HER1 R497K and HER2 I655V polymorphisms are linked to development of breast cancer

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Abstract.
BACKGROUND: Polymorphism of the genes of Human Epidermal growth factor receptor1 (HER1) and receptor2 (HER2) have been reported to be linked to pathogenesis of several malignant tumors but still there is contradiction regarding their association with breast cancer.

OBJECTIVE: In this case control study we aimed to analyze the frequency of HER1 R497K (rs 11543848) and HER2 I655V (rs 1136201) Polymorphisms in breast cancer.

SUBJECT AND METHOD: The frequency of HER1 Arg(R) 497Lys (K) and HER2 Ile (I) 655Val (V) polymorphisms were tested in 64 breast cancer patients and 86 normal control by polymerase chain reaction followed by restriction fragment polymorphism detection. Immunohistochemical analysis was done for HER2 protein on the available 18 malignant tissue samples.

RESULTS: HER1 497K and HER2 655V variant had significantly increased breast cancer risk (OR = 2.6, 95% CI 1.6–4.2, OR = 2.2, 95% CI 1.2–4.1, p < 0.05) respectively. Moreover, combined HER1 K497 and HER2 V655 variant was detected in 26.6% malignant in comparison to 8.14% of control group (OR = 4.1, 95% CI 1.58–10.57), but, no significant association was noticed between both Polymorphisms and clinicopathological features of the disease. As regard HER2 immunohistochemical expression no significant correlation was revealed with HER2 655V polymorphism.

CONCLUSIONS: Our findings suggest that HER1 497K and HER2 655V polymorphisms are potential risk factor for development of breast cancer.

Keywords: HER1 gene, HER2 gene, polymorphism, HER2 protein expression, breast cancer

1. Introduction

Recently enormous number of researches has been focused on identification of genetic predisposition to different complex diseases particularly cancer. The incidence of breast cancer has been progressively increased with estimated 229, 060 new diagnosis and 39, 510 deaths per year in the United States [1]. Molecular genetic analysis of breast cancer indicated the implication of various structural and functional genetic alterations in cancer development. Hence identification of molecular characteristics of breast cancer enables more accurate prediction of the course of the disease and response to chemotherapy [2].

Growth, differentiation, and motility of various tumor cells are remarkably related to growth factor receptors [3]. An important family of growth factor receptors is Epidermal Growth Factor Receptor EGFR, or erbB family, including four distinct transmembrane tyrosine kinase receptors, known as HER1 (EGFR, erbB1), HER2 (erbB2, neu), HER3 (erbB3), and HER4
(erbB4) [4]. Their activation leads to downstream effect in gene expression, cellular proliferation, cell growth, differentiation, and survival [5].

Alteration of genes and aberration of signaling pathway of HER1, and HER2 have been frequently implicated in carcinogenesis of a variety of solid tumors [6]. Uzan et al., 2009 found that HER1 and HER2 overexpression are associated with poor prognosis in breast cancer [4], as well as gene amplification of HER2 in 10 to 25% of invasive breast cancer and unfavorable effect on the course of the disease [7].

Single nucleotide polymorphisms (SNPs) are the commonest sources of human genetic variation which contribute to susceptibility to malignant transformation [8]. A SNP at codon 497 of HER1 gene leads to Arg (R) to Lys (K) substitution in the extracellular domain known as HER1 R497K (rs11543848) has been identified in breast cancer and other EGFR expressing tumors such as gliomas, lung and colorectal cancer [9].

Another SNP at codon 655 of HER2 indicates a isoleucine-to-valine substitution (I655V) in the transmembrane domain has been associated with risk of breast cancer [10]. The molecular mechanism of the involvement of HER2 I655V (rs1136201) polymorphism in the process of carcinogenesis is still under investigation. The specific concern about HER family comes from demonstrated improvement of overall survival in advanced HER2 overexpressing breast cancer patients who received HER2 monoclonal antibody trastuzumab which bind extracellular part of HER2 [11], and the clinical activity of antiHER1 was observed in tumors with low expression of HER1 [12].

Hsieh et al., 2012 reported that HER1 R497K polymorphism could be a key determinant of increased response to cetuximab-based chemotherapy and a longer survival in colorectal cancer patients [13]. Gerger et al., 2011 suggested that HER2 I655V polymorphism may identify patients with metastatic colorectal cancer likely to experience better outcome when treated with cetuximab [14]. In addition Williams et al., 2012 found potential predictive value of HER2 I655V polymorphism for overall survival in advanced cancer of the head and neck [15].

The proved interaction between different HER family has directed us to evaluate the possible link between HER1 R497K and HER2 I655V polymorphisms on development of breast cancer, and the possible influence of both polymorphisms on disease aggressiveness.

2. Subjects and methods

The current study has been performed between 2010 and 2012, it included 64 females pathologically proven as breast cancer with mean age (52.1 ± 13.8) selected from patients who were admitted in department of Surgery Ain Shams University hospitals. They were staged according to TNM classification of the American Joint Committee on cancer (AJCC) [16], and graded by the Nottingham grading system [17]. The study was performed in accordance with Declaration of Helsinki, and was approved by the Research Ethics Committee of Ain Shams University, Cairo, Egypt. An informed consent was obtained from all patients. The control group included 86 normal females with mean age (44.5 ± 8.1), all participates were subjected to history taking and withdrawal of 10 ml venous blood samples which were collected in EDTA containing tubes for DNA extraction. Fresh breast tissue samples were taken from tumors of 18 women subjected to surgical treatment and they were kept in 10% formaline for immunohistochemical analysis. An informed consent was taken from all participates. The clinicopathological features of patients were collected from patient’s pathological reports.

2.1. DNA extraction

Genomic DNA was extracted from white blood cell pellets of venous blood samples collected on EDTA tubes by salting out extraction method using wizard genomic DNA extraction kit from Promega UK. Red blood cell lysis was done by using red cell lysis buffer (20 mM Tris-CL pH 7.6) followed by centrifugation. Nuclei lysis was carried by cell lysis buffer (10 mM Tris-CL pH 8.0, 1 mM EDTA pH 8.0, 0.1% (w/v) SDS) and proteinase K (20 mg/ml) followed by centrifugation. Protein was precipitated by protein precipitation solution (60 ml of 5 M potassium acetate, 11.5 ml of glacial acetic acid, 28.5 ml of water) followed by centrifugation. Finally DNA was precipitated by isopropanol and then ethanol 70% and rehydrated in TE buffer (pH 7.6). The DNA purity and concentration were determined by spectrophotometer measurement of absorbance at 260 and 280 nm [18]. The extracted DNA was stored at −20°C.

2.2. Genotyping of HER1 R497K polymorphism

The R497K (G → A) polymorphism of the HER1 gene was examined by PCR-RFLP method as de-
cycles. PCR products after being digested by Bst\(\text{I}\) restriction enzyme (Promega UK) at 60\(\degree\)C, annealing for 1 min at 56\(\degree\)C and extension for 1 min at 72\(\degree\)C with a final extension for 7 min at 72\(\degree\)C. For RFLP analysis, 5 \(\mu\)L of each PCR product was digested with 4 \(\mu\)L BsmAI (Promega UK) at 55\(\degree\)C for 2 \(h\). BsmAI digestion gave fragments of 116 bp and 32 bp for the Val (GTC) allele and a single 148 bp fragment for the Ile (ATC) allele. Fragments digested with BsmAI were subjected to electrophoresis on a 3% agarose gel, stained with ethidium bromide and visualized under UV light [21].

### 2.4. Immunohistochemical protein expression of HER2

The protein expression level of HER2 was assessed using immunohistochemistry on formalin fixed, paraffin-embedded tumor tissues. Reactions were performed with anti-c-erbB-2 polyclonal antibody (DAKO, Glostrup-Denmark) using the streptavidin biotin peroxidase method along with DAB as chromogen by Ventana-NexES IHC automatic immunostainer.

HER2 was scored using a modification of the HercepTest 0–3 + scoring system [22]. HER2 was scored as 0 (absence of staining), 1 + (any amount of partial membranous staining), 2 + (weak to moderate complete membranous staining in more than 10\%, or strong complete membranous staining in 30\% or less), or 3 + (strong complete membranous staining in more than 30\%). The IHC staining results were correlated with multiple clinicopathologic variables. Clinical guidelines provide specific criteria for positive (3 +), equivocal (2 +), and negative (0 or 1 +) IHC staining results [23]. Ki67 immunostaining was performed to assess the proliferative activity of the tumor cells.

### 2.5. Statistical analysis

Data were tested for statistical significance by using Statistical Package for the Social Sciences software system SPSS–17 statistical software (SPSS, Chicago, IL). Chi-square or Fisher exact test was used to analyze differences in allele and genotype frequencies, as well as association between each of the SNP and clinicopathological parameters. Hardy-Weinberg equilibrium (HWE) was tested by comparing the observed and expected genotype frequencies contingency table analysis (\(\chi^2\)-test) for the cases and controls separately. The strength of association of the evaluated gene polymorph-

| Malignant samples | Pathological parameters of malignant patients |
|-------------------|---------------------------------------------|
| Age year (mean ± SD) | (52 ± 13.7) |
| Stage | IIA 14 (21.9) |
| | IIB 16 (25.0) |
| | IIIA 18 (28.1) |
| | III 4 (6.25) |
| | IV 12 (18.8) |
| Grade | I 6 (9.4) |
| | II 38 (59.4) |
| | III 20 (31.2) |
| Distant metastasis | Negative 52 (81.3) |
| | Positive 12 (18.7) |
| Lymph nodes | Negative 32 (50.0) |
| | Positive 32 (50.0) |
| Tumor size | ≤ 2 cm 46 (71.9) |
| | > 2 cm 18 (28.1) |
| Pathology | Duct carcinoma 44 (68.7) |
| | Lobular carcinoma 6 (9.7) |
| | Mixed duct and lobular 6 (9.4) |
| | Poorly differentiated tumor 8 (12.5) |
| HER2 expression | Weak (score 0, 1) 9 (50.0) |
| | Moderate (score2) 7 (39.0) |
| | Severe (score3) 2 (11.0) |

\(n\): number; SD: standard deviation.

scribed previously [19]. Briefly, 50–100 ng of genomic DNA was amplified in 50 \(\mu\)l reaction mixture containing, 20 pmol/L (forward primer, 5\(\prime\)-TGCTGTGACCC-ACTCTGTCT-3\(\prime\)) and reverse primer, 5\(\prime\)-CCAGAAGGTTCGACTCTGTCC-3\(\prime\)) and 25 \(\mu\)L of Master Mix containing 2.5 units of Taq DNA polymerase, 1 \(\times\) PCR buffer 50 mM Tris-HCl (pH 9.0); 50 mM NaCl; 5 mM MgCl\(\text{2}\); 200 \(\mu\)M of each dNTP. After initial denaturation at 95\(\degree\)C for 3 min, the reaction was carried out at 94\(\degree\)C denaturation for 1 min, 59\(\degree\)C annealing for 1 min, and 72\(\degree\)C extension for 1 min for a total of 35 cycles. PCR products after being digested by BstN1 restriction enzyme (Promega UK) at 60\(\degree\)C for 16 \(h\) were separated on 3% ethidium bromide – stained agarose gel, stained with ethidium bromide and visualized under UV light [21].

#### 2.3. Genotyping of HER2 V655 polymorphism

For the HER2 V655 polymorphism, 50–100 ng of genomic DNA was amplified in 50–\(\mu\)l reaction mixture containing, 20 pmol forward 5\(\prime\)-AGAGCGCCAGCCC-TCTGAGCTCCAT-3\(\prime\), and reverse 5\(\prime\)-TTGGCTTTCTGACACGTCTCCGC-3\(\prime\) [20] and 25 \(\mu\)L of Master Mix containing 2.5 units of Taq DNA polymerase, 1 \(\times\) PCR buffer 50 mM Tris-HCl (pH 9.0); 50 mM NaCl; 5 mM MgCl\(\text{2}\); 200 \(\mu\)M of each dNTP. The samples were denatured for 6 min at 94\(\degree\)C. This was followed by 35 cycles of denaturation for 30 \(s\) at 94\(\degree\)C, annealing for 1 min at 56\(\degree\)C and extension for 1 min at 72\(\degree\)C with a final extension for 7 min at 72\(\degree\)C. For RFLP analysis, 5 \(\mu\)L of each PCR product was digested with 4 \(\mu\)L BsmAI (Promega UK) at 55\(\degree\)C for 2 \(h\). BsmAI digestion gave fragments of 116 bp and 32 bp for the Val (GTC) allele and a single 148 bp fragment for the Ile (ATC) allele. Fragments digested with BsmAI were subjected to electrophoresis on a 3% agarose gel, stained with ethidium bromide and visualized under UV light [21].
Table 2

|                   | Cases n (%) | Control n (%) | OR (CI)            | p-value |
|-------------------|-------------|---------------|--------------------|---------|
| **HER1 R497K**    |             |               |                    |         |
| Codominant inheritance |            |               |                    |         |
| 497RR             | 11 (17.2%)  | 48 (55.8%)    | 1.0 (Reference)    |         |
| 497RK             | 50 (78.1%)  | 36 (41.9%)    | 4.9 (2.56–9.76)    | < 0.01* |
| 497KK             | 3 (4.7%)    | 2 (2.3%)      | 6.5 (0.97–43.9)    | > 0.05  |
| Recessive inheritance |         |               |                    |         |
| 497RK + RR vs KK  | 61 (95.3%)  | 84 (97.7%)    | 2.1 (0.33–2.70)    | 0.01*   |
| Dominant inheritance |          |               |                    |         |
| 497KK + RK vs RR  | 53 (82.8%)  | 38 (44.2%)    | 6.1 (2.79–13.21)   | < 0.01* |
| Allele frequency  |             |               |                    |         |
| 497 R allele      | 72 (56.0%)  | 132 (76.7%)   | 1.0 (Reference)    |         |
| Allele frequency  |             |               |                    |         |
| 497K allele       | 56 (44.0%)  | 40 (23.3%)    | 2.6 (1.56–4.21)    | < 0.01* |
| **HER2 I655V**    |             |               |                    |         |
| Codominant inheritance |        |               |                    |         |
| 655II             | 39 (60.9%)  | 67 (77.9%)    | 1.0 (Reference)    |         |
| 655IV             | 21 (32.8%)  | 18 (20.9%)    | 2.0 (0.95–4.21)    | > 0.05  |
| Recessive inheritance |       |               |                    |         |
| 655 IV + II vs VV | 60 (93.7%)  | 85 (98.8%)    | 5.7 (0.61–51.9)    | > 0.05  |
| Dominant inheritance |          |               |                    |         |
| 655 IV + VV vs II | 25 (60.9%)  | 19 (77.9%)    | 2.3 (1.11–4.62)    | < 0.05* |
| Allele frequency  |             |               |                    |         |
| 655 I allele      | 99 (77.3%)  | 152 (88.4%)   | 1.0 (Reference)    |         |
| 655V allele       | 29 (22.7%)  | 20 (11.6%)    | 2.2 (1.19–4.15)    | 0.01*   |
| **Combined HER1 R497K and HER2 I655V Mutation** | | | | |
| a) 497RR/655II    | 5 (7.8%)    | 37 (43.02%)   | 1.0 (Reference)    |         |
| b) 497K or 655V   | 42 (65.6%)  | 42 (48.84%)   | 7.4 (2.65–20.67)   | < 0.01* |
| c) 497K and 655V  | 17 (26.6%)  | 7 (8.14%)     | 17.9 (4.98–64.85)  | < 0.01* |
| c vs (a + b)      |            |               |                    |         |
| (c + b) vs a      |            |               |                    |         |

*p < 0.05 is significant, n = number, OR = Odd ratio, 95% CI = 95% confidence interval, K = lysine (mutant allele), R = arginine, V = valine (mutant allele), and I = isoleucine; a) Non mutated 497RR/655II genotype; b) Isolated mutation either 497K or 655V alleles; c) Combined 497K and 655V alleles.

The frequency of HER1 R497K and HER2 I655V alleles and genotypes in breast cancer patients (n = 64) and control groups (n = 86) was measured by the odd ratio (OR) and corresponding 95% confidence interval (95% CI) and two sided p value. P value < 0.05 was considered statistically significant.

3. Results

The present study was performed on venous blood samples withdrawn from 150 subjects (64 malignant cases with mean age (52 + 13.7) and 86 normal control individuals with mean age (44.6 + 7.9) with no significant difference between mean age of both groups P > 0.05. The clinicopathological criteria of malignant cases are shown in Table 1). All of the samples were subjected to analyses for two gene polymorphisms HER1 R497K, and HER2 I655V by using RFLP followed by agarose gel electrophoresis. Tissue samples of 18 cases were available for immunohistochemical analysis for HER2 protein expression.

![Fig. 1. Agarose electrophoresis for detection of HER1 Arg497Lys polymorphism: Lane 1 is 25 bp DNA ladder, lanes 3,6,8,10,13,14,15 are homozygous genotype Arg/Arg showing three bands 38, 50, 67 bp, lanes 3,4,5,7,11,12 are heterozygous genotype Arg/Lys showing four bands 38, 50, 67, and 117 bp, lane 9 is homozygous genotype Lys/Lys with two bands 38, 117 bp.](image1)

3.1. HER1 R497K polymorphism

The gel electrophoresis for HER1 R497K was shown in Fig. 1 after restriction with BstN1 enzyme, homozygous genotype Arg/Arg had three fragments with 38, 50, and 67 bp length; homozygous Lys/Lys had two fragments 38, 117 bp length and heterozygous genotype Arg/Lys had four fragments 38, 50, 67, and
### Table 3

Association of HER1 R497K and HER2 I655V polymorphisms with clinicopathological parameters of breast cancer patients

|                | RR genotype n (%)<sup>a</sup> | RK + KK genotype n (%)<sup>b</sup> | Total | OR (CI)  |
|----------------|-------------------------------|-----------------------------------|-------|---------|
| **Stage**      |                               |                                   |       |         |
| Late (IIA, B, IV) | 6 (17.6)                      | 28 (82.4)                         | 34    | 0.9 (0.26–3.43) |
| Early (IIA, IIB)   | 5 (16.7)                      | 25 (83.3)                         | 30    |         |
| **Distant metastasis** |                           |                                   |       |         |
| Positive        | 1 (8.3)                       | 11 (91.7)                         | 12    | 2.6 (0.30–22.7) |
| Negative        | 10 (19.2)                     | 42 (80.8)                         | 52    |         |
| **Grade**       |                               |                                   |       |         |
| High            | 2 (10)                        | 18 (90)                           | 20    | 2.3 (0.45–11.8) |
| Low             | 9 (20.5)                      | 35 (79.5)                         | 44    |         |
| **Tumor size**  |                               |                                   |       |         |
| > 2 cm          | 2 (11.1)                      | 16 (88.9)                         | 18    | 1.9 (0.37–10.03) |
| ≤ 2 cm          | 9 (19.6)                      | 37 (80.4)                         | 46    |         |
| **Lymph nodes** |                               |                                   |       |         |
| Positive        | 6 (18.8)                      | 26 (81.3)                         | 32    | 0.80 (0.22–2.95) |
| Negative        | 5 (15.6)                      | 27 (84.4)                         | 32    |         |
| **Age**         |                               |                                   |       |         |
| ≥ 45            | 8 (19)                        | 34 (81)                           | 42    | 0.67 (0.16–2.83) |
| < 45            | 3 (13.6)                      | 19 (86.4)                         | 22    |         |

|                | II genotype n (%)<sup>c</sup> | VV + IV genotype n (%)<sup>d</sup> | Total | OR (CI)  |
|----------------|-------------------------------|-----------------------------------|-------|---------|
| **Stage**      |                               |                                   |       |         |
| Late (IIA, B, IV) | 24 (70.6)                    | 10 (29.4)                         | 34    | 0.42 (0.15–1.16) |
| Early (IIA, IIB)   | 15 (50)                      | 15 (50)                           | 30    |         |
| **Distant metastasis** |                           |                                   |       |         |
| Positive        | 5 (41.7)                      | 7 (58.3)                          | 12    | 2.64 (0.74–9.53) |
| Negative        | 34 (65.4)                     | 18 (34.6)                         | 52    |         |
| **Grade**       |                               |                                   |       |         |
| High            | 15 (75)                       | 25% (5)                           | 20    | 0.43 (0.15–1.26) |
| Low             | 24 (54.5)                     | 45.5% (20)                        | 44    |         |
| **Tumor size**  |                               |                                   |       |         |
| > 2 cm          | 12 (66.7)                     | 6 (33.3)                          | 18    | 0.70 (0.22–2.22) |
| ≤ 2 cm          | 27 (58.7)                     | 19 (41.3)                         | 46    |         |
| **Lymph nodes** |                               |                                   |       |         |
| Positive        | 23 (72)                       | 9 (28)                            | 32    | 0.39 (0.13–1.10) |
| Negative        | 16 (50)                       | 16 (50)                           | 32    |         |
| **Her2 gene expression** n = 18 |               |                                   |       |         |
| Strong + medium | 4 (44.4)                      | 5 (55.6)                          | 9     | 1.56 (0.24–10.03) |
| Weak            | 5 (55.6)                      | 4 (44.4)                          | 9     |         |
| **Age**         |                               |                                   |       |         |
| ≥ 45            | 25 (59.5)                     | 17 (40.5)                         | 42    | 1.46 (0.49–4.32) |
| < 45            | 15 (68.2)                     | 7 (31.8)                          | 22    |         |

All parameters showed p > 0.05 which is non-significant, n = number, OR = Odd ratio, CI = 95% confidence interval; a: number of cases with RR genotype = 11; b: number of cases with RK + KK genotype = 53; c: number of cases with II genotype = 39; d: number of cases with VV + IV genotype = 25.

Fig. 2. Agarose gel electrophoresis for detection of HER2 Ile655Val polymorphism: Lane 1 is Val/Val homozygous genotype with single band at 116 bp, lanes 2,3,4,6,10 are Ile/Ile homozygous genotype with one band 148 bp, lanes 5,8,9 are Ile/Val heterozygous genotype with two bands 116, 148, and lane 7 is 50 bp DNA ladder.

117 bp length. The frequency of HER1 R497K genotypes were consistent with Hardy Weinberg equilibrium (HWE) in control group (χ² = 2.57, p² = 0.59, 2pq = 0.35, q² = 0.05), but malignant group was deviated from HWE (χ² = 22.8, p² = 0.32, 2pq = 0.49, q² = 0.19). As shown in Table 2) the frequency of K allele was significantly higher in malignant cases (44%) than in control group (23.3%), p < 0.01. Moreover the frequency of HER1 R497K heterozygous genotype R/K and K containing genotypes (KK + KR) versus homozygous RR were significantly higher in malignant compared to control group (p < 0.01).

#### 3.2. HER2 I655V polymorphism

As shown in Fig. 2, agarose gel electrophoresis of HER2 I655V polymorphism after restriction with BsmA1 enzyme. Homozygous Val/Val (V/V) genotype has single band with 116 bp length. Homozygous Ile/Ile (I/I) genotypes has one band 148 bp, and heterozygous I/V has two bands 116 and 148 bp. The frequency of HER2 I655V genotypes was not deviated from HWE both in control group (χ² = 0.77, p² = 0.01) and malignant group (χ² = 0.21, p² = 0.14). As indicated in Table 2), HER2 I655V genotypes was significantly higher for V containing genotypes (V/V + I/V) compared to homozygous I/I genotype in malignant group than in control group (p < 0.05). Similarly,
3.3. Combined HER1 R497K and HER2 I655V polymorphisms

As seen in Table 2 the group at which there was combined variants (497K/655V) showed marked increased risk of breast cancer, it was detected in 26.6% of malignant in comparison to 8.14% of control (OR = 4.1, 95% CI 1.58–10.57, $P$ value < 0.01), when compared to other combinations of genotypes.

3.4. The relation of HER1 R497K and HER2 I655V polymorphisms to clinicopathological parameters

No significant association could be detected between the frequency of polymorphisms and any of the evaluated parameters (Table 3). Regarding histopathological types there was significant difference for HER2 655V allele and genotype which was higher in poorly differentiated tumors in comparison to other types (Table 4).

3.5. Immunohistochmical expression of HER2 protein

As shown in Figs 3–5 the expression was categorized into three degrees, strong expression have given

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**Table 4**

| Polymorphism | Histopathological types | | | |
|--------------|-------------------------|---|---|---|
|              | Duct carcinoma | Lobular carcinoma | Mixed duct and lobular | Poorly differentiated tumor |
| HER1 R497K   | | | | |
| 497RR        | 6(13.6) | 0(0) | 3(50.0) | 2(25) |
| 497KK + RK   | 38(86.4) | 6(100) | 3(50.0) | 6(75) |
| $P$ value    | > 0.05 | | | |
| HER1 allele  | 497R 47/88(53.4) | 6/12(50.0) | 9/12(75) | 10/16(62.5) |
| 497K 41/88(46.6) | 6/12(50.0) | 3/12(25) | 6/16(37.5) |
| $P$-value    | > 0.05 | | | |
| HER2 I655V   | | | | |
| 655II        | 25(56.8) | 6/100 | 6/100 | 4/50.0 |
| 655VV + VI   | 19(43.2) | 0(0) | 0(0) | 4/50.0 |
| $P$-value    | < 0.05* | | | |
| 655I allele  | 65/88(73.9) | 12/12(100) | 12/12(100) | 10/16(62.5) |
| 655V allele  | 23/88(26.1) | 0/12(0) | 0/12(0) | 6/16(37.5) |
| $P$-value    | 0.01* | | | |

*p < 0.05 is significant.
score 3 (Fig. 3) and it was detected in 2 samples out of the analyzed 18 malignant specimens (11%) of malignant, moderate expression with score 2 was found in 7 samples (39%) (Fig. 4), and weak expression with score 0, 1 found in 9 samples (50%) (Fig. 5). No significant association was found between HER2 overexpression (score 3) and HER2 I655V Polymorphism as overexpression was found in 5 samples (55.6%) of 655V containing genotypes as compared to 4 samples (44.4%) of homozygous 655II genotype ($p > 0.05$) (Table 3).

4. Discussion

Genetic mutation has been contributed to the susceptibility of malignant transformation including breast carcinomas [10,24]. Genetic polymorphism and protein over-expression of erbB (HER) signaling system has been widely studied and proved to be associated with pathogenesis of many tumors [25]. Indeed, an interaction between different HER receptors was confirmed in malignant alteration and had been implicated in responsiveness of adjuvant cytotoxic chemotherapy [26,27]. Identification of new genetic and environmental factors that affect susceptibility to breast cancer would yield further insight into breast tumorigenesis, and provide target for development of new therapeutic approaches, in this respect HER receptor genes are plausible candidate genes to recognize tumors that are likely to respond to HER directed therapies [28].

HER1 (EGFR) was the first tyrosine kinase receptor to be directly linked with human cancer. The HER1 gene maps to 7p11.2-p2 and encodes a 170 kDa trans-
membrane protein [29]. HER1 gene amplification has been described in oligodendrogliomas [30], glioblastomas [31], lung carcinomas [32], gastric carcinomas [33], and breast carcinomas [34,35]. The HER2 gene maps to chromosome 17q21 and encodes a 185 kDa glycoprotein. It is reported to be amplified and overexpressed in several types of human tumors, including about 30% of all breast carcinomas [35–37].

In the current study we analyzed the frequency of HER1 497K and HER2 655V variants in breast cancer patients, our results revealed significant increase of both variants in comparison to control group (p < 0.05) with increased risk of breast cancer (OR = 2.6, 95% CI 1.6–4.2/OR = 2.2, 95% CI 1.2–4.1) for HER1 497K allele, and HER2 655V allele respectively.

Some of earlier reports have revealed that the presence of HER2 655V polymorphism is associated with increased risk of breast cancer including a meta-analysis performed by Tao et al., 2009 on 20 eligible reports including 10,642 cases and 11,259 controls showed higher frequency of V allele in breast cancer cases (OR = 1.1, 95% CI 1–1.2, p = 0.04) [38], a further meta-analysis study by Lu et al., 2010 included 11504 cases and 12538 controls, in subgroup analysis by ethnicity they found significant association among Africans (OR = 8.6, 95% CI 1.9–38.5), and Asians (OR = 1.2, 95% CI 0.01–1.38) [39]. Xie et al., 2000 recorded very high risk of HER2 I655V polymorphism in relation to breast cancer in Chinese population (OR 14.1, 95% CI 1.8–113.4) of the V/V versus I/I genotype [40]. Kruszyna et al., 2010 suggested that HER2 I655V polymorphism may be susceptibility biomarker of breast cancer among older women [41], while Millikan et al., 2003 reported a twofold increased risk of breast cancer associated with V/V or V/I genotype compared with I/I genotype among women living in North Carolina (United States) who were both less than 45 years of age and reported a positive family history of breast cancer (OR 2.3 95% CI 1–5.3) [42]. Similarly, the results of the present study was correlating with previous reports of Baxter and Campbell 2001; Mackean Cowdin et al., 2001; Montogomery et al., 2003; Lee-Hoeflich et al., 2008; and Ozturk et al., 2012 [43–46,21].

Several studies have shown conflicting results with the majority of them showing no risk association with HER2 655V polymorphism as those of Montgomery et al., 2003; Kara et al., 2010; Dahabreh and Murray 2011; and Ma et al., 2011 [47–49,45]. These studies were performed on different ethnic groups at which same polymorphisms have different somatic variations, therefore they may not be reliable to compare to our study which has investigated HER polymorphisms in Arabic Egyptian subjects.

Actually, limited number of studies have been focused on HER1 497K polymorphism in cancer patients, and we couldn’t find any record in breast cancer, on the other hand; the impact of and HER2 655V polymorphisms on progression of breast cancer is another controversial point supported by study of Naidu et al., 2008 who found significant positive correlation between HER2 655V polymorphism and lymph node involvement in breast cancer patients; alternatively [50], Ozturk et al., 2012 and Akisik and Dalay 2004 have observed lack of association between HER2 V allele and clinicopathological parameters as tumor type, grade, and stage [46,21].

In the current study there was lack of association between the frequency of HER1 497K polymorphism and all evaluated clinicopathological parameters; nevertheless, for HER2 655V polymorphism there was significant difference between histopathological types as the frequency of V allele was significantly higher (p < 0.05) in poorly differentiated tumors (37.5%) as compared to 26.1% of duct carcinoma, and complete absence in lobular and mixed carcinomas. These findings may imply the potential effect of HER2 655V polymorphism on tissue differentiation which is considered a poor prognostic feature of the disease.

Regarding HER1 497K polymorphism our findings disagreed with those of Khalil et al., 2009 who observed lack of risk association between HER1 497K allele and breast cancer in 148 Tunisian patients, but they found positive correlation with both tumor grade and nodular status [51].

The comparison of HER1 497K and HER2 655V genotypes and alleles in the present study have shown a pronounced increased frequency of combined HER1 497K and HER2 655V allele variants in 26.6% of malignant cases versus 8.1% of control individuals (OR = 4.1, 95% CI 1.58–10.57, p < 0.01), this elevation of combined mutation may reflect a possible genetic background of active HER receptor co-expression and communication which have great bear on the process of carcinogenesis.

At the present study we were able to test immunohistochemical expression of HER2 receptor protein, with over expression recorded in 11% of the analyzed breast cancer tissues with a relevant lack of association with HER2 655V polymorphism. Previous literatures have proved that over-expression of HER2 protein is almost always accompanied by HER2 DNA amplification [52,
53], but we couldn’t find recorded results for association with HER2 655V polymorphism.

Most likely HER2 receptor induce tumorigenesis through number of signaling pathways [54], the simplest mechanistic model is through elevation of kinase activity and over phosphorylation of itself and cellular substrates. The second model is increase of HER2 hetero-dimerization with HER1 and HER3 [55,56] that interfere with HER1 endocytic degradation with subsequent increase of signaling duration and potency [57]. A third mechanism through alternative HER2 transcript named Δ HER2 which increase ligand independent signaling activity and increase transforming potency [58]. The last known mechanism is activation of interacting SH2 containing protein (src kinase) which acts as second messenger of HER2. Elevated activity of src kinase is seen in many tumors including breast cancer with or without HER2 over-expression [59].

The results of current study suggest that implication of HER2 655V polymorphism in carcinogenesis may preferentially occur through functional modification rather than increase protein expression by increasing the formation of active HER2 heterodimer causing alteration of tyrosine kinase activity. Indeed, several limitations should be considered in the present study, as limited number of samples, lack of data about some prognostic markers as hormonal receptors, moreover, disease free survival which have not been examined interfered with reliable evaluation of prognostic value of investigated SNPs in breast cancer.

Finally, we concluded that HER1497K and HER2 655V polymorphisms may be considered susceptibility genetic markers for the risk of breast carcinogenesis but not suitable indicators of disease aggressiveness. HER2 655V polymorphisms may have qualitative rather than quantitative stimulatory effect on HER2 protein that needs further exploration, with concern of other erbB receptors.

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