Original
ISSN 2413-0516

Study of the Genetic Polymorphisms of ABCB1 3435G>A in Postmenopausal Women Breast Cancer on Paclitaxel Chemotherapy

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(Submitted: 13 February 2023 – Revised version received: 28 February 2023 – Accepted: 22 April 2023 – Published online: 26 June 2023)

Abstract

Objectives: The aim of study was to determine the relationship between this SNPs in postmenopausal women with breast cancer 3435G>A (rs1045642) polymorphism of the ABCB1 gene, and serum concentrations of Paclitaxel chemotherapy in Iraq.

Methods: This study included 100 patients (45–75 years) with a clinical diagnosis of breast cancer who were going to start (paclitaxel) chemotherapy. Patients at the oncology center at Imam al-Hussain medical city in Kerbala Iraq, were sorted into groups based on their postmenopausal age, disease duration, and treatment length for this study, which was conducted between July 2022 and October 2022. Serum concentrations of Estradiol and Ca 15.3 were measured in breast cancer patients who had been taking Paclitaxel. Allele-Specific PCR assay determined gene polymorphisms 3435G>A (rs1045642) and HPLC measured paclitaxel drug concentration.

Results: The results found There is an inverse relationship between Ca15.3 concentration and patient response to paclitaxel chemotherapy, and when compared to the genotyping frequency of ABCB1 3435G>A (rs1045642) genetic polymorphism, patients with allele AA in their chromosomes respond better (15.56 ± 6.89) than those with alleles GG and GA.

Conclusion: After paclitaxel chemotherapy, there were statistically significant differences ($P < 0.05$) in the reduction of drug concentration among serum patients with the AA genotype for ABCB1 3435G>A (rs1045642).

Keywords: ABCB1, breast neoplasms, polymorphism, chromatography, high pressure liquid, paclitaxel

Introduction

Breast cancer is a malignant tumor that has developed from cells of the breast. The disease occurs mostly in women, but does occur rarely in men.[1]

Some breast cancers are called insitu, because they do not spread beyond the area where they begin, while others are invasive cancer. The majority of insitu tumors will not progress to become an invasive tumor, and at this stage nearly all of these cancers can be cured.[2]

Among women, breast cancer has remained the commonest tumor in Iraq, it forms 22.3% of all malignant tumor and 37% of the registered female cancers with a sharp increase in the incidence of this tumor in younger age group. It forms 5% of those under 30 years and 75% of those older than 40 years and highest number of cases is between 40 and 50 years, and the incidence looks to be increasing.[3]

Delay in the diagnosis of breast cancer is an area of increasing litigation for medical practitioners, both in primary care and in hospital practice. Whether delay in the diagnosis of breast cancer affects the outcome has been uncertain, but some studies have found evidences of reduced survival.[4] Chemotherapy is a cancer treatment where medicine is used to kill cancer cells. There are many different types of chemotherapy medicine, but they all work in a similar way. They stop cancer cells reproducing, which prevents them from growing and spreading in the body.[5]

Paclitaxel belongs to the family of cytoskeletal drugs that target tubulin. As a result, paclitaxel treatment leads to abnormality of the mitotic spindle assembly, chromosome segregation, and consequently defects of cell division. By stabilizing the microtubule polymer and preventing microtubules from disassembly, paclitaxel arrests cell cycle in the G0/G1 and G2/M phases and induces cell death in cancer. It has been known that inhibition of mitotic spindle using paclitaxel usually depends on its suppression of microtubule dynamics.[6]

ATP binding cassette (ABC) multidrug transporters such as P-glycoprotein (P-gp, ABCB1) and BCRP (ABCG2) confer resistance against anticancer drugs and can limit their oral availability, thus contributing to failure of chemotherapy. Like P-gp and BCRP, another ABC transporter, MRP2 (ABCC2), is found in apical membranes of pharmacologically important epithelial barriers and in a variety of tumors. MRP2 transports several anticancer drugs and might thus have a similar impact on chemotherapy as P-gp and BCRP.[7] The most widely studied variant of ABCB1 is a commonly synonymous C to T transition at nucleotide position 3435 in exon 26 (3435 G > A), an exon 26 of the MDR1 gene is responsible for the up regulation of this gene's activity, which in turn effluxes a wide variety of chemicals throughout the plasma membrane (Ozen et al. 2011).[8] The study aimed to detect the genetic polymorphism of ABCB1 3435G>A (rs1045642) in participated breast cancer women and to investigate the effect of genetic polymorphism on Paclitaxel drug concentration.

Materials and Methods

A cross-sectional study was carried out at the Imam Al-Hussein oncology center in Kerbala city in Iraq. The sample collection was carried out between July and December of 2022 and in the University of Kerbala's College of Pharmacy laboratories.

Patients were randomly assigned to receive paclitaxel intravenously once a week for 1 or 3 hours.

Postmenopausal women having a histologically proven diagnosis of breast cancer were the patients seen at Imam Hussein Hospital. They were admitted for endocrine, radiation, or

cytotoxic treatments. While Controls consisted of age-matched, breast-cancer-free women.

Inclusion Criteria

Women with histologically confirmed breast cancer and post menopause, they were required to be non-pregnant, all patients were required to have clinically or radiographically measurable disease and to have adequate renal and hepatic function normal, and postmenopausal women aged range (45–75 years).

Exclusion Criteria

Patients were ineligible if they had diabetic mellitus or any other underlying medical condition that would hinder study participation, those with child-bearing potential who did not implement adequate contraceptive measures were also ineligible.

Genotyping for ABCB1 Polymorphisms Detection

In our research, we have shown that allele-specific amplification using polymerase chain reaction (PCR) is widely utilized for genotyping single-nucleotide polymorphisms (SNPs). However, the manufacturer (Add bio/korea) reports that the isolation of genomic DNA from whole blood that is PCR-compatible is the standard method of use. Primer 3 plus creates primer pairs that are specific to the template, and the specificity testing software looks for primer-target matches in databases containing ABCB1 3435G>A (rs1045642) polymorphisms using BLAST websites (Table 1). The quantities of nuclease-free water added to each primer to achieve a concentration of 100 pmol/l (represent a stock solution). Lyophilized primers were dissolved with the appropriate volume of nuclease-free water per manufacturer instructions.

Premixed PCR tubes (AccuPower® PCR PreMix/korea) were made with a final volume of 20 µl for PCR sample collection. PCR protocol was consist of 1.5 µl of forward primer, 1.5 µl of reverse primer, 4 µl of DNA template, and 12 µl of distal water, the PCR amplification program consist of initial denaturation at 95°C for 5 minutes, the 35 cycles include a 30-second denaturation at 95°C, a 45-second anneal at 60°C, a 30-second extension at 72°C, and a 5-minute extension at 72˚C.

Detected PCR products were run on a DNA gel electrophoresis as follows. On a 1.5% agarose gel (1.5 g/100 ml 1X TBE support), 3 µl of loading buffer and 5 µl of product were loaded and the gel was run at 100 volt for 35 minutes. Gel was recolored by adding ethidium bromide (0.5 g/ml). DNA bands were photographed using a UV trans illuminator, and the molecular weights of the bands were determined using a DNA ladder (100–1500 bp).

Table 1 **Primers sequences of ABCB1 3435G>A (rs1045642) polymorphisms**

Allele specific	Primer sequence (5′->3′)	Product size
Reverse allele A	5-GGGTGGTGTCACAGGAAGAGATT-3	
Reverse allele G	5-GGGTGGTGTCACAGGAAGAGATC-3	400 bp
Forward Common	5-TAAGGCTGACAAAGGTGGAGCC-3	

Measurement of Drug Concentration of Paclitaxel in Patient Serum

To prepare the sample for HPLC analysis of Paclitaxel concentration, 0.1 mL of human plasma was added to the extraction tube. Next, I combined 4 mL of acetonitrile with 1 mL of ethyl-acetate (v/v ratio of 4:1). A 3500 rpm freeze centrifuge was used. After then, the organic layer inside the glass tube was evaporated by the nitrogen stream. After centrifuging at 3500 rpm, 200 uL of 50% MeOH is used to dissolve the dry residue, which is then transferred to an auto sampler vial. Injecting a 100 uL aliquot of material into the HPLC. The mobile phase was a 45:55 (v/v) mixture of formic acid and MeOH (0.1%). The rate of the mobile phase was 1.1 mL/min. The column was C18-ODS (25 cm × 4.6 mm), and the detector was a UV-Vis at 305 nm (Ankit Jian, 2013).[9]

Statistical Analysis

This study employed mean ± standard error, Anova, and *t* tests to significantly compare means. Two-tailed Fisher's exact probability (P) tests were used to compare alleles genotyping percentage frequencies in breast cancer patients and controls, various statistical methods were used to analyze data, chi-square (χ^2) test for contingency tables to find the statistical difference between cases and controls about the risk factors.

Results

The studied population included 100 female patients with breast cancer. Based on the findings of the study. The majority of women diagnosed with breast cancer were recorded at 85% of the total number of postmenopausal women having surgical involvement. The results also found that all women with breast cancer have taken chemotherapy (100%) as well as 5% of women who have breast cancer have received radiotherapy treatment. The results also found that rare cases of postmenopausal women (6% of the total) had recurrences of breast cancer (Table 2).

Amplification Reaction of A BCB1 3435G>A (rs1045642) Polymorphism

The results of genotype ABCB1 3435G>A (rs1045642) genetic polymorphism was a clear band with a molecular size 400 bps (Figure 1). The size of amplicon was determined by compare

Table 2. **External affect of status for women with breast cancer**

		Percentage (%)
Surgery	Yes	85%
	No.	15%
Chemotherapy	Yes	100%
	No.	0%
Radiotherpy	Yes	5%
	No	95%
Recurrence	Yes	6%
	No	94%

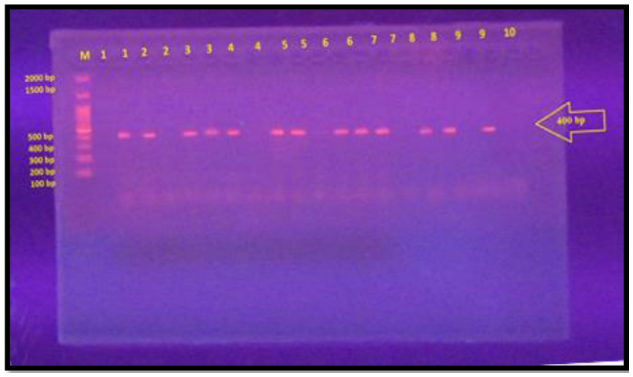

Fig. 1 **Detection o fABCB1 3435G>A (rs1045642) genetic polymorphism by Allele specific PCR with three possible genotype (GG, AA, and GA); Allele-specific PCR reaction yielded a specific fragment of 400 bp in those tubes where the mutant allele (1, 6 and 9 wells) was present. (2, 4 and 7 wells) indicates a wild-type homozygous. (3, 5 and 8 wells) indicates a heterozygous samples.**

Table 3. **Distribution of ABCB1 3435G>A (rs1045642) genotypes in breast cancer patients**

Variables	Group	Frequency	Percentage
Genotype	GG (Wild)	28	28%
	AA(Homo)	36	36%
	GA (Hetero)	36	36%

Data presented by numbers and percentage.

with DNA ladder 100–1000 bp, genotype of rs1045642 which were classified into three genotypes:

1. The major genotype group (GG) for the allele G.
2. The homozygous genotype group (AA) for the allele A.
3. Heterozygous (GA).

The result of comparison between observed and anticipated value for ABCB1 3435G>A (rs1045642) tested population were shown in Figure 1 and Table 3. The distribution and percentage of individuals having rs1045642 differ from those expected under Hardy–Weinberg equilibrium {number of observed vs expected were: GG (28); GA (36); AA (36) (goodness-of-fit χ^2 for rs1045642; 7.5824, $P < 0.001$) and therefore it was statistically significant.

Relationship between Concentration of Chemotherapy Paclitaxel of Chemotherapy Intake and ABCB1 3435G>A (rs1045642) Genetic Polymorphism

Table 4 shows that there is an inverse relationship between the concentration of drug with the therapeutic response of the patient when using paclitaxel chemotherapy and when compared with the level of genotyping frequency among ABCB1 3435G>A (rs1045642) genetic polymorphism, we noticed that patients who have allele GG in their chromosomes respond to treatment (192.8 ± 16.33) better than patients who have alleles GA and AA, respectively. LSD value was recorded 11.16 among the genes and indicated the existence of significant differences ($P < 0.05$) between the three alleles.

Table 4. **Relationship between drug concentration of chemotherapy intake and ABCB1 3435G>A (rs1045642) genetic polymorphism**

Genotype "rs1045642" Mean ± SD	*N*	Drug Concentration No-Responder	Drug Concentration Responder	*P*-value
GG	28	176.82±14.46	192.8±16.33	00.037
GA	36	168.45±16.39	184.91±13.76	00.053
AA	36	167.36±14.8	165.26±15.7	00.247
LSD	100	9.37	11.16	-

Discussion

This study included 100 women stratified by age, BMI, and paclitaxel duration intake. The clinical demographic data and laboratory parameters of patients group were, the mean age of participants which was within a mean age of (54.36 ± 4.21) years old, number of birth (3 ± 1) times. They are many adjusted other self-reported characteristics and risk factors responsible for breast cancer. These characteristics included BMI, marital status, family history, lymph node involvement, number of patients who had surgery or chemotherapy, disease duration and diagnosis, cancer location, and histochemical test results, some of studies showed the majority of breast cancers are found in women over the age of 50 years old, and some women will develop breast cancer despite having no other known risk factors. The presence of a risk factor does not guarantee the presence of the disease, and not all risk factors have the same effect.[10]

The results of our current study showed that 85% of women with cancer had surgical intervention, breast surgery, also known as a wide local excision, is a type of operation in which the region of cancer in the breast that needs to be removed is cut out surgically. The cancerous tissue and a margin of healthy tissue all the way around it are removed by the surgeon, they do so while preserving the maximum amount of healthy breast tissue feasible.[11] A total breast removal, sometimes known as a mastectomy, may be necessary for some women. They also have the option of undergoing this surgical procedure. The breast tissue, including the skin and nipple, as well as the tissues that protect the chest muscles, are both removed by the surgeon during the procedure.[12] Extremely infrequently, the surgeon will also remove the muscles that make up the chest wall. This type of mastectomy is known as a radical mastectomy.[13]

The study observed five women with breast cancer who were users of radiotherapy, breast cancer radiation therapy involves the use of X-rays, protons, or other high-energy particles to eradicate cancer cells.[14] Radiation therapy is more effective against rapidly dividing cells, such as cancer cells, than it is against stationary ones. There is no discomfort or visual impact from the X-rays or particles. After treatment, you are no longer radioactive and can safely be in close proximity to others, including children (Duma et al., 2019).[15]

Breast cancer at nearly any stage may be treated with radiation treatment. After breast cancer surgery, radiation therapy is an effective method of lowering the likelihood of a recurrence. In addition, it is frequently used to alleviate the discomfort associated with metastatic breast cancer.[14]

According to Figure 1 and Table 3 display the results of a comparison between the observed value and the expected value for the ABCB1 3435G>A (rs1045642) tested population. It was statistically significant since the distribution and proportion of individuals who had rs1045642 varied from those expected under Hardy–Weinberg equilibrium. The number of observed vs expected were: GG (28); GA (36); AA (36) (goodness-of-fit 2 for rs1045642; 7.5824, $P = 0.001$).

In this way, Mutations, gene flow, and other factors can all upset the Hardy-Weinberg equilibrium. For instance, rs1045642 polymorphism introduce novel alleles into a population, which might shift the balance of existing allele frequencies.[16] This investigation concurred with the findings of (Barliana et al., 2021),[17] who discovered the Hardy–Weinberg equilibrium was used to conduct an investigation of the frequency of alleles for each gene. The genetic profiles of ABCB1 rs1045642 were found to be significantly different from equilibrium in the population of Indonesia.

Table 4 shows that there is an inverse relationship between drug concentration and patient therapeutic response when using paclitaxel chemotherapy, and when compared with the level of genotyping frequency among ABCB1 3435G>A (rs1045642) genetic polymorphism, the study noticed that patients with allele GG respond to treatment (192.816.33) better than patients with alleles AA and GA, respectively. The LSD value was 11.16 among the genes, indicating that there were substantial differences ($P < 0.05$) between the three alleles, The study found that the mutant gene A allele played a role as a protective factor in order to maintain the concentration of the drug Paclitaxel in the patient's serum for a period of time, unlike the other two genetic polymorphism AA and GA. This study differed from what was explained by (Su et al., 2021)[18] when he stated that not all mutated genes play a role as a protective factor, as there are some mutated genes that were pathological in women with metastatic colon cancer.

This Study conclude that patients whose chromosomes contained the allele GG responded to treatment more favorably than patients whose chromosomes contained the alleles AA and GA for ABCB1 3435G>A (rs1045642) genetic polymorphism.

Conflict of Interest

None.

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