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The Prognostic Value of HER1 R497K in the Management of Patients With Breast Cancer

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Introduction: Breast cancer is the most common cancer among the women. Despite the advances in the diagnosis of metastatic cancers, there are still many challenges. Hence, there is a need to use a sensitive, fast and cheap method for diagnosis. Tumor markers are one of the methods that are used extensively for this purpose. Despite extensive efforts to identify novel prognostic and predictive clinical biomarkers, a very small number of markers have been reported as risk stratification biomarkers (e.g., BRCA1/2 and HER2). The substitution of arginine with lysine in codon 497 of HER1 497 has been suggested as a potential marker in breast cancer. This study attempted to explore the association between HER1 497 gene polymorphisms with pathological and clinical information of breast cancer patients.

Methods: 110 breast cancer patients were recruited in this study. Genomic DNA extraction and genotyping were performed using Taqman-PCR and sequencing, respectively. The association between this genetic variant with breast cancer risk and pathological information of patients was evaluated.

Results: Findings showed that 9.43% of cases had AA genotype, while these frequencies in AC and CC genotypes were 77.35% and 13.20%, respectively. Moreover, we assessed that 78.4% of breast cancer patients with M0 had AA+AC genotypes, while 21.6% of CC cases had M0 status. Furthermore, 22.7% of these cases with CC genotype had N0/1. We observed that most patients with CC genotype had lower HER2 expression.

Conclusions: Our study indicated the potential association of CC genotype of HER1 497 with the prognosis of patients with breast cancer.

INTRODUCTION

Breast cancer (BC) is the second leading cause of cancer death in women [1]. It has been estimated that breast cancer constitutes 76% of cancer types in women, and 190,000 new cases are diagnosed each year. The prevalence of BC in the United Kingdom is one in 12 [1, 2] and in the United States of America is one in eight [3]. Several factors are involved in BC development, including lifestyle, alcohol and smoking, postmenopausal obesity, hormone level, pregnancy, and genetic factors [4]. Different studies have investigated the effect of genetic variants, family history, and cancer risk [5, 6]. Human growth factor receptors are remarkably involved in proliferation and differentiation of tumor cells [7]. The human epidermal growth factor receptor (EGFR) is one of the key dysregulated pathways in BC, which has four members such as HER2 or ERBB2 [8]. There is growing body of data showing the association between genetic variants in HER1 and HER2 and increased risk of BC [9-11]. Among the genetic variants, HER1 R497K (rs11543848) and HER2 I655V (rs1136201) are among the promising variants, which are involved in altered expression of HER1 and HER2 expression [12]. It has been shown that valine substitution, with respect to isoleucine, changes the hydrophobic domain configuration in HER2 and affects the transmembrane domain stability [13]. In the presence of isoleucine in HER2 transmembrane domain, the signal transduction pathway is reduced due to malfunction in dimerization of 655Ile, compared to 655Val variant [14], although several studies have reported a controversial data about these two variants in BC. In particular Wang et al., showed the association of HER1 K497R and risk of BC [15], which is in line with several other studies about other EGFR members in colorectal, lung, and breast cancers [16]. In Caucasian women, the variant HER2 Valine allele has been imputed to increase BC capability, while the Val-Val homozygosity in African women was reported as an increased BC risk factor [11]. Xie et al., showed that the 655Val was the major risk factor among females with BC, compared to healthy controls (16% vs. 11%) [16]. Baxter at al. in a case-control study showed no association between 655Val allele and BC risk [17]. Similarly, in another study by Wang-Gohrke et al., no association was found between valine 655 SNP and risk of BC in young females (≤45 years) of a population from Germany [18]. Fleishman et al., showed the different function of HER2 Ile655Val as an opposing effect on oncogenic activity, termed HER2 equilibrium shifts [14]. Since there is much controversy over the role of these candidate genetic variants, in the present study, we investigated the relationship between HER1 R497K (rs11543848) with HER2 expression and pathological information of patients with BC..

METHODS

Patients

Totally 110 age-matched Iranian women patients (41 breast cancer patients and 69 healthy controls) participated in the study. The patients were recruited based on histologically confirmed diagnosis of locally advanced or metastatic breast cancer from Omid Hospital affiliated to MUMS. The samples were taken before radiotherapy. Moreover, patients with history of other cancers, infectious diseases, absence of the history of stroke, myocardial infarction, and diabetes mellitus were excluded from the study. Individuals in the control group were recruited from Mashhad-Stroke and Heart-Atherosclerotic-Disorders cohort study. They had no known history of cancers, infectious diseases, family history of stroke, myocardial infarction, and diabetes mellitus. The sample size was estimated by on line (https://epitools.ausvet.com.au/casecontrolss), and the study power was considered to be 80. The patients included in this study had histologically confirmed BC between 2013 and 2014. The BC patients were graded by the Nottingham grading system [19] and were classified according to the TNM classification of American Joint Committee on cancer [20]. The average age of patients was 52±13 years old (Table 1). Our study was performed according to the Helsinki declaration. Informed consent was obtained from all participants using protocols approved by the Ethics Committee of Mashhad University of Medical Sciences (License number: IR.MUMS.MEDICAL.REC.1397.309).

Table 1: Pathological Parameters of Malignant Patients

	Value
Age, y (mean±SD)	52±13.3
Stage	
IIA	25.3
IIB	20.1
IIIA	27.7
IIIC	18.9
IV	8.0
Grade	
I	8.2
II	59.0
III	32.8
Tumor Size	
<2 cm	70.9
≥2 cm	28.1
Distant Metastasis	
Negative	70.3
Positive	29.7

DNA Extraction and Genotyping

Genomic DNAs were extracted from peripheral blood leukocytes using by Yekta Tajhiz Azma (Cat No.YT9030) according to the manufacturer's protocol. The concentration and purity of DNA samples were determined using the NanoDrop®-1000-Detector (NanoDrop-Technologies, Wilmington, USA). Genotype analysis of HER1 R497K polymorphism was carried out using Taqman®-probes-based assay; PCR reactions were carried out in 12.5 μL total volume, using 20 ng of DNA in TaqMan® Universal Master Mix with specific primers ((forward primer, 5-TGCTGTGACCCACTCTGTCT-3 and reverse primer, 5-CCAGAAGGTTGCACTTGTCC-3) and probes (C-901792-10 and C-790057-10; Applied Biosystems, Foster City, CA, USA). The ABIPRISM-7500 instrument equipped with the SDS version-2.0 software was utilized to determine the allelic content of the samples [21, 22]. Amplification was performed in a program with primary denaturation at 95°C for 5 min and pursued by 35 cycles of denaturation at 95°C for 30 sec, annealing at 57-60°C for 30 sec and extension at 72°C for 30 sec, with a final extension of 7 min at 72°C.

DNA Sequencing

DNAs were amplified as described above, sequenced with Sanger sequencing and then analyzed with DNA Analyzer (Sequetech, USA) to confirm the genotypes obtained.

Statistical Analysis

Demographic and clinical information were compared across mutations using Pearson's χ^2 tests [23]. Continuous variables were evaluated using Student's t-tests [24, 25]. The observed genotype frequencies of polymorphism were assessed with χ^2 tests. The Hardy–Weinberg equilibrium assumption was assessed by comparing the genotype frequencies using the Pearson χ^2 distribution. The associations between the risk of BC for the CC and CT genotypes, relative to the risk genotype TT homozygote under the recessive genetic model, were assessed by logistic regression [21, 26]. Data were analyzed using SPSS-20 software (SPSS Inc., IL, USA). All the analyses were two-sided, and statistical significance was set at $P<0.05$.

RESULTS

Association of the Genetic Variant With Clinical Characteristics of the Population

In order to explore whether there was an association between HER1 R497K polymorphism and BC, genotyping was performed. Genotyping was completed in the vast majority of DNA samples (Figure 1). We then characterized our population based on patients' genetic information for age, body mass index, SA, tumor size, nodal status, distant metastasis, TNM stage, HER-2/neu status, CEA, and CA153 (Table 2). 78.4% of the patients in stage M0 showed the genotype AA+AC, and 21.6% at this stage were related to genotype CC. In stage M1, all the patients showed the genotype AA+AC. Furthermore, 77.3% of involved lymph node of patients with genotype AA+AC was in stage N 0+1, and 82.4% was in stage N 2+3. According to tumor size, the results showed that 75% of patients with genotype AA+AC were in stage T 1+2, and the remaining 25% showed the genotype CC. Moreover, 90.9% of patients with genotype AA+AC were in stage T3+4, and the remaining 9.1% showed the genotype CC (Table 1). According to the recessive genetic inheritance model, we found that the CC genotype was associated with HER-2 expression (Table 3).

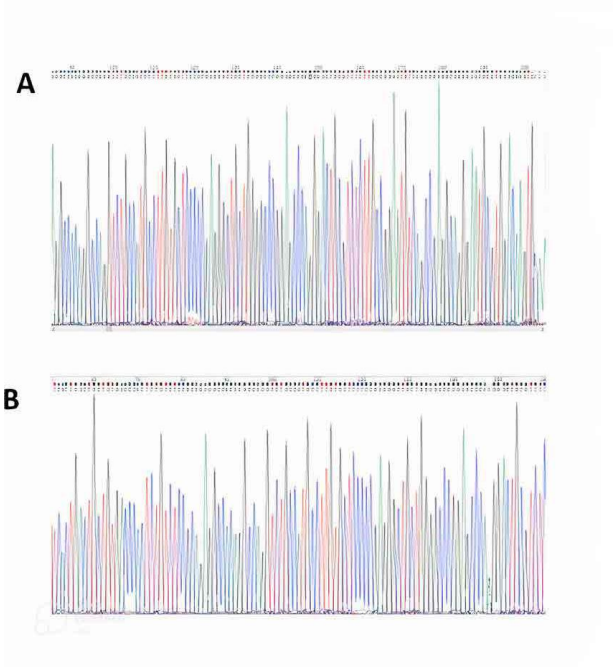

Figure 1: The Sequence of the Amplified Fragment in HER1 R497K (rs11543848)
A) Homozygous; B) AG heterozygote; DNAs were amplified as described above, sequenced with Sanger sequencing and then analyzed with DNA Analyzer.

Table 2: Association Between the HER1 R497K (rs11543848) With Serum Her2, T, N and Cancer Stage Under Recessive+Genetic Model

	OR [b,c] (95% CI)	P Value
Her2[a]	3.6 (0.4-34)	0.2
Tumor Size	3 (0.3-31)	0.8
Node	1.5 (0.2-10)	0.6
Stage	1.2 (0.2-7.5)	0.8

[a] Abbreviation: Her2; human epidermal growth factor receptor 2
[b] Adjusted for age, BMI
[c] + Recessive model; CC genotype (n=8) vs. AA/AC genotype (n=33)

The Relationship Between HER1 R497K Polymorphism With Her2, Estrogen Receptor, Tumor Size, Number of Lymph Nodes, and Stage

The logistic regression analysis under recessive genetic model showed that CC carriers had a higher risk of BC (OR=3.6, 95%CI: 0.4-34), with respect to CC/CG genotypes, although this association was statistically significant. Similar results were also detected for tumor size, node, and stage of disease (Table 3).

Table 3: Baseline and Clinical Characteristics of Case Subjects Under Recessive Model

	CC	AA+AC
Age, y (mean±SD)	48±13	47±11
BMI, kg/m²	22.3	26.9±5
Metastasis		
0	21.6	78.4
1	0.0	100.0
Node, %		
0+1	22.7	77.3
2+3	17.6	82.4
Tumor Size, %		
1+2	25.0	9.1
3+4	75.0	90.9
Estrogen Receptor, %		
0	12.5	87.5
1	20.0	80.0
Stage, %		
0	0.0	0.0
1	0.0	100.0
2	30.8	69.2
3	22.2	77.8
4	0.0	100.0
Her2[a,b], %		
0	12.5	87.5
1	60.0	40.0
2	14.3	85.7
3	10.0	90.0
CEA[c]	2 (1.3)	2 (2.7)
CA153[c]	15 (18.7)	18 (11.7)
P53[c]	1 (0)	1 (0.75)

[a] Abbreviation: Her2, human epidermal growth factor receptor 2
[b] $P<0.05$
[c] Data were reported as med (IQR).

DISCUSSION

Genetic mutation has been contributed to the susceptibility of malignant transformation, including breast carcinomas [10, 25]. 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 [21]. 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 transmembrane protein [26]. HER1 gene amplification has been described in many various cancers [10-15]. 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. In the current study, we analyzed the frequency of HER1 497K and HER2 655V variants in BC patients. Findings of the study demonstrated that a small number of patients with CC genotype were positive for HER2, and most of the cases with AA+AC genotypes were HER-2 positive, suggesting its value as the prognosis of BC patients with CC genotype. There is a growing body of evidence showing the overexpression of HER-2 with poor prognosis in BC patients. The overexpression of HER has been associated with the pathogenesis of different tumors [27] such as lung carcinoma [21], oligodendroglioma [22], and breast carcinomas [23]. The gene map of HER2 (EGFR2) is 17q21, encoded 185 kDa transmembrane glycoprotein, initiates the downstream signaling pathways such as PI3K and RAS-MAPK, which play vital roles in the cell proliferation, differentiation, angiogenesis, and tumor progression [24]. Uzan et al., found that overexpression of HER1 and HER2 is associated with poor prognosis in BC [25]. The overexpression of HER2 has been observed in 10%-25% of non-aggressive BC [21]. Xie et al., showed that the HER2 I655V polymorphism was in relation to breast carcinoma in Chinese women [16], while Kruszyna et al., observed that the gene polymorphism of HER2 I655V can be considered as the susceptible marker in patients with BC [28]. A case-control study by Millikan et al., showed that the risk of breast carcinoma associated with the V/I or V/V genotype compared to I/I increased twofold in patients with breast carcinoma who were younger than 45 years old [29]. In line with our observation, AbdRaboh et al., also showed that HER1 497K polymorphism was associated with the risk of developing BC [12]. Although we observed a significant difference between HER2 expression and the polymorphism in the present study, our findings showed the potential association of CC genotype of HER1 497 with the prognosis of patients with BC.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to disclose.

ETHICS APPROVAL

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study, using protocols approved by the Ethics Committee of Mashhad University of Medical Sciences (License number: IR.MUMS.MEDICAL.REC.1397.309).

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