Genetic Polymorphisms of the Efflux Transporter Gene ABCB1 and Their Effects on the Anastrozole Response in Iraqi Breast Cancer Patients

Hiba Salah Mahdi[1], Ahmed Salih Sahib[2], Hassan Mahmood Mousa Abo Almaali[3], Karrar Kadhim Mohsin[4]

[1]Bachelor in Pharmacy, Department of Pharmacology and Toxicology, College of Pharmacy, University of Kerbala, Iraq, [2]Professor, Department of Pharmacology and Toxicology, College of Pharmacy, University of Kerbala, Iraq, [3]Assistant Professor, Department of Clinical Laboratory Sciences, College of Pharmacy, University of Kerbala, Iraq, [4]Diploma, Imam Al-Hussein Hematology and Oncology Center, The Holy City of Kerbala, Iraq

Abstract

Background: The ABCB1 gene (ATP-binding cassette) encodes the ABCB1 transporter. Anastrozole is a substrate for the ABCB1 efflux transporter. The objective of the study was to detect the genetic polymorphisms of this efflux transporter in Iraqi breast cancer patients and their effects on the anastrozole response.

Method: Blood samples were taken from breast cancer patients, and a portion of each sample was used for biochemical analysis (measuring estradiol and cancer antigen 15.3 levels), while the remaining portion was used for genetic analysis. Genotyping demonstrates that the mutant genotype being the most frequent type for both C1236T and C3435T SNPs.

Results: The result indicated nonsignificant differences in the serum levels of estradiol or cancer antigen 15.3 among different genotype groups of the ABCB1 gene.

Conclusion: It is concluded that the ABCB1 gene is highly polymorphic in Iraqi women with breast cancer. This study indicated that ABCB1 gene may not affect the response to anastrozole.

Keywords *Anastrozole, ABCB1 gene, estradiol, cancer antigen CA15.3, arthralgia.*

Introduction

Breast cancer (BC) is one of the most frequent cancers worldwide, with an elevated incidence rate in all countries [(1)]. In Iraq, BC is the first among the top ten malignancies affecting society. In 2016, hundreds of females died from this disease, which is recorded as the leading cause of cancer-related mortality among Iraqi women [(2)]. Of the risk factors that have been associated with BC, such as family history, genetic mutations, lifestyle and others, estrogen is a major factor in breast cancer pathogenesis. Factors such as obesity, age at menarche and menopause, and hormone replacement therapy have been demonstrated to increase the risk of BC [(3)].

Tumor markers are biomarkers that are produced at higher levels by cancer cells or by the host in response to cancer. One of these markers is cancer antigen (CA) 15-3, which is used to determine breast cancer prognosis and to monitor the efficacy of therapy [(4)].

Because of the role of estrogen in carcinogenesis, hormonal therapy targeted at decreasing estrogen production or its action has been applied in various ways to manage BC [(3)]. Decreasing the quantity of estrogen by inhibiting its production can be achieved with aromatase inhibitors (AIs), such as anastrozole [(5)]. Anastrozole is a nonsteroidal inhibitor that inhibits aromatase enzyme competitively by binding to the heme group of the enzyme, thus decreasing estrogen biosynthesis in the breast and periphery [(6)].

One study showed that anastrozole is a substrate for ATP-binding cassette B1 (ABCB1) transporter [(7)], an energy-dependent xenobiotic efflux pump that is expressed at the apical membrane in tissues that are involved in excretion or form blood-tissue barriers.

Several studies have confirmed the notable impact of drugs transport via ABCB1 on the pharmacokinetics of these drugs in humans [8]. Additionally, the ABCB1 gene has been identified to have multiple single nucleotide polymorphisms (SNPs) that effect on drug disposition and clinical outcomes [9]. Of these mutations, the most well studied are two synonymous transitions (C1236T and C3435T) located in exons 12 and 26, respectively [10].

Patients and Methods

Study samples

This cross-sectional observational study included Iraqi females with breast cancer taking 1 mg of anastrozole by tablet daily. This study was carried out at the Oncology Center in Kerbala at Imam AL-Hussein Medical City.

The protocol of the study was approved by the Ethical Committee of Pharmacy College at Kerbala University, and each participant was given a written informed consent form for their participation.

The study was conducted on 100 females aged 34-76 years, who had estrogen receptor and/or progesterone receptor (ER, PR)-positive breast cancer.

The exclusion criteria for this study involved: taking other adjuvant endocrine therapies, taking anastrozole therapy concomitantly with either adjuvant chemotherapy or adjuvant radiotherapy (or both), females with a history of gastrointestinal surgery or disorders, women who took CYP3A4/5 or UGT1A4 inducers or inhibitors or any drugs affecting ABCB1 transporter, and females who were pregnant or lactating.

Clinical data collection

The clinical data were taken from the medical records of the patients and from the women themselves and included weight, height, age, workplace, academic achievement, marital status, breast feeding, pre- or postmenopause, family history of breast cancer, date of breast cancer and duration, site, grade and stage of breast cancer, presence of liver or any other diseases, and time and duration on anastrozole.

Sample collection and analysis

A 5 ml peripheral blood sample was taken from each female, 3ml of the blood was placed in a gel tube and used to measure the biochemical parameters, and 2 ml was placed in an EDTA tube for the genetic assay.

Biochemical parameters

Estradiol level (E2)

E2 levels in the serum of breast cancer patients were determined by using a CL-series chemiluminescence immunoassay (CLIA) analyzer (Mindray/China). This assay is a competitive binding immunoenzymatic assay [11]. The method was performed according to a kit.

Normal values:

Postmenopausal (<25-84 pg/ml)

Follicular phase (20-138 pg/ml)

Ovulation phase (100-440 pg/ml)

Luteal phase (31-317 pg/ml)

Tumor marker CA15.3

Levels of the serum tumor marker CA15.3 in breast cancer patients were determined by using a Minividas (Biomerieux/France) instrument, which utilizes the enzyme linked fluorescent assay (ELFA) technique [12, 13]. The method was performed according to a kit.

Normal value: 0-30 U/ml.

Genotyping

Genomic DNA was extracted from blood samples using the G-DEX™IIb kit (iNtRON/Korea).

ABCB1 gene polymorphism genotyping

C1236T and C3435T were detected using amplification refractory mutation system PCR (ARMS PCR). Primers (synthesized by Macrogen/Korea) were used for C1236T and C3435T identification, and the detection of alleles is shown in Table (1).

Table (1) Primer sequences for determining the genotypes of C1236T and C3435T based on product size (14).

SNPs	Primer sequences	Product size
1236P1	5' AAT GTT CAC TTC AGT TAC CCA TCT CG 3'	508
1236P2	5' AAT GAT TTC CCG TAG AAA CCT TAC 3'	
1236C	5' TGG TAG ATC TTG AAG CGC 3'	305
1236T	5' TGC ACC TTC AGG TTC TGA 3'	238
3435P1	5' TGC TGG TCC TGA AGT TGA TCT GTG AAC 3'	300
3435P2	5' GGC CAG AGA GGC TGC CAC AT 3'	
3435C	5' GTG TCA CAG GAA GAG TTC 3'	126
3435T	5' TCC TTT GCT GCC CTC TCA 3'	209

The PCR mixture was prepared in an AccuPower® PCR PreMix (Bioneer/Korea) tube by adding 1 µl of each primer at 10 pmol/µl, and 5 µl of DNA, and the volume was brought to 20 µl with distilled water. PCR was carried out with the following program: initial denaturation for 3 minutes (min) at 94ºC, followed by 35 cycles of denaturation at 94ºC for 30 seconds (s), annealing at 60ºC and 58 ºC for C1236T and C3435T respectively for 30 s, and extension at 72ºC for 55 s. Final extension was performed at 72ºC for 5 min. Amplified segments were separated by electrophoresis on a 1.5% agarose gel, stained with ethidium bromide and observed under ultraviolet (UV) light.

Statistical Analysis

For statistical analysis, SPSS software for Windows (version 15.0 USA) was used. Single-factor ANOVA was performed to examine the differences in the mean of parameters tested within genotype groups. Odds ratios and the corresponding 95% CIs were used to examine associations. For all tests, p-values of <0.05 were considered statistically significant.

Results

Study Population

The general characteristics of the participants: the mean age of women who participated in this study was 57.51 (range: 34-76); the percentages of women who were married and unmarried were 94% and 6%, respectively; the menopausal status of the patients was 95% postmenopausal and 5% premenopausal; the proportion of women who depended on lactation to feed their babies was 64%, while 19% did not lactate and 17% undertook mixed feeding; the percentages of females who had and did not have a family history of breast cancer were 12% and 88%, respectively; some patients presented with breast cancer on the right side (48%), others on the left side (49%) and only 3% presented with breast cancer on both sides; the percentage of patients who were both ER- and PR-positive was 94%, while only 6% of patients were ER- or PR-positive; and the proportion of women who suffered from arthralgia (as a side effect of anastrozole) was 89%, while 11% did not have arthralgia.

Frequency and distribution of ABCB1 gene polymorphisms

The percentage of ABCB1 genotypes detected in 100 Iraqi breast cancer patients are shown in Table (2). For C1236T, the frequencies of patients who carried CC, CT, and TT were 28%, 18%, and 54%, respectively. For C3435T, 9% patients had the wild genotype (CC), 76% had the mutant genotype (TT), and only 15% patients carried the heterozygote genotype (CT). The most frequent genotype for both SNPs was TT.

Table (2): The percentage of C1236T and C3435T detected in Iraqi breast cancer patients.

SNPs	Genotypes	Percentage
C1236T	CC (wild type)	28%
	CT (heterozygote)	18%
	TT (mutant type)	54%
C3435T	CC (wild type)	9%
	CT (heterozygote)	15%
	TT (mutant type)	76%

SNPs: single nucleotide polymorphisms, C: cytosine, T: thiamine.

Effect of ABCB1 gene polymorphisms on estradiol levels (E2)

Table (3) shows the mean and standard deviation (SD) of estradiol levels in the detected genotypes for C1236T and C3435T SNPs. For C1236T, patients with CC had a lower mean level of E2 (19.482 ± 15.06 pg/ml), while those with CT had the highest mean level of E2 (22.656 ± 8.02 pg/ml), and the mean level in those carrying the TT genotype was 19.716 ± 9.717 pg/ml.

In C3435T, the mean and SD values of E2 in patients who had the CC, CT, and TT genotypes were 15.275 ± 6.817 pg/ml, 23.023 ± 18.803 pg/ml, and 20.2 ± 9.45 pg/ml, respectively. There were nonsignificant differences in the mean levels of E2 and genotype groups for both SNPs.

Table (3) Mean and standard deviation of E2 levels in the detected ABCB1 (C1236T and C3435T) genotypes.

SNPs	Genotypes	E2 level Mean ± SD Pg/ml	P value
C1236T	CC	19.482 ± 15.06 a	0.585
	CT	22.656 ± 8.02 a	
	TT	19.716 ± 9.717 a	
C3435T	CC	15.275 ± 6.817 a	0.26
	CT	23.023 ± 18.803 a	
	TT	20.2 ± 9.45 a	

P value derived from ANOVA test, $p < 0.05$ is significant, $p > 0.05$ is nonsignificant. Same letters indicate nonsignificant differences. SD: standard deviation.

Effect of ABCB1 gene polymorphisms on cancer antigen CA15.3 levels

Table (4) shows the mean and standard deviation values of CA15.3 levels in different genotype groups for C1236T and C3435T. For C1236T, the mean and SD of CA15.3 in patients with the wild genotype was 15.616 ± 6.26 pg/ml which is the lowest mean among the different genotypes. The highest mean of CA15.3 appeared in the heterozygous genotype (23.235 ± 14.73 pg/ml). The mean and SD of CA15.3 in patients with

the mutant genotype was 20.507 ± 16.446 pg/ml. There were significant differences in the mean levels of CA15.3 between the wild type and heterozygote genotypes, while there were nonsignificant differences between the wild type and mutant or between the heterozygote and mutant genotypes.

For C3435T, there were nonsignificant differences between the mean CA15.3 and genotype groups. The mean and SD values of CA15.3 for CC, CT, and TT were 14.438 ± 5.43 pg/ml, 14.882 ± 6.42 pg/ml, and 21.417 ± 15.93 pg/ml, respectively.

Table (4) Mean and standard deviation of CA15.3 levels for the detected genotypes of ABCB1 gene polymorphisms.

SNPs	Genotypes	CA15.3 level Mean ± SD	P value
C1236T	CC	15.616 ± 6.26a	0.163
	CT	23.235 ± 14.73b	
	TT	20.507 ± 16.446ab	
C3435T	CC	14.438 ± 5.43a	0.09
	CT	14.882 ± 6.42a	
	TT	21.417 ± 15.93a	

SNPs: single nucleotide polymorphisms, CA15.3: Cancer Antigen 15.3, SD: standard deviation. P value derived from ANOVA test, $p< 0.05$ is significant, $p> 0.05$ is nonsignificant. The same letters indicate nonsignificant differences, and different letters indicate significant differences.

Association of ABCB1 gene polymorphisms and the elevation of CA15.3 levels

The association between the genetic polymorphisms in ABCB1 and the elevation in serum levels of CA15.3 was determined by using odds ratios (Table (5)). For both SNPs, there was a nonsignificant association ($p> 0.05$) between ABCB1 gene polymorphisms and the elevation in CA15.3.

Table (5) Association of ABCB1 gene polymorphisms (C1236T and C3435T) with the elevation of CA15.3 levels.

SNPs	Odds ratio (CI-95)	P value
C1236T	1.56 (0.427-5.721)	0.499
C3435T	1.477 (0.296-7.364)	0.633

CI-95: confidence interval 95%, $p< 0.05$ is significant.

Association of ABCB1 gene polymorphisms with the occurrence of arthralgia

Table (6) shows the association between ABCB1 gene polymorphisms and the onset of arthralgia. For C1236T and C3435T, there were nonsignificant associations with the development of arthralgia ($p> 0.05$).

Table (6) Association of ABCB1 gene

polymorphisms (C1236T and C3435T) with the occurrence of arthralgia.

SNPs	Odds ratio (CI-95)	P value
C1234T	0.401 (0.099-1.611)	0.1979
C3435T	1.214 (0.295-4.994)	0.787

CI-95: confidence interval. $p< 0.05$ is significant.

Discussion

An in vitro study introduced by Miyajima et al. indicated that anastrozole is a substrate for ABCB1 transporters [7]**.** Because ABCB1 is a highly polymorphic transporter, variability in this gene has been associated with changes in drug response, disposition, and toxicity [9]. Therefore, to obtain an effective therapeutic response and lower the side effects of anastrozole, it is important to study the effects of genetic polymorphisms of ABCB1 on the therapeutic response (by measuring serum E2 and CA15.3 levels) in Iraqi women with breast cancer treated with anastrozole.

The association between the development of breast cancer and the continued increase in serum estrogen levels has been studied by several researchers. These studies suggest that estrogen is a mammary gland carcinogen [15]. Therefore, the use of aromatase inhibitors is a common strategy to treat women with hormone receptor-positive breast cancer [16]. Although anastrozole was used as an aromatase inhibitor, this study found a detectable quantity of E2 in the serum of breast cancer patients that was still within normal limits (Table 3), which suggests that there may be other pathways for estrogen synthesis. These results were in agreement with a study introduced by Abd-Allateef et al. (2016), who found a detectable concentration of estrogen in females with breast cancer treated with anastrozole despite complete inhibition of the aromatase enzyme [17]. In this study, both SNPs (C1236T and C3435T) exhibited nonsignificant differences in the mean E2 among genotype groups, with those who carried the heterozygote genotype CT (in both SNPs) having the highest mean E2 level, although it was still within normal limits. A study conducted on females with breast cancer found that patients who carried TT genotypes had significantly lower ABCB1 expression [18]. Accordingly, the capacity of ABCB1 transporters may be lowered, so patients with TT may respond better to treatment than those with wild genotypes, although this was not indicated in our results[20].

Serum tumor markers are soluble molecules present in the blood that are discharged from tumor cells, and they are usually used to determine the response to anticancer drugs or determine prognosis because their concentrations may indicate the presence of hidden metastasis or reflect the extent of the tumor mass [19,21]. In the present study, there were nonsignificant associations between serum CA15.3 and the genotype groups for both SNPs, except for the CT genotype of C1236T, which showed a significant difference in the mean CA15.3 levels relative to the CC genotype (Table 4). [19]

This study revealed that there were nonsignificant associations between different genotype groups of the ABCB1 gene and the elevation of CA15.3 (Table 5), which may be because these SNPs (C1236T and C3435T) are synonymous and do not alter amino acids or affect activity [10].

Arthralgia, one of the common adverse effects of anastrozole, usually affects quality of life and may lead to the patient discontinuing treatment [22]. In the present study, most women suffered from arthralgia, but there was nonsignificant association between the occurrence of this side effect and ABCB1 gene polymorphisms (Table 6). This supports the findings of a study by Gervasini et al. (2017), who did not report a statistically significant association between C1236T and the occurrence of arthralgia, although the same study found that C3435T was inversely associated with arthralgia development [23].

Conclusions

It can be concluded that the ABCB1 gene is highly polymorphic, with mutant genotype TT being the most frequent for both SNPs in Iraqi breast cancer patients. This study revealed that ABCB1 gene polymorphisms may not impact the anastrozole response.

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Ethics approval: This study was approved by the ethical committee of the College of Pharmacy at the University of Kerbala.

Conflict of Interest - The authors declare no conflict of interest.

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