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Investigating the impact of CYP1B1 polymorphism on the response to tamoxifen in women with Breast cancer women

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ABSTRACT

Objectives Primary Objective Determine how CYP1B1 variants (rs1056836 and rs1056827) affect the response to tamoxifen in women with breast cancer.

Secondary objectives

1. To explore the linkage between cyp1b1 genotypes (rs1056836 -rs1056827) on clinical outcomes (e.g., recurrence of the disease, survival rates)

2. Test whether there is interaction between cyp1b1 genotypes (rs1056836 and rs1056827) and clinical factors (e.g., hormone receptor status, menopausal status).

Methods The study involved 139 female breast cancer patients aged 45 and above at Imam Al-Hussein Medical City in Kerbala. Data was collected from clinical data, including weight, age, family history, and health problems. Blood samples were analyzed for biochemical parameters, including estradiol levels, tumor markers, and lipid profiles. The study aimed to investigate treatment responses and hormone levels in breast cancer patients, and also estimated high-density and low-density lipoprotein concentrations.

Results The study revealed that there was a prevalence of excess weight, and most of them were overweight. The duration of Tamoxifen use was also a major attribute, with 86.3% taking it in 3-60 months. The majority of the patients had a family history of the disease, with the contribution of only 1.4% by smoking and hormone therapy. In breast cancer, the researchers also determined that there was a lack of findings that there was a strong genetic correlation between the genotype of the hormone receptor subtypes and the rs1056827 genotype.

Conclusion: genetic variation in CYP1B1 is shown to play a major role in tamoxifen response and treatment outcomes and affecting patient adherence to the drug.

Keywords - tamoxifen, CYP1B1, breast cancer, personalized medicine, pharmacogenomics..

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INTRODUCTION

Breast cancer is a widespread cancer and a major health issue of concern among the populace. It causes the most cancer-related deaths in women, and in 2020, it took about 2.3 million new cases around the world, comprising 11.7% of all diagnosed cancers (Bray et al., 2021). The disease is described as an uncontrolled proliferation of the cells within the breast tissue, which might extend to the other tissues of the body and spread to other parts, such as the bones, liver, and lungs (Hanahan and Weinberg, 2011; Harris et al.,2012).

Breast cancer is a heterogeneous disease, which is categorized into various subtypes in accordance with molecular and histological features. The two major ones include invasive ductal carcinoma (IDC), which starts in the milk ducts, and the second, invasive lobular carcinoma (ILC), which starts in the milk-producing lobules and occupies around 10 to 15 percent of the cases (Rakha et al., 2010). Subtyping by receptor status, namely, the presence or absence of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) has facilitated even more specific treatment approaches (Perou et al., 2000; Schettini et al.,2016)

Breast cancer is a multifactorial etiological phenomenon that is a product of both genetic and environmental factors. The

BRCA1 and BRCA2 genes are the most common examples of germline mutations that are known to be risk factors that present high risk of breast and ovarian cancer throughout the lifetime (Narod and Foulkes, 2004). Additional hormonal influences, which are essential, include long-term exposure to estrogens caused by early menarche, late menopause, or hormone replacement therapy (Key et al., 2001; Dubey et al.,2005). Besides, other lifestyle risk factors such as alcohol use, obesity, and lack of exercise also expose people to the risk of disease (McTiernan, 2003).

Early detection and diagnosis have contributed a lot to breast cancer outcomes. Mammography is the gold standard of screening and has been established to have the ability to prevent mortality by early detection (Tabár et al., 2011; Lashof et al.,2001). There are other imaging procedures like the breast MRI and ultrasound, which have improved the level of diagnosis, especially in thick breast tissues (Saslow et al., 2007) (Na and Houserkovaa,2007). Public sensitization efforts have also been very crucial in promoting the practice of screening and self-examination.

The breast cancer treatment is multimodal and depends on the type of tumor, the stage, and the receptors. The initial treatment is often by surgery, which can be lumpectomy and mastectomy, with adjuvant treatments in the form of radiation, chemotherapy, hormone therapy, and targeted therapy. Targeted therapies (e.g., trastuzumab in breast tumors with HER2, CDK4/6 in tumors with the ER, etc.) have transformed treatment, providing a better survival rate and fewer side effects (Johnston et al., 2020).

Regardless of these innovations, there are still disparities in access to screening and treatment, especially in the low- and middle-income countries where the resources are scarce (Unger-Saldaña, 2014) (Barragan-Carrillo et al.,2025). Further studies are aimed at learning the biology of tumors and finding new biomarkers, as well as personalized treatment, to make the results even better (Waks and Winer, 2019) (Wolfet al.,2022).

To conclude, breast cancer is an irreversible and heterogeneous disease that is affected by genetic, hormonal, and lifestyle. Research, education, and equity in healthcare are essential efforts that will play an important role in minimizing the global burden of breast cancer, even though much progress has been made in the detection, diagnosis, and treatment of breast cancer.

The study aimed to evaluate the impact of CYP1B1 polymorphism on the response to tamoxifen

MATERIALS AND METHODS

2.1Study Design and Participants

A cross-sectional observational study took place between September 2024 and December 2024 at the Oncology Center in Kerbala, located at Imam Al-Hussein Medical City in Iraq. This study was done on 139 female breast cancer patients. The researchers were interested in the response to the development of treatment and the level of hormones in such patients. The participants were separated into two groups depending on the estradiol plasma levels and tumor marker outcomes. The paper did not consider patients who were tamoxifen users who were undergoing chemotherapy or radiation, or individuals who had gastrointestinal problems that were likely to interfere with tamoxifen absorption. Clinical data were gathered via direct reporting, whereby aspects that included weight, age, family history, and birth-related information were put into consideration. Participants were sampled by taking blood, which was then analyzed with biochemical parameters such as calcium level, estradiol, tumor markers, and lipid profiles. The scientific and ethical approval of the research protocol was obtained by the College of Pharmacy of the University of Kerbala.

2.2Blood sample collection

The participants had to provide the venous blood samples of about 5 ml, which were later split into two parts. Molecular examination was done using 2 mL of blood in an EDTA tube. The serum type of the three milliliters of blood was separated, and three milliliters were taken in a gel tube to analyze the biochemical parameters that involved the level of calcium, the level of estradiol and tumor markers, and lipid profiles.

2.3Biochemical analysis

The chemiluminescence immunoassay is the method used to quantitatively detect the cancer antigen 15.3 in the human serum with the help of the double antibody sandwich detection scheme. The reagent test is separated into three parts, namely R1, R2, and R3. The system involves the use of magnetic particles that carry estradiol analogue, acridinium ester-labeled estradiol antibody, and buffer solution that has a releasing agent. The relative light units are detected in an inverse relationship to estradiol content in the sample. The Mindray BS series analyzer is used in estimating the plasma calcium levels by a photometric Arsenazo III method. Enzymatic colorimetric tests are used to determine the total cholesterol (CHO) levels in vitro. An enzymatic quantitative assay is used to measure the triglyceride (TG) levels. Identification of the level of the high-density lipoprotein (HDL) is directly measured in the serum by the use of a blocking reagent that reacts with apo-B-containing lipoproteins in the test specimen. The chemiluminescence immunoassay represents an important technique that can be used to determine the occurrence of different biochemical markers in human serum and plasma samples.

2.4Genetic analysis

2.4.1Extraction of genomic DNA from blood samples

Favor Prep Genomic DNA Mini kit from Favorgen delivered an efficient and expedient DNA extraction process for blood and biological materials, and generated DNA ready for both preservation and subsequent use.

Sample preparation

About 200 µl of blood was transferred into a 1.5 ml microcentrifuge tube.

30 µl Proteinase K was introduced into the sample and mixed by vortex. Incubated at 60°C for 15 mins. (vortex occasionally

during incubation)

Cell lysis

We added 200 microliter FABG to the sample mixture and mixed thoroughly by pulse-vertexing. Incubated at 70°C for 15 minutes

The tube received a brief spinning motion to remove liquid drops accumulating inside the lid.

DNA binding

The next step involved adding 200 microliters of ethanol 100% to the sample and vertexing for 15 seconds

Placed FABG Mini Column into a Collection Tube. The mixture with all mineral precipitates was transferred cautiously to the FABG Mini Column. A centrifugal force of 18,000 x g full speed rotation occurred for one minute, after which I moved the FABG Mini Column to an unused Collection Tube.

Column washing

A total of 400 µl W1 Buffer was added to the FAbG Mini Column. Complete high-speed centrifugation for 30 seconds resulted in the disposal of the flow-through.

600 µl Wash Buffer was introduced to the FABG Mini Column. Full speed centrifugation lasted 30 seconds, followed by the disposal of the flow-through.

Application of full-speed centrifugation for 3 additional minutes dried up the column. Eliminating residual liquid is the purpose of this step.

Elution

Placed the column into a new 1.5 ml microcentrifuge tube

Placed 100 µl of preheated Elution Buffer onto the membrane surface of the FATG Mini Column. Allowed the FATG Mini Column to stand for 10 minutes at 37°C. The process of effective elution requires the elution solution to be dispensed directly onto the membrane center until complete absorption occurs.

DNA elution occurred after one minute of full-speed centrifugation.

2.4.2Quantitation of DNA by the spectrophotometric method

DNA concentration and isolated purity assessment occur through nanodrop by using Nano spectrophotometry. A micro detector was cleaned from the blank while 1µL of sample was applied onto the nanodrop micro detector, followed by equipment measurement of DNA concentration and A260/A230 ratio.

2.4.3Polymerase chain reaction

This research employed amplification refractory mutation system polymerase chain reaction (ARMS-PCR) and PCR amplification of specific alleles (AS-PCR) to identify rs1056827 and rs1056836 CYP1B1 gene SNPs.

2.4.4Primers design

Primer-BLAST software on the https://WWW.ncbi.nlm.nih.gov website generated the primers, which Macrogen in Korea provided as dried products. Tables (2-1) and (2-2) display the primer sequences dedicated to amplifying the CYP1B1 gene to identify SNPs.

Table 2-1 Primers sequences for rs1056827 G>T

Primers	Primers sequences	Primer size (bp)	Product size (bp)
Forward	CTCGTTCGCTCGCCTGGCGC	20	250
Reverse	GAAGTTGCGCATCATGCTGT	20	250
G Forword	GGCCTTCGCCGACCGGCCGG	20	130
T Forword	GGCCTTCGCCGA CCGGCCGT	20	130

Table 2-2 Primers sequences for rs1056836 C>G

Primers	Primers sequences	Primer size (bp)	Product size (bp)
Forward	ATGCGCTTCTCCAGCTTTGT	20	625
Reverse	TATGGAGCACACCTCACCTG	20	625
G Reverse	TCCGGGTTAGGCCACTTCAC	20	150
C Reverse	TCCGGGTTAGGCCACTTCAG	20	150

The lyophilized primer solutions needed specific water amounts to form 100 pmol/µl stock solutions before use. A diluted work solution required adding 90µl nuclease-free water to 10µl of each stock solution to reach (10 pmol/ µL) as its final concentration (table 2-3). The solution needed to be subjected to 20 °C for storage.

Table 2-3 Quantities of nuclease-free water served to dilute the primers to achieve a concentration of 100 pmol/µl

Primers		Volumes of nuclease free water added in µL
rs1056827 G>T	rs1056836 C>G	
F	F	250
R	R	250
T Forword	G Reverse	250
G Forword	C Reverse	250

2.4.5Optimization of PCR Conditions

Several testing procedures led to the optimization of PCR conditions to identify optimal temperatures for both annealing reactions and cycle repetitions Table 2-6.

Table 2-4 PCR mixture for genotyping rs1056827

Component	Volume (µl)
If	2
Ir	2
Of	2
Or	2
DNA templet	5
Nuclease free water	14
Smart mix	25
Total	50

Table 2-5 PCR mixture for genotyping rs1056836

Component	Volume (µl)
If	2
Ir	2
Or	2
Of	2
DNA templet	5
Nuclease free water	14
Smart mix	25
Total	50

Table 2-6 PCR condition for genotyping rs1056836 and rs1056827

step	Temperature C	Minutes: seconds	cycles
Initial denaturation	95	03:00	1
denaturation	95	00:30	35
annealing	61	00:30	
extension	75	01:00	
Final extension	75	05:00	1

Table: - (3.1) Descriptive statistics of demographic characteristics of the studied

Variable		N	Percent
Age	32-50	98	70.5
	51-80	41	29.5
Menarche	8-12	50	36.0
	13-16	89	64.0
BMI	Under weight	0	0.0
	Normal	15	10.8
	Over weight	60	43.2
	Obese	64	46.0
Duration of disease (months)	3-60	112	80.6
	61-120	21	15.1
	121-180	6	4.3
Duration of tamoxifen (months)	3-60	120	86.3
	61-120	13	9.4
	121-168	6	4.3
Women social activity	B.C. history	135	97.1
	Smoking	2	1.4
	B.C.+ HT	2	1.4
Tumor site	Right	68	48.9
	Left	68	48.9
	Bilateral	3	2.2
Types of surgery	Mastectomy	118	84.9
	Br. conserve	21	15.1
Adjuvant therapy	Chemo+Radio	52	37.4
	Chemo	86	61.9
	Radio	1	0.7
Receptor status	Es+Pr	44	31.7
	Es	92	66.2
	Es+Her2	3	2.2
Hypertension	No	130	93.5
	Yes	9	6.5
Diabetes	No	130	93.5
	3.Yes	9	6.5

breast cancer women (n=139)

Table (3.2a): Hardy–Weinberg equilibrium for, rs1056836 in patients.

Variable			Frequency	Percent	Alleles		Hardy–Weinberg equilibrium X^2 test
rs1056836	CC wild	Observed expected	84 82.37	60.4 59.26	C	G	
	CG hetero	Observed expected	46 49.27	33.1 35.44	214 76.98%	65 23.02%	**X^2 =0.6109** **P-value=0.4344**

Table (3-2b) : Hardy–Weinberg equilibrium for, rs1056827 in patients.

Variable			Frequency	Percent	Alleles		Hardy–Weinberg equilibrium X^2 test
rs1056827	GG wild	Observed expected	99 98.84	71.2 70.85	G	T	
	GT hetero	Observed expected	36 37.04	25.9 26.64	234 84.17%	44 15.83%	**X^2 = 0.1088** **P-value= 0.7416**

Table (3.3): Multiple response crosstab analysis comparing reported symptoms across genetic variations of rs1056836.

Genotype rs1046836	**Blurred vision**	**Arthralgia**	**Hot flashes**	**P value**
CC	31	70	61	0.001
	19.1%	43.2%	37.7%	
CG	25	38	39	0.166
	24.5%	37.3%	38.2%	
GG	3	6	6	0.549
	20.0%	40.0%	40.0%	
Total	59	114	106	

*Percentages and total are based on responses.

Table (3.4): Multiple response crosstab analysis comparing reported symptoms across genetic variations of rs1056827.

Genotype rs1056827	**Blurred vision**	**Arthralgia**	**Hot flashes**	**P value**
GG	44	81	76	0.002
	21.9%	40.3%	37.8%	
GT	13	29	27	0.037
	18.8%	42.0%	39.1%	
TT	2	4	3	0.717
	22.2%	44.4%	33.3%	
Total	59	114	106	

*Percentages and total are based on responses.

Table: (3.5) Correlation of genetic variation with Ca15.3 level.

	Parameter	Ca15.3
Spearman's (rho)	rs1056836	0.153
	P- value	0.072
	rs1056827	0.082
	P- value	0.337

2.4.6Running the polymerize chain reaction

The PCR mixture preparation occurred in a microcentrifuge tube through the addition of 25 µl Smart Mix (BIONEER/Korea) along with 2 µl each primer and 5 µl DNA, followed by nuclease-free water up to 50 µl to reach the final volume(table2-4) and (table2-5). The electrophoresis apparatus separated amplified segments through a gel made with 1.5% agarose containing ethidium bromide, followed by UV trans-illuminator observation. The DNA bands were photographed with a UV trans illuminator after an initial DNA ladder (100–1500 bp) was employed to determine band molecular weights.

2.4.7Agarose gel electrophoresis

Preparations started with dissolving 1.5g agarose powder in 100ml 1x TBE buffer under normal conditions (Tris-Borate-EDTA). The needed buffer involves mixing 10ml 10x TBE buffer with 90ml distilled water.

The solution boiled on a hot plate for several minutes to reach transparency without forming bubbles.

After the solution had cooled down, an addition of three microliters of ethidium bromide was made to the gel.

PCR product loading wells were prepared using the comb that connected to the tray finishes.

Remove the comb using caution from the agarose gel when it reached its solid state, which required 20 minutes to obtain after carefully pouring it into the plastic tray.

The gel operation requires a gel electrophoresis tank for installation. The tank received 1X TBE-electrolysis buffer until it reached a level that exceeded the gel surface by three to five millimeters.

A well-received five microliters of DNA ladder, while each of the other wells received five microliters of the PCR products.

Dry tanks are connected to electrophoresis chambers where the sample tray stays in place.

The set distance between the cathode and anode required adjustment of the electrophoresis apparatus voltage to generate a 5 v * cm-1 electrical field.

A UV transilluminator with a wavelength range from 320 to 336 nm was used to identify run-time bands at the conclusion.

The digital camera gel recorded the gel photographs.

2.5Statistical analysis

The Statistical Package for the Social Sciences (version 26) analyzed data stored in an Excel sheet. The research findings were displayed as frequencies and percentages alongside mean and standard deviation using suitable graphical representations and tables. The analysis included an independent t-test combined with ANOVA and post hoc testing, followed by the least significant differences test (LSD) for the detection of relationships between relevant study variables. The chi-square (Fisher's exact test) statistic compares the observed values to the expected values. Statistical significance of differences between observed and expected values is examined using this test statistic. A statistical Pearson correlation test helped determine any existing connections between the investigated parameters. A significant p-value of 0.05 or 0.01 (p-value $\leq$ 0.05, 0.01) was established for statistical associations.

RESULTS

3.1 demographic characteristics of the study population

The table (3.1) gives insights into different factors with the descriptive statistics as highlighted: age, menarche, BMI, disease duration, tamoxifen duration, social activity, tumor site, types of surgeries, adjuvant therapy, receptor status, and other comorbidities like hypertension and diabetes mellitus.

The women studied were between the ages of 32 to 80 years, whereby 70.5 percent in the range of 32 to 50. The rest 29.5 per cent fell in the age bracket of 51-80 years. This shows that young females were mostly afflicted with breast cancer in this cross-sectional study.

The age of onset of menarche ranges between 816 years. A larger percentage of women (64.0) menstruated when they were aged 13-16 years, whereas 36.0 experienced menarche at a younger age of 8-12 years. These data could be useful to know about the hormonal factors of breast cancer risk.

Explanation of body mass index (BMI) classification indicated that not all the women were underweight. Nevertheless, 10.8 percent had normal BMI, and most of them were overweight (43.2 percent) or obese (46.0 percent). This finding is notable in the

prevalence of excess weight in breast cancer patients, which has been established as a risk factor for the disease.

Breast cancer duration has a wide range among military personnel, between 3 to 180 months. The largest proportion of women (80.6%) lived with the disease for 3-60 months, 15.1% had 61-120 months, and only 4.3% had the duration of disease between 121-180 months. This is indicative of most cases as being a fairly new diagnosis.

Similarly, the duration of tamoxifen ranged between 3 and 168 months in duration. Seventy-nine per cent of them (86.3%) were receiving tamoxifen for 3-60 months, 9.4% between 61-120 months and 4.3% between 121-168 months. This indicates how tamoxifen is widely used as a long-term treatment

Social activity portrays that 97.1 of women were characterized by a family history of breast cancer, and this implies a definite family association. Smoking and the combination of breast cancer and hormone therapy were unusual as they individually contributed only millstones of the study, i.e., 1.4 percent.

In terms of the location of the tumor site, there was an equal distribution of 48.9 percent of the tumor sites on the right and left breasts, respectively, with bilateral breast cancer being seen only among 2.2 percent of the participant women patients.

One of the treatment methodologies was surgery. Most (84.9%) of them receive mastectomy, whereas 15.1% prefer breast-conserving surgery. This is a desire to receive a mastectomy, which may be caused by the disease stage or its severity.

Depending on the adjuvant therapy, which is different among the women. Chemotherapy and radiotherapy were a combination given to only 37.4% of patients, whereas chemotherapy was the most given treatment to 61.9% with radiotherapy being used in only 0.7 per cent of cases.

The receptor status of the tumors indicated that 66.2 per cent of women had estrogen receptor-positive tumors, whereas 31.7 per cent had tumors that tested positive for both the estrogen and progesterone receptors. The outcomes indicated a low percentage (2.2) of displays of tumors that showed positive effects to estrogen and HER2.

The percentage of women who had hypertension and diabetes was low. Having hypertension was only 6.5% and the same proportion (6.5%) had diabetes. These comorbidities were not prevalent in the majority of the respondents (93.5%), implying that they were not prominent risk factors for breast cancer in this study

3.2 Genetic Analysis

3.2.1 genotyping of rs1056827 and rs1056836

The following successfully conducted Polymerase Chain Reaction (PCR) was used to amplify the genomic position harboring the SNP rs1056836 in the gene CYP1B1. Primers were composed in order to generate a fragment of 150 base pairs (bp).

As indicated in Figure 3.1, all ten samples (lanes 1-10) gave distinct bands of the anticipated size of 150 bp. The estimated band size was confirmed by the DNA ladder (lane M), and the amplification products were visualized by 2% agarose gels run through electrophoresis with ethidium bromide as a stain and visualized under UV lamp.

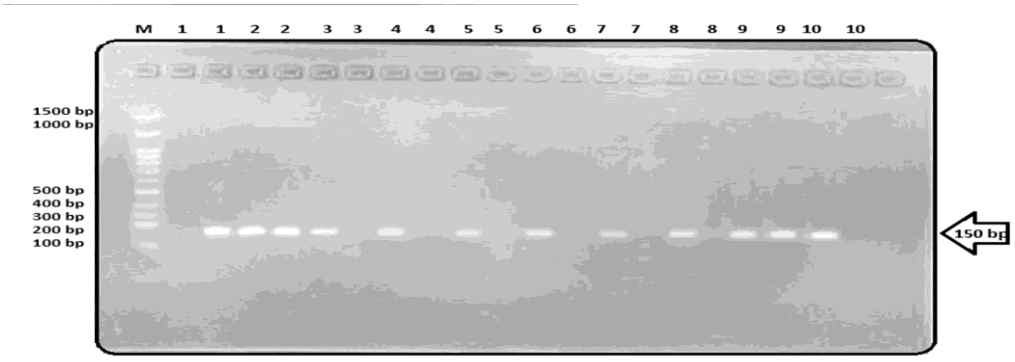

Figure 3.1 PCR result for rs1056836

There were no nonspecific bands or contamination, which means that the primers are specific and the PCR reaction is successful. The existence of one smell candidate band in each sample implies that all the target areas were definitely amplified.

This effective amplification provides the basis to be used by later genotyping methods, e.g., restriction fragment length polymorphism analysis (RFLP analysis), or DNA sequencing to find out the definitive genotype of the rs1056836.

Measurement of the rs1056827 SNP in the CYP1B1 gene by PCR was successfully done. Under the 2% agarose gel with ethidium bromide staining, all the samples produced only one distinct band with an estimated size of 130 base pairs (bp) (Figure 3.2). It shows that the target region in which the polymorphic point of insertion is rs1056827 has been amplified successfully. There were no nonspecific multiplications and primer-dimer formation.

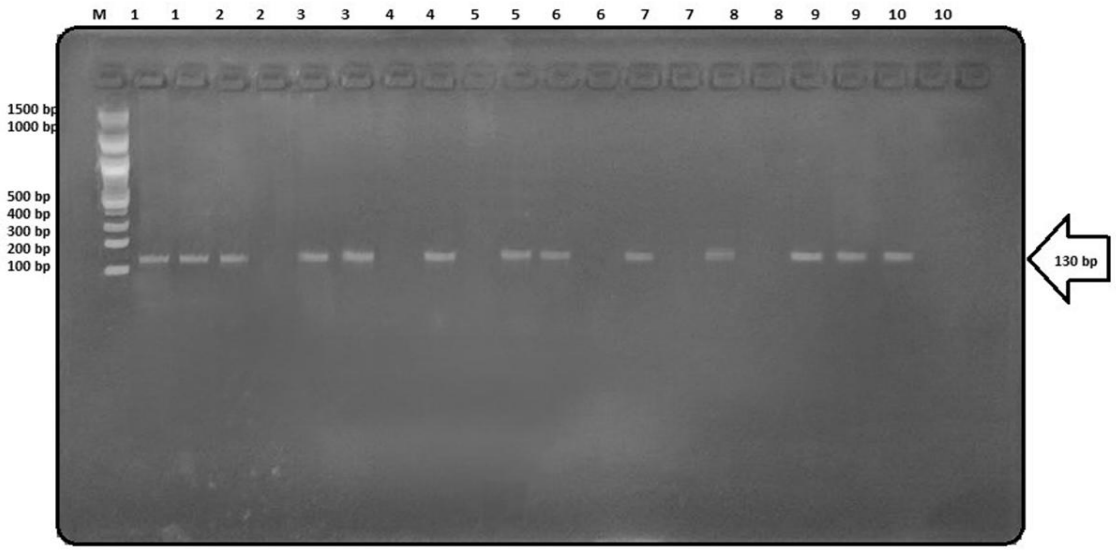

Figure 3.2 PCR result for rs1056827

The gel electrophoresis results indicated using the gels alone that all the samples contained uniform amplicons, that is, they had the uniform length required as PCR product length.

3.2.2 Hardy–Weinberg equilibrium

Hardy-Weinberg. If a population is not changing its genes, the reasons behind the genetic variation in the population come into a mathematical equation referred to as the Hardy-Weinberg principle. It presupposes the constancy of the allele frequency between generations when dealing with a large and random-mating population as long as the following conditions are achieved: no mutation, no gene flow or natural selection, and random mating.

Here, the inquiry on the rs1056836 polymorphism with three genotypes, including CC (homozygous wild type), CG (heterozygous), and GG (homozygous mutant). Table 3.2) shows how the observed and expected frequencies of these genotypes are as explained therein.

The chi-square test is applied to determine whether the observed frequencies of genotypes are significantly different from the anticipated genotype frequencies under the Hardy-Weinberg equilibrium conditions. The chi-square value that has been computed was 0.6109, and the p-value that it poses is 0.4344. The value of chi-square (0.6109) is small, showing that there is an insignificant difference between observed and expected frequencies.

The p-value 0.4344 is higher than the usual meaning, which is proportional to 0.05. The above implies that there is no statistically meaningful difference between expected and observed genotype.

The study does not reject the null hypothesis that the population is in Hardy-Weinberg disengagement about the rs1056836 polymorphism because the p-value is 0.4344. This shows that the allele frequencies of this patient population are stable and not substantially affected by any evolutionary force, including selection, mutation, and migration.

The Hardy-Weinberg law equips a mathematical formula to comprehend the genetic variation of a non-evolving population. It supposes that a population is large, that genes are randomly mated in each generation, and that it is randomly mating (that is, there is no mutation, no flow of genes into or out of the population, and no natural selection).

In the present case, the analyses of the rs1056827 polymorphism, which has three alleles: GG (wild type), GT (heterozygote), and TT (mutant). These are the possible and anticipated frequencies of the genotypes as exemplified in Table 3.8.

The chi-square test is applied to ascertain whether the frequencies of the observed genotype vary significantly from the expected frequencies obtained when the Hardy-Weinberg equilibrium holds. The obtainable chi-square value is 0.1088, and its p-value is 0.7416. Such a chi-square of 0.6109 is small, and the difference between the observed and expected frequencies is small.

The p-value is 0.7416, which exceeds the standard level of significance that is normally set at 0.05. This implies that there is no statistically significant dissimilarity between the observed genotype frequencies and expectations.

The study does not reject the null hypothesis about the people being in Hardy-Weinberg equilibrium with regard to the rs1056836 polymorphism, since the p-value is 0.7416. This implies that the allele frequencies in this patient group are constant, which is not much affected by the processes of evolution, including selection, mutation, and migration (Bourgain et al.,2004).

3.3 Association between adverse effects and genetic variation in rs1056836

Single-nucleotide polymorphisms or genetic variations are able to determine the likelihood of some symptoms or diseases by means of influence on the individual (Bell,2002). The given crosstab analysis considers the correlation between the rs1056836 genotype (CC, CG, GG) to three reported symptoms: blurred vision, arthralgia, and hot flashes, as well as to determine whether the Pearson chi-square test was significant at a p-value table 3-3.

The most prevalent cases of arthralgia, hot flashes, and blurred vision were reported among those having the CC variant (43.2, 37.7, and 19.1%, respectively). The conclusion concerning the result of the p-value (0.001) entails that there is a statistically significant relationship between the CC genotype and the symptom distribution, which implies that the gene, or rather the genetic variation, is possibly involved with the symptoms.

The CG genotype was more evenly distributed between the symptoms, as 38.2 percent experienced hot flashes, 37.3 percent arthralgia, and 24.5 percent blurred vision. The p-value of 0.166, however, indicates that the observed results might not be statistically significant.

There were a few people with the GG variant, and 40 percent of them had problems with arthralgia and hot flashes, whereas 20 percent of them experienced blurred vision. The p-value (0.549) is very large; hence, there is no significant relationship between the variant and the reporting of the symptoms.

There is a close correlation between the CC genotype and symptom manifestation, mostly arthralgia. It may mean that the rs1056836-CC carriers may be more susceptible to inflammatory or pain-related diseases.

According to the analysis, there is evidence that the CC type of rs1056836 may highly contribute to the development of the probability of arthralgia and hot flushes, but the CG and GG types have no high correlations. The outcomes may also apply to personalized medicine because with genetic screening of rs36, there may be opportunities to prognosticate the risk of developing symptoms.

3.4 Association between adverse effects and genetic variation in rs1056827

Crosstab analysis is plotted to investigate the relationship between three reported symptoms: blurred vision, arthralgia, and hot flashes, with genotypes, that is, GG, GT, and TT, of the rs1056827 genetic variation. The p-values that were provided in Table 3.4 explained the number of individuals who had each symptom in each genotype group, as well as the percentages of individuals who had each symptom in each genotype group, as to whether a given result showed a p-value less than or equal to a p-value of 0.05.

In the case of the GG genotype as the most frequent symptom is arthralgia (40.3%), in 2d place there are hot flashes (37.8%) and blurred vision (21.9%). The p-value of 0.002 indicates that the association between the GG genotype and symptoms distribution is significant, and therefore, this genetic variant is likely to determine the risk of having such symptoms.

In the same manner, arthralgia was frequently reported (42.0%), followed by hot flashes (39.1 %), and blurred vision was the least occurrence (18.8) of GT variation. The significance level of 0.037 shows that the association is not that high, but is lower than GG. This means that the GT genotype might not be as involved in the manifestation of the symptoms that are definite symptoms, but there is a possibility

3.5 Correlation of genetic variation with Ca15.3 level.

Table (3.5) presents the findings of the Spearman rank test procedure looking into the relationship between two genetic variant variables (rs1056836 and rs1056827) and the level of Ca15.3, a common test that is applied in monitoring breast cancer. Non-normally distributed data or ordinal variables were suitable to utilize in the assessment of the strength and direction of the monotonic relationship between these variables using Spearman's rho (rho).

The rs1056836 correlation coefficient is 0.153, which assumes that it has a weak positive relation to Ca15.3 levels. It implies that an increasing genetic variation in rs1056836 is associated with a tendency to increased Ca15.3 levels as well. But due to a p-value of 0.072, this correlation is not statistically significant at the standard level of $p < 0.05$.

Even worse, rs1056827 shows a correlation coefficient of 0.082, which again is not too high. The positive number is an indication of a slight tendency towards an increase in Ca15.3 levels with variation of rs1056827; however, the p-value of 0.337 does not say anything, and it is not significant. The negotiated correlation of the delta of 0.0029 with the associated p-value of 0.8942 is weak and non-significant, thus suggesting that the value of rs1056827may not produce a significant effect on levels of Ca15.3 in this group.

DISCUSSION

4.1. Impact of demographic characteristics on biomarker profiles and treatment outcomes:

It was discovered that the prevalence of breast cancer is mostly prevalent among younger women, and its highest prevalence was among females below 50 years. Most of the patients experienced menarche between the ages of 13-16, and obesity was high. The majority of patients were below 5 years and had been taking tamoxifen treatment for the same period. The location of the tumor was nearly equivalent in both breasts, and only 2.2 of % bilateral tumors were localized. Most of the patients received chemotherapy (61.9%), and 37.4% chemo-radiotherapy. Estrogen receptor+ tumors prevailed, 66.2% of all were expressed alone, and this may have an effect on tamoxifen treatment. There were no reports of comorbidities, and 6.5% were hypertensive or diabetic. In disease progression, there were no significant changes in the levels of calcium and CA15-3 in the laboratory biomarkers. Nevertheless, the vitamin D3 levels decreased with the duration of the disease, which could be a sign of vitamin D deficiency. The level of triglycerides and VLDL was higher in those patients who had had longer disease durations, and not in total cholesterol, HDL, and LDL. Epidemiological findings suggest that obesity is a risk factor that increases the chances of developing breast cancer. Such an association is generally enhanced in obese post-menopausal women who are likely to develop estrogen-receptor-positive breast cancer (Łukasiewicz et al.,2021).

4.2. Frequency of CYP1B1 (rs1056836) gene polymorphism in Iraqi breast cancer patients:

Analysis of CYP1B1 gene polymorphisms showed that wild (CC), heterozygous (CG), and homozygous (GG) genotypes were the most common in the case of the patient of the study who had been treated using the records of CYP1B1: the heterozygous (CG) and homozygous (GG) genotypes were the most common types of the CYP1B1 genotype exhibited by the patient. The GG genotype was widespread (71.2%) in the case of rs27 patients; heterozygous (GT) and homozygous (TT) genotypes were found (24.9). Such findings indicate that CYP1B1 polymorphs in these populations are also similar, i.e., wild-type alleles are prevalent. This implies that CYP1B1 polymorphs may have an influence on tamoxifen metabolism and response that may have an impact on therapeutic efficacy and toxicity. Cross tabulation of the CYP1B1 polymorphisms of the rs1056836 and rs1056827 showed that the majority of the patients who had the CC genotype had the GG genotype, with low numbers having the GT and TT genotypes. This indicates that there is a significant overlap of wild-type alleles in this population, and it may affect tamoxifen metabolism and toxicity. The researchers recommend that the genotype of the receptor status does not have any significant predictors of the receptor status, but it would be applicable in pharmacogenetic research to study the efficacy and side effects of tamoxifen (Ibrahem et al,2021

4.3. Hardy-Weinberg Equilibrium

4.3.1. HardyWeinberg Equilibrium rs1056836

The genotype distributions of CYP 1B1 rs1056836 in breast cancer patients align with the Hardy-Weinberg equilibrium (HWE), indicating stable allele frequencies. This confirms the accuracy of genotyping data and supports the validity of genetic data. HWE should be maintained in pharmacogenetic reports to avoid complications in determining relationships between CYP1B1 variations and tamoxifen response or adverse effects.

4.3.2. HardyWeinberg Equilibrium rs1056827

The genotype of CYP1B1 rs1056827 of the breast cancer patients studied was consistent with the Hardy-Weinberg equilibrium (HWE) (X 2 = 0.1088, P = 0.7416). The observed frequencies of GG, GT, and TT genotypes were very close to the expected frequencies, which is an indication that the alleles were well inherited in this population, and there are no instances of selection bias, genotyping errors, or stratification of the population that influenced this locus.

This result confirms the suitability of genotyping data and makes any further analysis to identify the relationships between rs1056827 and laboratory biomarkers, receptor status, and treatment outcomes credible and explainable. HWE is an important maintenance in pharmacogenetic research: associations of drug response and adverse effects can be confounded by deviations.

Comprehensively, the validation of HWE of rs1056827 indicates the genetic purity of the cohort and the believability of the associations to be seen with tamoxifen metabolism and therapeutic outcomes

4.4. Correlation between rs1056836 Genotype and Adverse Drug Reactions

Cross-tab analysis of multiple responses showed that the CC genotype of rs1056836 had a significant association with reported adverse drug reactions, especially arthralgia (43.2%) and hot flashes (37.7) (P = 0.001). On the contrary, CG and GG genotypes did not exhibit statistically significant correlations to any of the recorded symptoms (P = 0.166 and 0.549, respectively). The blurred vision had no significant relationship with any genotype.

These results indicate that patients with CC wild-type genotype could be more vulnerable to tamoxifen-induced musculoskeletal and vasomotor adverse effects (Thorne,2007; Park et al,2013).. The absence of any significant connections in CG and GG carriers could be explained by the smaller sample sizes or by diverged enzymatic activity affecting the metabolism of tamoxifen. This is in line with the past reports that polymorphs of CYP1B1 have the capacity to control the adverse effects of drugs by influencing estrogen metabolism. Such results support the findings of earlier studies according to which musculoskeletal and vasomotor symptoms are the most prevalent and clinically significant adverse effects of tamoxifen therapy (Sung et al., 2022) (Mieog et al,2012).

These findings, in general, indicate the potential existence of a pharmacogenetic pathway between the activity of the rs1056836 and the predisposition to ADRs that could be taken into account in individual management approaches to tamoxifen treatment (Golmohammadzadeh et al.,2019)

4.5. Relationship between the Tamoxifen Duration and Lipid Profile

Our findings suggest that the duration of tamoxifen use does not have a significant effect on lipid metabolism in this group of cohorts. This is in line with certain past research findings that the effects of tamoxifen on lipids are possibly small and changing, as they may vary depending more on personal metabolic and BMI and genetic polymorphisms, and not necessarily as a result of the length of the treatment (Sara et al., 2022; Markopoulos et al.,2006)

On the whole, although it is known that tamoxifen therapy has complicated effects on lipid metabolism, the present results point to the fact that the duration of therapy is not a significant determining factor of the changes in the lipid profile, and it is important to consider the effects of treatment on the individual lipids, and not just assume that the effect of prolongation of treatment will be linear.

4.6. Genetic Variations are correlated with Ca15.3

The rank correlation analysis of Spearman showed no significant correlation between the CYP1B1 polymorphism of 36 (= 0.153, = 0.072) or 27 (= 0.082, = 0.337) with the Ca15.3 concentration, which is one of the tumor biomarkers of breast cancer. Even though there was a weak positive correlation in the case of rs36, which indicated a slight inclination towards the rise in the Ca15.3 levels with this genotype, the correlation between the two was not statistically significant. Correlation with rs27 was even less and also non-significant.

These results indicate that Ca15.3 levels in this group of patients are not significantly affected by CYP1B1 genetic variations. It is consistent with the prior research, which states that although CYP1B1 polymorphism has the potential to influence the metabolism of tamoxifen and the chances of its undesirable side effects, its direct effect on tumor markers, including Ca15.3, might be minimal.

Altogether, these findings suggest that Ca15.3 monitoring in the context of tamoxifen treatment should be based on such variables as clinical disease progression and treatment response, but not the genotype of CYP1B1 of the patient.

Ethical Declaration: The written informed consent form operated as an entry requirement to the study, with approval given by Kerbala University's ethical and scientific committee of the College of Pharmacy.

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Authors' Contributions

All the authors played equal roles in the conception and design of the study, the acquisition of data, the analysis, and the interpretation. The involvement of all the authors in drafting and critically reviewing, as well as providing final approval to the version to be published, was also required, and the authors agreed to be responsible for all issues related to the work.

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