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ASSOCIATION BETWEEN MDR1 (C3435T) GENE POLYMORPHISM AND RISK OF BREAST CANCER: AN IRANIAN CASE-CONTROL STUDY

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Abstract – *Objective:* *Breast cancer is known as the most prevalent cancer among women and the second most common cancer in the world. Different studies mentioned the chemoresponsiveness potential of breast cancer among solid tumors. Though resistance against chemotherapy drugs is a major problem in cancer therapies that could lead to treatment failure. Multidrug resistance proteins (MDRs) are one of the main members of transporting protein that play a crucial role in pharmacokinetics. P-glycoprotein (P-gp) is the product of MDR1 gene, which is responsible for absorption, distribution, metabolism, excretion (ADME), and drug-drug interaction (DDI) of drugs in humans. The role of MDR1 3435C>T variation on alteration of P-glycoprotein expression and its contribution in risk of breast cancer is proven. Therefore, we decided to examine the role of 3435C>T polymorphism on breast cancer risk in Mazandaran, Iran.*

Patients and Methods: *A case-control study involving 196 breast cancer patients and 98 healthy subjects was conducted. Genomic DNA was isolated from the whole blood sample by a column-based method. The polymerase chain reaction/restriction fragment length polymorphism (PCR-RFLP) technique was used for genotyping of MDR1 3435C>T polymorphism.*

Results: *Distributions of the TT, CT, and CC genotypes of MDR1 were 34.7, 50, and 15.3%, respectively, in controls, and 32.2, 48.9, and 19% in breast cancer patients. There were no significant differences between the cases and controls.*

Conclusions: *Genetic polymorphism of MDR1 C3435T was not found to be associated with increased risk of breast cancer. Further studies on different larger population are needed to confirm these data.*

KEYWORDS: *MDR1 C3435T, Gene polymorphism, Breast cancer, PCR-RFLP.*

INTRODUCTION

According to Globocan 2012, breast cancer is the most common female malignancy and the second most common cancer worldwide[1]. This type of cancer is considered as a significant issue in women's health worldwide[2]. In Iran, breast cancer with increasing rate of mortality has become a major health problem[3]. Development of breast cancer is a multistep process, arising from genetic alterations[4]. MDR1 gene has approximately 200 kb length and 28 exons that are located on the long arm of human chromosome 7[5]. Over-expression of the MDR1 gene is associated with drug resistance[6]. Despite preoperative chemotherapy has great importance in the treatment of breast cancer[7], alteration in expression of MDR1 could adversely affect chemotherapy treatment in cancer patients. MDR1 gene is highly polymorphic gene and among 50 reported MDR1 polymorphisms, 38 single nucleotide polymorphisms (SNPs) are detected in the coding region[8]. The presence of polymorphisms in this gene may result in gene expression, the sequence of amino acids in the constitution of proteins, protein functions, and response to treatment[9]. There is a high geographical variation in the field of cancer[10] and the paucity of information about the risk of developing breast cancer risk and MDR1 changes in Iran. Therefore

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this investigation has focused on the possible association between C3435T MDR1 gene polymorphism and breast cancer in Mazandaran Province in North of Iran.

PATIENTS AND METHODS

The present study was approved by the Ethics Committee of Mazandaran University of Medical Sciences, Sari, Iran (Approval No. IR.MAZUMS.IMAMHOSPITAL. REC. 95.2721). Written informed consent was obtained from all subjects following full disclosure of the study objectives and procedures.

Study population

The current project was conducted in 196 breast cancer patients who referred for receiving chemotherapy medications at Gastrointestinal Cancer Research Center, Mazandaran University of Medical Sciences, Sari, Iran from July 2016 to September 2017. The included subjects were aged from 15 to 78 years old. Normal controls (N=98) were selected among healthy women attending for a routine checkup.

DNA Isolation and RFLP

The genomic DNA was purified from peripheral blood samples derived from normal donors and breast cancer patients using spin column-based DNA isolation kit (DENAzist, Mashhad, Khorasan, Iran). The concentration of genomic DNA samples was determined by Nanodrop spectrophotometer (WPA, Cambridge, UK) and the quality of genomic DNA samples was analyzed by agarose gel electrophoresis (1%). The exon 26 related to *MDR*1 was amplified by the PCR using specific forward and reverse primers (Table 1). PCR was performed in a total volume of 25 microliters under the following conditions: denaturation at 94°C for 5 min, followed by 40 cycles at 94°C (for 30 s), 56°C (for 30 s), 72°C (for 30 s) and a final extension at 72°C for 5 min. Sufficient PCR products were used for Restriction Fragment Length Polymorphism (RFLP). The PCR products were digested by *Mbo*I restriction enzyme (Fermentase, Lithuania) according to the manufacturer's protocol. The PCR product of the 207 bp in TT genotype was digested into fragments of 145 and 62 bp for CC genotype. The presence or absence of digested fragments was detected by the agarose gel electrophoresis 3% containing DNA green fluorescent dye (Parstous, Tehran, Iran).

Statistical Analysis

Data were analyzed in SPSS 19 8IBM, Armonk, NY, USA), by x^2-test and *t*-test. In order to determine the correlation between MDR1 polymorphisms and incidence of breast cancer, the odds ratio (OR) and 95% confidence intervals (CI) were calculated. A *p*-value < 0.05 was considered to be statistically significant.

RESULTS

A case-control study was conducted in 196 breast cancer patients and 98 healthy controls to determine whether the MDR1 genetic polymorphism is associated with the development of this cancer. The mean ages of breast cancer patients and controls were 49.83 and 39.16 years old (Table 2). Patients were stratified into three groups based on the age at diagnosis, under 50 years, patients between the age of 51 and 59, and those over 60 years old. There was a significant difference between patients and control groups in age at diagnosis (p <0.000). Demographic characteristics of patients with breast cancer are shown in Table 2. The proportion of patients with lower tumor size (≤ 30 mm) and patients with ductal histology were 64.9% and 94.9%, respectively. The percentage of patients with positive estrogen receptor (ER), positive progesterone receptor (PR), HER2 (human epidermal growth factor receptor 2), and Ki67 index were 70.3%, 62.6%, 76.7%, and 97.6% respectively. 35.8% of the included patients were postmenopausal (Table 2). Analysis of MDR1 C3435T polymorphism revealed that homozygote wild-type allele was found in 37 (18.9%) patients with breast cancer and in 15 (15.3%) controls. The TT genotype was found in 65 (33.2%) patients and in 34 (34.7%) control subjects. The difference in genotype frequencies between controls and patients of MDR1 (rs1045642) was not found to be statistically significant (p=0.751) (Table 3 and Table 4). The distribution of MDR1 C3435T polymorphism in breast cancer patients and controls is shown in Figure 1. Figure 2 illustrates the results obtained by MDR1 genotyping: one band (207 bp) for homozygous TT genotype, two bands (145 and 62 bp) for homozygous CC genotype and three bands (207, 145, and 62 bp) for heterozygous CT genotype.

TABLE 1. Specific forward and reverse primers.

SNP	*Position*	*rs name*	*Primers sequence*	*PCR product size*
C3435T	87509329	rs1045642	Forward: TTGATGGCAAAGAAATAAAGC Reverse: CTTACATTAGGCAGTGACTCG	207 bp

TABLE 2. Clinic and Demographic Characteristics of Participants.

Characteristics	*Case N (%)*	*Control N (%)*	*Total N (%)*	*p-value*
Gender				
Men	2 (1.1)	0	2 (0.7)	0.287
Women	196 (98.9)	98 (100)	294 (99.3)	
Ages (year)				
≤ 50	99 (50.5)	77 (78.6)	166 (61.0)	<0.000
51 – 59	55 (28.1)	11 (11.2)	58 (21.3)	
≥ 60	42 (21.4)	10 (10.2)	48 (17.6)	
Mean ± SD	49.83 ± 10.80	36.16 ±13.77	45.99 ± 12.99	
Tumor size				
≤ 30 mm	50 (64.9)			0.931
31 – 40 mm	19 (24.7)			
≥ 41 mm	8 (10.4)			
Total	77 (100)			
Tumor type				
Ductal	74 (94.9)			0.954
Lobular	4 (1.5)			
Total	78 (100)			
ER				
Positive	64 (70.3)			0.144
Negative	27 (29.7)			
Total	91 (100)			
PR				
Positive	57 (62.6)			0.044
Negative	34 (37.4)			
Total	91 (100)			
Menopausal status				
Pre-menopausal	52 (64.2)			0.077
Post-menopausal	29 (35.8)			
Non-postmenopausal	96 (55.2)			
Her2/neu				
Positive	69 (76.7)			0.483
Negative	21 (23.3)			
Total	90 (100)			
Ki-67				
Positive	82 (97.6)			0.692
Negative	2 (2.4)			

Data are expressed as mean ± standard deviation (SD).
Abbreviations: ER, Estrogen receptor; PR, Progesterone receptor;

TABLE 3. Genotype frequencies of MDR1 (C3534T) gene polymorphism in breast cancer patients.

Models	*Cases (freq) N (%)*	*Controls (freq) N (%)*	*Total N (%)*	*p-value*
Genotype				
CC	37 (18.9)	15 (15.3)	48 (17.6)	0.736
CT	64 (48)	49 (50.0)	134 (49.3)	
TT	65 (33.2)	34 (34.7)	90 (33.1)	

TABLE 4. Genotype frequencies of MDR1 (C3534T) gene polymorphism in breast cancer patients.

Models	*Cases (freq) N (%)*	*Controls (freq) N (%)*	*Total N (%)*	*p-value*	*OR (95% CI)*
Allele					
C	168 (42.9)	79 (40.3)		0.595	0.9 (0.63- 1.27)
T	224 (57.1)	117 (59.7)			

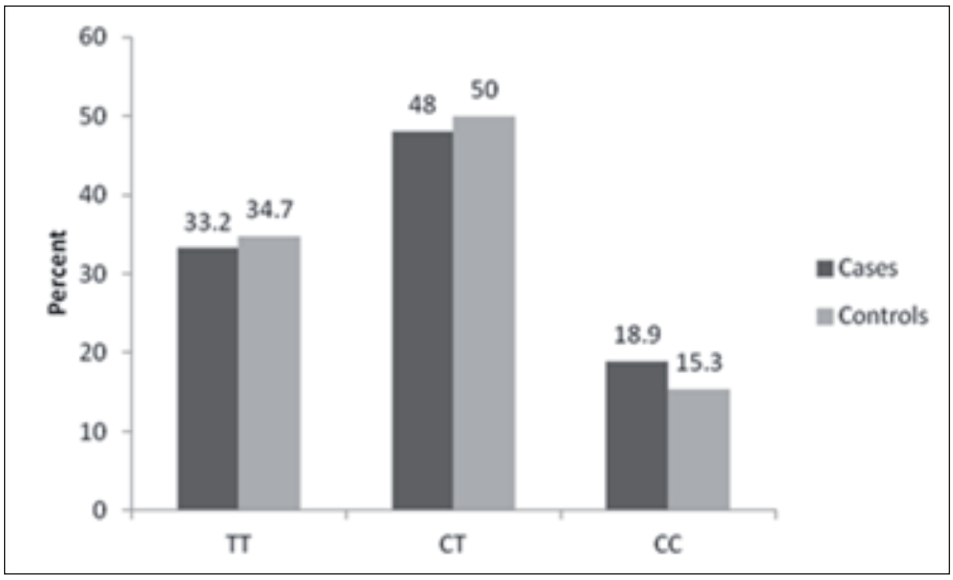

Fig. 1. Distributions of MDR1 variants. Bar charts show the distribution of MDR1 polymorphism in breast cancer patients and cancer-free controls.

DISCUSSION

Breast cancer is one of the most common cancer among females and more than one-third of women experience it in their lifetime[11]. Different types of treatment options such as mastectomy, chemotherapy, hormonal therapy, anti- Her2 therapy, axillary dissection, and radiation therapy are used in the treatment of breast cancer patients[12]. Resistance against anticancer drugs remains a major issue for achieving the best clinical response. Multidrug resistance (MDR) is the main resistance way that may interfere with cancer treatment options or affect the recurrent phase. Several studies have shown evidence of the role of gene polymorphisms in relation to drug metabolism via molecular pathways[13]. The ATP-binding cassette (ABC) transporters play an important role in drug metabolism, uptake, and efflux. P-glycoprotein (ABCB1), a member of this family, is encoded by the multidrug resistance 1 (MDR1) gene[14]. At first MDR1 gene was known as multidrug resistance (MDR) of cancer cells. Then other transporters were discovered to be resistant against drug entrance especially anticancer drugs, thereby causing treatment failure[15]. In case of anticancer chemotherapy drugs, genetic variations deeply affect the drug efficacy, their toxicity, and therapeutic response[13]. MDR1 is expressed in both normal and tumor cells. It mainly acts as a cellular metabolite transporter. Knockout *in-vitro* analysis proved its role in drug metabolism via a high level of drugs bioavailability and drugs elimination in absence and presence of MDR1 homologs[15]. The expression of human P-glycoprotein is associated with the metabolism of breast cancer chemotherapy drugs and alters their absorption. Several types of research proved the relationship between MDR1 regulation and P-glycoprotein expression in different types of cancers[14]. Polymorphisms in the MDR1 gene are associated with variation in P-glycoprotein expression levels and change the role of P-glycoprotein as a drug transporter (16). MDR1 gene is known as a highly polymorphic gene; therefore, different

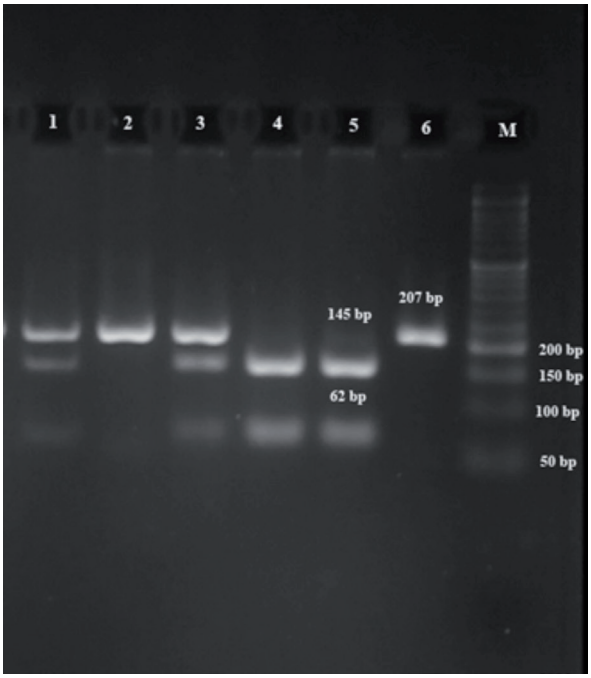

Fig. 2. PCR-RFLP patterns of the MDR1 3435C>T with Mbo I. 1 and 3 lanes: CT genotype (heterozygous samples); 2 and 6 lanes: TT genotype; 4 and 5 lanes: CC genotype; and M: Ladder 50 bp.

SNPs such as 1236C>T, 2677G>T/A, and 3435C>T are suggested to affect the P-glycoprotein permeability and the metabolism of anticancer drugs[17].

Here, we investigated the C3435T *MDR*1 polymorphisms in breast cancer patients and healthy controls. Our results found no significant differences in genotype and allele distribution between cases and controls. Similar results were obtained by Ghafouri et al[18]. They found no association between ABCB1 C3435T variant and risk of breast cancer among the Kurdish population. They also reported that these polymorphisms were not associated with tissue expression of ER, PR, Her2/neu, and Ki67 (18). Whereas in the current study progesterone receptor expression was found to be significantly different between the cases and controls (p= 0.044). In a study on Iranian population, no association was detected in genotype and allele frequencies between the patients and controls. Furthermore, the risk factors for breast cancer were not related to TT, CT, and CC genotypes[19]. Another case-control study among 150 breast cancer patients and 140 healthy controls showed that T allele was associated with breast cancer risk[20]. The PCR-RFLP results of a study in 2006-2007 showed no correlation between C3435T polymorphism of *MDR*1 and response to treatment[21]. Lu et al[22] reported that ABCB1 C3435T polymorphism was not associated with increased risk of breast cancer. George et al[23]) showed the significant prevalence of TT genotype in exon 26 of the *MDR*1 gene in breast cancer patients. A case-control study in Jordan came to a conclusion that *MDR*1 C3435T polymorphisms increased the risk of breast carcinoma[24]. According to another study, TT homozygotes seems to be at higher risk of breast cancer (OR = 2.12; 95% CI = 1.12-4.03). They also found significant differences in allelic and genotype distribution between patients and controls[25]. No significant differences were observed in the C3435T variant in a meta-analysis conducted in Moroccan population. No differences were observed in allele distribution between patients and controls (p = 0.84)[26]. In a silico-analysis performed by Kheirkhah and Karimian[27], no significant association was observed between *MDR*1 c.3435T>C and risk of breast cancer in the Asian population. It was revealed that the 3435T>C variant was correlated with the risk of breast cancer among the Iranian population. It was also suggested that this transition mutation may affect the RNA structure and its splicing arrangement. No association was found between the allelic and genotypic distribution of ABCB1 3435 C/T in breast cancer patients and controls[28]. It was reported that T allele carriers in combination with another risk factor may be correlated with the development of breast cancer[28]. The same results came from another study of Czech breast cancer patients[29]. They described that ABCB1 3435C>T SNP reduced the expression of ABCB1, altered the function of P-glycoprotein, and affected breast cancer prognosis [29]. MDR1 C3435T genotyping by PCR-RFLP method showed a high frequency of T allele in patients compared with that in controls[16]. Their findings determined that T allele carriers are more susceptible for development of breast cancer [OR = 1.5 (95% CI: 1.09 -1.96)]. Moreover, according to Turgut et al[16] significant differences were observed in genotype frequencies of MDR1 C3435T[16]. Different meta-analysis studies examined the possible association between C3435T variant and risk of breast cancer. Wang et al[30] suggested that the MDR1 C3435T polymorphism is highly associated with the risk of developing breast cancer. In the current study, we found no relationship between C3435T MDR1 polymorphism and risk of breast cancer. With notice to the presence of genetic differences in different communities[31] and significant correlation in another region, C3435T variant may be ethnic-dependent.

CONCLUSIONS

Further investigations with larger sample sizes from different regions may prove the role of ethnicity in pharmacokinetic variations. This study suggests that C3435T polymorphism of the MDR1 gene does not have any association with breast cancer in the north of Iran.

Conflict of Interest:

The authors declare that there is no conflict of interests regarding the publication of this paper.

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