one case of patient.

overexpression with Ki-67 high expression, p53 positive, PR negative and ER negative indicates that there may be some regulatory relationship between Her-2 and these genes in signaling transduction pathways.

Role of Her-2, PR, ER, Ki-67 and p53 in breast cancers

In this study, it was uncovered that Her-2 overexpression is connected with a higher incidence rate of infiltrating ductal carcinoma, a lower incidence rate of ductal carcinoma *in situ*, larger primary tumor diameters, a higher incidence rate of axillary lymph node metastasis, a higher histological grading, and a later pTNM staging in breast cancer patients, which is in agreement with previous reports.[1,37-47] Our observations support that Her-2 overexpression leads to aggressive forms of breast cancer.[11] P53 is a transcription factor that is encoded by the Tp53 gene and regulates the cell cycle as a tumor suppressor. Mutations in the p53 genes cause the formation of stabilized proteins, which accumulate and can be analyzed by immunohistochemistry.[15,16] Our finding that p53 positive caused breast carcinoma to progress to a higher histological grade, which is in agreement with previous reports[37,48,49] and suggests that mutation in p53 genes have a tumor promotion role in breast cancer patients,[39] which is supported by previous reports.[37] Ki-67 is a nuclear protein that is commonly used for the detection and quantification of proliferating cells because an increase in its expression is associated with cell growth.[27] Consistent with the observations of others,[6,37,50-53] our observation that Ki-67 positive led breast carcinoma progress to higher histological grade in breast patients implicated that Ki-67 high expression promoted tumor growth in breast cancer patients.[50] Our observation that both PR positive and ER positive connected with a slower histological progress in breast cancer, which was in agreement with the observations by Shapochka et al.,[37] suggests that PR and ER might have an inhibitive role in breast cancers. Both PR and ER facilitate cell growth through nuclear pathways and non-nuclear pathways.[54] ER expression was found to be positively correlated with Her-2 expression in Her-2 non-overexpressing breast cancers.[55] The connection of ER positive and a slower histological progress in breast cancer observed in this study might not be due to an ER-mediated inhibitive effect, but instead may be a result of negative associations between ER and Her-2 positive in breast cancers[56] caused by the down-regulation of ER,[57] higher ER protein turnover, and lower ER protein expression mediated by Her-2 overexpression through the PI3K/Akt signaling pathway.[32,58] The connection of PR positive and a slower histological progress in breast cancer observed in this study might also be a result of a negative association between PR and Her-2 positive in breast cancers[56] caused by the loss of PR protein due to Her-2 overexpression through the PI3K/Akt signaling pathway.[59]

Relationship of Her-2, PR, ER, Ki-67 and p53 in signaling transduction pathways

In this study, it was found that Her-2 overexpression was negatively connected with PR and ER expression and positively connected with Ki-67 positive and p53 positive, which was consistent with previous reports[30,32,37,44,46,49,53,59,60] This suggests that there might be some relationship among Her-2 and these genes in signal transduction pathways. Through MAPK and PI3K signaling pathways, Her-2 prevents apoptosis and promotes cell proliferation.[9] Both ER and PR, after activation by ligand binding, act on the transcription of their target genes in the nuclei, or through non-genomic pathways.[54] Her-2, ER and PR may mediate their effects on breast cancer through crosstalk among Her-2, ER and PR pathways.[54] Our observed negative connection among Her-2 overexpression, ER and PR expression might be the result of downregulation of ER,[57] higher ER protein turnover, lower ER protein expression mediated by Her-2 overexpression through the PI3K/Akt signaling pathway,[32,58] and loss of PR protein mediated by Her-2 overexpression through the PI3K/Akt signaling pathway.[59] P53 is a transcription factor that is encoded by gene Tp53. Mutation in the p53 genes causes the formation of stable proteins that can be analyzed by immunohistochemistry.[15,16] The detection of mutations in p53 genes in germline[30] suggests that they may be genetically inherited. Both the p53 gene and the Her-2 gene are located in chromosome 17.[30] It is not clear why p53 mutations are associated with Her-2 gene amplification.[39,40] Ki-67 is a nuclear protein that is commonly used for the detection and quantification of proliferating cells.[28] In agreement with the observations by others,[35,50,51] it was found that Ki-67 positively connected with Her-2 overexpression in this study, suggesting that Her-2 overexpression might upregulate the expression of Ki-67.

Her-2, PR, ER, Ki-67 and p53 as breast cancer biomarkers

Our finding that Her-2 overexpression is connected with a higher incidence rate of infiltrating ductal carcinoma, a lower incidence rate of ductal carcinoma *in situ*, bigger diameters of the primary tumors, a higher incidence rate of axillary lymph node metastasis, a higher histological grading, and a later pTNM staging in breast cancer patients further confirmed the observation that Her-2 positive breast cancer patients had a lower survival rate than patients without Her-2 overexpression,[12] and supports the use of Her-2 as a predictive and prognostic biomarker of breast cancer.[12] In agreement with the observation by Kobayashi *et al.*,[61] our finding that p53 positive is connected with the progression of carcinoma to a higher histological grade and Her-2 overexpression in breast cancer patients suggests that p53 positive could be used as a prognostic biomarker for breast cancer[56,62-64] in addition to being used as a diagnostic biomarker.[38] Our findings that Ki-67 positive is connected with the progression of carcinoma to a higher histological grade as well as Her-2 overexpression in breast cancer patients are consistent with the previous observations[5,35,50,61] and increase the reliability of this emerging biomarker[38] as a predictor of breast cancer prognosis outcomes. Our observation that both PR positive and ER positive are connected with a slower histological progress and are negatively connected with Her-2 overexpression in breast cancer supports that PR positive and ER positive are good biomarkers for predicting better prognosis and response to endocrine therapy than patients that lacked these receptors.[26]

It is concluded that Her-2 overexpression is related with invasive breast cancer. Her-2 overexpression is positively connected with p53 and Ki-67 expression, supporting that in addition to Her-2 and p53, Ki-67 is a prognosis biomarker for breast cancer.

Patients and methods

Patients

A cohort of 260 female breast cancer patients of northern China was included in the study. 160 of the patients were surgically and pathologically diagnosed to be primary breast cancer patients, and histochemically confirmed to be Her-2 positive; these patients had a mean age of 52.5 y (range 26–79, median 49 years). 100 cases were Her-2 negative patients, with a mean age of 53 y (range 28–78, median 50 years) (Table 1).

Methods

This was a comparative study retrieving the clinical records of 260 breast cancer patients for information about pathologic type, histological grade, vascular cancer embolus, axillary lymph node status, clinical tumor stage, Her-2 expression, p53 expression, ER expression, PR expression and Ki-67 expression. The patients were further grouped according to the TNM cancer staging system by the American Joint Committee on Cancer (AJCC)[65] and the ASCO-CAP guideline for the pathological diagnosis of breast cancer.[66] CerbB-2 was tested with immunochemistry and FISH. Her-2 (+++) was designated as Her-2 positive while Her-2 (++) was designated as Her-2 positive only when there was amplification of Her-2 gene detected with FISH. Estrogen receptor (ER), progesterone receptor (PR), p53 and Ki-67 were tested with immunochemistry. The percentage of ER positive cells or PR positive cells greater than 1% was designated as ER positive or PR positive. The percentage of Ki-67 positive cells less than 14% was designated as low expression, while greater than or equal to 14% was medium/high expression. For p53, cells were p53 positive if there were brown granules in the nuclei. Ten microscopic fields (x400) were examined in each section, and the percentage of p53 positive cells $\leq$ 5% was designated as negative while $>$ 5% as positive.

Data analysis

Statistical analysis was performed using SPSS 14.0 software. T-test was performed for normal distribution measurement data, and presented as mean $\pm$ SE Rank sum test was performed for abnormal distribution measurement data, and presented as median (maximum, minimum). X^2 test (Chi-square test) or Fisher's exact probability analysis was performed for enumeration data and presented as the number of cases n (%). Rank sum test was performed for rank variable data and presented as the number of case n (%). $p < 0.05$ was considered statistically significant.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

References

[1] Ullah MF, Aatif M. The footprints of cancer development: Cancer biomarkers. Cancer Treatment Rev 2009; 35:193-200; PMID:19062197; https://doi.org/10.1016/j.ctrv.2008.10.004

[2] Shin HR, Masuyer E, Ferlay J, Curado MP. Asian Contributors to CI. Cancer in Asia - Incidence rates based on data in cancer incidence in five continents IX (1998–2002). Asian Pacific J Cancer Prevention 2010; 11(Suppl 2):11-6; PMID:20553065

[3] Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer J Int Du Cancer 2010; 127:2893-917; PMID:21351269; https://doi.org/10.1002/ijc.25516

[4] Wang LW, Yang GF, Chen JM, Yang F, Yuan JP, Sun SR, Chen C, Hu MB, Li Y. A clinical database of breast cancer patients reveals distinctive clinico-pathological characteristics: a study from central China. Asian Pacific J Cancer Prevention 2014; 15:1621-6; PMID:24641378; https://doi.org/10.7314/APJCP.2014.15.4.1621

[5] Madani SH, Payandeh M, Sadeghi M, Motamed H, Sadeghi E. The correlation between Ki-67 with other prognostic factors in breast cancer: A study in Iranian patients. Indian J Med Paediatr Oncol 2016; 37:95-9; PMID:27168707; https://doi.org/10.4103/0971-5851.180136

[6] Zhou SJ, Guo H. Ki-67 expression and significance of different molecular subtypes of breast invasive ductal carcinoma. Zhonghua Yi Xue Za Zhi 2013; 93:2895-7; PMID:24373403

[7] Misek DE, Kim EH. Protein biomarkers for the early detection of breast cancer. Int J Proteomics 2011; 2011:343582; PMID:22084684

[8] Strauss B. Best hope or last hope: access to phase III clinical trials of HER-2/neu for advanced stage breast cancer patients. J Adv Nursing 2000; 31:259-66; PMID:10672081; https://doi.org/10.1046/j.1365-2648.2000.01302.x

[9] Kaufmann R, Muller P, Hildenbrand G, Hausmann M, Cremer C. Analysis of Her2/neu membrane protein clusters in different types of breast cancer cells using localization microscopy. J Microscopy 2011; 242:46-54; PMID:21118230; https://doi.org/10.1111/j.1365-2818.2010.03436.x

[10] Petit T, Borel C, Ghnassia JP, Rodier JF, Escande A, Mors R, Haegele P. Chemotherapy response of breast cancer depends on HER-2 status and anthracycline dose intensity in the neoadjuvant setting. Clin Cancer Res 2001; 7:1577-81; PMID:11410493

[11] Arteaga CL, Sliwkowski MX, Osborne CK, Perez EA, Puglisi F, Gianni L. Treatment of HER2-positive breast cancer: current status and future perspectives. Nat Rev Clin Oncol 2012; 9:16-32; https://doi.org/10.1038/nrclinonc.2011.177

[12] Faltus T, Yuan J, Zimmer B, Kramer A, Loibl S, Kaufmann M, Strebhardt K. Silencing of the HER2/neu gene by siRNA inhibits proliferation and induces apoptosis in HER2/neu-overexpressing breast cancer cells. Neoplasia 2004; 6:786-95; PMID:15720805; https://doi.org/10.1593/neo.04313

[13] Slamon DJ, Leyland-Jones B, Shak S, Fuchs H, Paton V, Bajamonde A, Fleming T, Eiermann W, Wolter J, Pegram M, et al. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. N Eng J Med 2001; 344:783-92; PMID:11248153; https://doi.org/10.1056/NEJM200103153441101

[14] Ross JS, Fletcher JA. The HER-2/neu oncogene in breast cancer: prognostic factor, predictive factor, and target for therapy. Stem Cells 1998; 16:413-28; PMID:9831867; https://doi.org/10.1002/stem.160413

[15] Hosoda M, Yamamoto M, Nakano K, Hatanaka KC, Takakuwa E, Hatanaka Y, Matsuno Y, Yamashita H. Differential expression of progesterone receptor, FOXA1, GATA3, and p53 between pre- and postmenopausal women with estrogen receptor-positive breast cancer. Breast Cancer Res Treatment 2014; 144:249-61; PMID:24549642; https://doi.org/10.1007/s10549-014-2867-0

[16] Lee DS, Kim SH, Suh YJ, Kim S, Kim HK, Shim BY. Clinical implication of p53 overexpression in breast cancer patients younger than 50 years with a triple-negative subtype who undergo a modified radical mastectomy. Japanese J Clin Oncol 2011; 41:854-66; PMID:21719749; https://doi.org/10.1093/jjco/hyr066

[17] Lane DP, Brown CJ, Verma C, Cheok CF. New insights into p53 based therapy. Discov Med 2011; 12:107-17; PMID:21878188

[18] Chen F, Wang W, El-Deiry WS. Current strategies to target p53 in cancer. Biochem Pharmacol 2010; 80:724-30; PMID:20450892; https://doi.org/10.1016/j.bcp.2010.04.031

[19] Smeenk L, Lohrum M. Behind the scenes: unravelling the molecular mechanisms of p53 target gene selectivity (Review). Int J Oncol 2010; 37:1061-70; PMID:20878053

[20] Wright PE, Dyson HJ. Intrinsically disordered proteins in cellular signalling and regulation. Nat Rev Mol Cell Biol 2015; 16:18-29; PMID:25531225; https://doi.org/10.1038/nrm3920

[21] Varna M, Bousquet G, Plassa LF, Bertheau P, Janin A. TP53 status and response to treatment in breast cancers. J Biomed Biotechnol 2011; 2011:284584; PMID:21760703; https://doi.org/10.1155/2011/284584

[22] Wei CL, Wu Q, Vega VB, Chiu KP, Ng P, Zhang T, Shahab A, Yong HC, Fu Y, Weng Z, et al. A global map of p53 transcription-factor binding sites in the human genome. Cell 2006; 124:207-19; PMID:16413492; https://doi.org/10.1016/j.cell.2005.10.043

[23] Petitjean A, Achatz MI, Borresen-Dale AL, Hainaut P, Olivier M. TP53 mutations in human cancers: functional selection and impact on cancer prognosis and outcomes.

Oncogene 2007; 26:2157-65; PMID:17401424; https://doi.org/10.1038/sj.onc.1210302

[24] Hollstein M, Sidransky D, Vogelstein B, Harris CC. p53 mutations in human cancers. Science 1991; 253:49-53; PMID:1905840; https://doi.org/10.1126/science.1905840

[25] Anderson E, Clarke RB. Steroid receptors and cell cycle in normal mammary epithelium. J Mammary Gland Biol Neoplasia 2004; 9:3-13; PMID:15082914; https://doi.org/10.1023/B:JOMG.0000023584.01750.16

[26] Brisken C. Reply to is progesterone a neutral or protective factor for breast cancer? Nat Rev Cancer 2014; 14:146; PMID:24442144; https://doi.org/10.1038/nrc3518-c2

[27] Milde-Langosch K, Karn T, Muller V, Witzel I, Rody A, Schmidt M, Wirtz RM. Validity of the proliferation markers Ki67, TOP2A, and RacGAP1 in molecular subgroups of breast cancer. Breast Cancer Res Treatment 2013; 137:57-67; PMID:23135572; https://doi.org/10.1007/s10549-012-2296-x

[28] Denkert C, von Minckwitz G. Reply to Ki67 in breast cancer: a useful prognostic marker. Annals Oncol 2014; 25:542-3; PMID:24431345; https://doi.org/10.1093/annonc/mdt564

[29] Yerushalmi R, Woods R, Ravdin PM, Hayes MM, Gelmon KA. Ki67 in breast cancer: prognostic and predictive potential. Lancet Oncol 2010; 11:174-83; PMID:20152769; https://doi.org/10.1016/S1470-2045(09)70262-1

[30] Melhem-Bertrandt A, Bojadzieva J, Ready KJ, Obeid E, Liu DD, Gutierrez-Barrera AM, Litton JK, Olopade OI, Hortobagyi GN, Strong LC, et al. Early onset HER2-positive breast cancer is associated with germline TP53 mutations. Cancer 2012; 118:908-13; PMID:21761402; https://doi.org/10.1002/cncr.26377

[31] Rudolph P, Olsson H, Bonatz G, Ratjen V, Bolte H, Baldetorp B, Ferno M, Parwaresch R, Alm P. Correlation between p53, c-erbB-2, and topoisomerase II α expression, DNA ploidy, hormonal receptor status and proliferation in 356 node-negative breast carcinomas: prognostic implications. J Pathol 1999; 187:207-16; PMID:10365096; https://doi.org/10.1002/(SICI)1096-9896(199901)187:2%3c207::AID-PATH223%3e3.0.CO;2-U

[32] Konecny G, Pauletti G, Pegram M, Untch M, Dandekar S, Aguilar Z, Wilson C, Rong HM, Bauerfeind I, Felber M, et al. Quantitative association between HER-2/neu and steroid hormone receptors in hormone receptor-positive primary breast cancer. J Natl Cancer Inst 2003; 95:142-53; PMID:12529347; https://doi.org/10.1093/jnci/95.2.142

[33] Hayes DF, Thor AD. c-erbB-2 in breast cancer: development of a clinically useful marker. Semin Oncol 2002; 29:231-45; PMID:12063676; https://doi.org/10.1053/sonc.2002.32899

[34] Nicholson RI, McClelland RA, Finlay P, Eaton CL, Gullick WJ, Dixon AR, Robertson JF, Ellis IO, Blamey RW. Relationship between EGF-R, c-erbB-2 protein expression and Ki67 immunostaining in breast cancer and hormone sensitivity. Eur J Cancer 1993; 29A:1018-23; PMID:8098946; https://doi.org/10.1016/S0959-8049(05)80215-1

[35] Alco G, Bozdogan A, Selamoglu D, Pilanci KN, Tuzlali S, Ordu C, Igdem S, Okkan S, Dincer M, Demir G, et al. Clinical and histopathological factors associated with Ki-67 expression in breast cancer patients. Oncol Lett 2015; 9:1046-54; PMID:25663855

[36] Weigel MT, Dowsett M. Current and emerging biomarkers in breast cancer: prognosis and prediction. Endocrine-Related Cancer 2010; 17:R245-62; PMID:20647302; https://doi.org/10.1677/ERC-10-0136

[37] Shapochka DO, Zaletok SP, Gnidyuk MI. Relationship between NF-kappaB, ER, PR, Her2/neu, Ki67, p53 expression in human breast cancer. Exp Oncol 2012; 34:358-63; PMID:23302996

[38] Sana M, Malik HJ. Current and emerging breast cancer biomarkers. J Cancer Res Therapeutics 2015; 11:508-13; PMID:26458575; https://doi.org/10.4103/0973-1482.163698

[39] Meng S, Tripathy D, Shete S, Ashfaq R, Haley B, Perkins S, Beitsch P, Khan A, Euhus D, Osborne C, et al. HER-2 gene amplification can be acquired as breast cancer progresses. Proc Natl Acad Sci U S A 2004; 101:9393-8; PMID:15194824; https://doi.org/10.1073/pnas.0402993101

[40] Espinosa AB, Tabernero MD, Garcia-Macias MC, Primo D, Bernal AG, Cruz JJ, Ramos M, Font de Mora J, Gomez Alonso A, Orfao A. Her-2/neu gene amplification in familial vs sporadic breast cancer. Impact on the behavior of the disease. Am J Clin Pathol 2003; 120:917-27; PMID:14671981; https://doi.org/10.1309/5H778W5K75CMM03W

[41] Beckmann MW, Niederacher D, Massenkeil G, Tutschek B, Beckmann A, Schenko G, Schnurch HG, Bender HG. Expression analyses of epidermal growth factor receptor and HER-2/neu: no advantage of prediction of recurrence or survival in breast cancer patients. Oncology 1996; 53:441-7; PMID:8960138; https://doi.org/10.1159/000227618

[42] Bebenek M, Bar JK, Harlozinska A, Sedlaczek P. Prospective studies of p53 and c-erbB-2 expression in relation to clinicopathological parameters of human ductal breast cancer in the second stage of clinical advancement. Anticancer Res 1998; 18:619-23; PMID:9568187

[43] Radovic S, Babic M, Doric M, Balta E, Kapetanovic E, Secic S. Correlation of the HER-2 protein expression and other clinicopathological features of ductal infiltrative breast cancer. Bosn J Basic Med Sci 2005; 5:3-7; PMID:15771595

[44] Selvarajan S, Wong KY, Khoo KS, Bay BH, Tan PH. Over-expression of c-erbB-2 correlates with nuclear morphometry and prognosis in breast carcinoma in Asian women. Pathology 2006; 38:528-33; PMID:17393979; https://doi.org/10.1080/00313020601024060

[45] Chen ST, Lai HW, Tseng HS, Chen LS, Kuo SJ, Chen DR. Correlation of histologic grade with other

clinicopathological parameters, intrinsic subtype, and patients' clinical outcome in Taiwanese women. Jpn J Clin Oncol 2011; 41:1327-35; PMID:22071339; https://doi.org/10.1093/jjco/hyr157

[46] Patnayak R, Jena A, Rukmangadha N, Chowhan AK, Sambasivaiah K, Phaneendra BV, Reddy MK. Hormone receptor status (estrogen receptor, progesterone receptor), human epidermal growth factor-2 and p53 in South Indian breast cancer patients: A tertiary care center experience. Indian J Med Paediatr Oncol 2015; 36:117-22; PMID:26157289; https://doi.org/10.4103/0971-5851.158844

[47] Elkablawy MA, Albasry AM, Hussainy AS, Nouh MM, Alhujaily A. Molecular profiling of breast carcinoma in Almadinah, KSA: Immunophenotyping and clinicopathological correlation. Asian Pac J Cancer Prev 2015; 16:7819-24; PMID:26625804; https://doi.org/10.7314/APJCP.2015.16.17.7819

[48] Sekar P, Bharti JN, Nigam JS, Sharma A, Soni PB. Evaluation of p53, HoxD10, and E-Cadherin status in breast cancer and correlation with histological grade and other prognostic factors. J Oncol 2014; 2014:702527; PMID:24634677; https://doi.org/10.1155/2014/702527

[49] Sirvent JJ, Salvado MT, Santafe M, Martinez S, Brunet J, Alvaro T, Palacios J. p53 in breast cancer. Its relation to histological grade, lymph-node status, hormone receptors, cell-proliferation fraction (ki-67) and c-erbB-2. Immunohistochemical study of 153 cases. Histol Histopathol 1995; 10:531-9; PMID:7579801

[50] Nishimura R, Osako T, Okumura Y, Hayashi M, Toyozumi Y, Arima N. Ki-67 as a prognostic marker according to breast cancer subtype and a predictor of recurrence time in primary breast cancer. Exp Therapeutic Med 2010; 1:747-54; PMID:22993598; https://doi.org/10.3892/etm.2010.133

[51] Wang B, Wang X, Wang J, Xuan L, Wang Z, Wang X, Gao J, Zhang H. Expression of Ki67 and clinicopathological features in breast cancer. Zhonghua Zhong Liu Za Zhi 2014; 36:273-5

[52] Golmohammadi R, Pejhan A. The prognostic value of the P53 protein and the Ki67 marker in breast cancer patients. J Pak Med Assoc 2012; 62:871-5; PMID:23139965

[53] Elkablawy MA, Albasri AM, Mohammed RA, Hussainy AS, Nouh MM, Alhujaily AS. Ki67 expression in breast cancer. Correlation with prognostic markers and clinicopathological parameters in Saudi patients. Saudi Med J 2016; 37:137-41; PMID:26837394; https://doi.org/10.15537/smj.2016.2.12285

[54] Arpino G, Wiechmann L, Osborne CK, Schiff R. Crosstalk between the estrogen receptor and the HER tyrosine kinase receptor family: molecular mechanism and clinical implications for endocrine therapy resistance. Endocrine Rev 2008; 29:217-33; PMID:18216219; https://doi.org/10.1210/er.2006-0045

[55] Pinhel I, Hills M, Drury S, Salter J, Sumo G, A'Hern R, Bliss JM, Sestak I, Cuzick J, Barrett-Lee P, et al. ER and HER2 expression are positively correlated in HER2 non-overexpressing breast cancer. Breast Cancer Res 2012; 14:R46; PMID:22417870; https://doi.org/10.1186/bcr3145

[56] Perin T, Canzonieri V, Massarut S, Bidoli E, Rossi C, Roncadin M, Carbone A. Immunohistochemical evaluation of multiple biological markers in ductal carcinoma in situ of the breast. Eur J Cancer 1996; 32A:1148-55; PMID:8758245; https://doi.org/10.1016/0959-8049(96)00037-8

[57] Pietras RJ, Arboleda J, Reese DM, Wongvipat N, Pegram MD, Ramos L, Gorman CM, Parker MG, Sliwkowski MX, Slamon DJ. HER-2 tyrosine kinase pathway targets estrogen receptor and promotes hormone-independent growth in human breast cancer cells. Oncogene 1995; 10:2435-46; PMID:7784095

[58] Creighton CJ, Fu X, Hennessy BT, Casa AJ, Zhang Y, Gonzalez-Angulo AM, Lluch A, Gray JW, Brown PH, Hilsenbeck SG, et al. Proteomic and transcriptomic profiling reveals a link between the PI3K pathway and lower estrogen-receptor (ER) levels and activity in ER+ breast cancer. Breast Cancer Res 2010; 12:R40; PMID:20569503; https://doi.org/10.1186/bcr2594

[59] Kim HJ, Cui X, Hilsenbeck SG, Lee AV. Progesterone receptor loss correlates with human epidermal growth factor receptor 2 overexpression in estrogen receptor-positive breast cancer. Clin Cancer Res 2006; 12:1013s-8s; PMID:16467118; https://doi.org/10.1158/1078-0432.CCR-05-2128

[60] Barbati A, Cosmi EV, Sidoni A, Collini P, Porpora MG, Ferri I, Luthy M, Lauro V, Bucciarelli E. Value of c-erbB-2 and p53 oncoprotein co-overexpression in human breast cancer. Anticancer Res 1997; 17:401-5; PMID:9066684

[61] Kobayashi T, Iwaya K, Moriya T, Yamasaki T, Tsuda H, Yamamoto J, Matsubara O. A simple immunohistochemical panel comprising 2 conventional markers, Ki67 and p53, is a powerful tool for predicting patient outcome in luminal-type breast cancer. BMC Clin Pathol 2013; 13:5; PMID:23384409; https://doi.org/10.1186/1472-6890-13-5

[62] Yamamoto M, Hosoda M, Nakano K, Jia S, Hatanaka KC, Takakuwa E, Hatanaka Y, Matsuno Y, Yamashita H. p53 accumulation is a strong predictor of recurrence in estrogen receptor-positive breast cancer patients treated with aromatase inhibitors. Cancer Sci 2014; 105:81-8; PMID:24118529; https://doi.org/10.1111/cas.12302

[63] Loskutova KS, Kirillina MP, Lushnikova EL, Nepomnyashchikh LM. Immunohistochemical study of apoptotic marker p53 as a prognostic factor in breast cancer. Bull Exp Biol Med 2014; 158:84-7; PMID:25403404; https://doi.org/10.1007/s10517-014-2698-3

[64] Payandeh M, Sadeghi M, Sadeghi E, Madani SH. Expression of p53 breast cancer in Kurdish women in the West of Iran: a reverse correlation with lymph node metastasis. Asian Pac J Cancer Prev 2016;

17:1261-4; PMID:27039757; https://doi.org/10.7314/APJCP.2016.17.3.1261

[65] Singletary SE, Allred C, Ashley P, Bassett LW, Berry D, Bland KI, Borgen PI, Clark GM, Edge SB, Hayes DF, et al. Staging system for breast cancer: revisions for the 6th edition of the AJCC cancer staging manual. Surgical Clin N Am 2003; 83:803-19; PMID:12875597; https://doi.org/10.1016/S0039-6109(03)00034-3

[66] Hammond ME. ASCO-CAP guidelines for breast predictive factor testing: an update. Applied Immunohistochem Mol Morphol 2011; 19:499-500; https://doi.org/10.1097/PAI.0b013e31822a8eac