AMJ
ISSN: 0005-2523

ISSN: 0005-2523
Volume 62, Issue 08, November 2022

INVESTIGATION OF CYP2C19 GENETIC POLYMORPHISM IN POPULATION OF IRAQI BREAST CANCER WOMAN ON TAMXIFEN

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Abstract

Background: Tamoxifen is drug choice for female breast cancer in both pre and postmenopausal women as adjuvant treatment for hormone sensitive breast cancer. CYP2C19 play important role in the formation of a proportion of tamoxifen metabolites, including the conversion of 4-OH-TAM to endoxifen.

Objective: in order to study the prevalence of CYP2C19*3 (G > A, rs4986893) and CYP2C19*17 (C > T, rs11188072) genetic polymorphism and their impact on tamoxifen therapy response in Iraqi breast cancer woman.

Methods: This study was across-sectional observational study carried out at Imam AL-Hussein Medical City/Oncology center in kerbala and laboratories of College of Pharmacy / University of Kerbela, during the period between (November 2021 and Augest 2022). The study was conducted on total 100 females Iraqi women with (ER and /or PR) positive breast cancer who are taken tamoxifen (at least since 3 months). After taking blood sample from each patient genomic DNA was extracted from each blood sample by using the protocol and manufacturers instruction of Favorgen Bio Tech /China for blood genomic DNA extraction kit.

Results: The mean age of participants in this study wass (51.08) years old. The distribution and percentage of individuals having ((C > T, rs 11188072) differ from those expected under Hardy–Weinberg equilibrium {number of observed vs expected were: CC (71, 63.2); TT (12, 4.2); CT (17, 32.6) (goodness-of-fit $\chi 2$ for rs 11188072; 22.891, $P < 0.001$} and therefore it was statistically significant. The distribution and percentage of individuals having (G > A, rs 4986893) differ from those expected under Hardy–Weinberg equilibrium {number of observed vs expected were: GG (20, 10.6); AA (25, 45.6); GA (25, 43.9) (goodness-of-fit $\chi 2$ for rs 93 18.507, $P < 0.001$} and therefore it was statistically significant.

Conclusion: For this cross sectional study in Iraqi breast cancer woman, we observed that genotypes frequencies of CYP2C19*3 (G > A, rs4986893) and CYP2C19 *17 (C > T, rs 11188072) be consistent with Hardy–Weinberg equilibrium and the results were statistically significant and wild type (CC) of rs 11188072 and hetrozogyous (GA) of rs4986893 are more predominant genotype in these population .

Key words: Breast cancer, Tamoxifen , CYP2C19 gene.

Introduction

Carcinogenesis is a multifactorial process that is stimulated by both genetic and environmental causes. Breast cancer is currently one of the most prevalently diagnosed cancers and the 5th cause of cancer-related deaths with an estimated number of 2.3 million new cases worldwide according to the GLOBOCAN 2020 data [1]. Breast cancer (BC) is the leading cause of cancer-related mortality in females and thus accounts for approximately 684,996 deaths annually [2]. BC is complex and highly heterogeneous disease and is composed of distinct subtypes associated with different clinical outcomes [3]. These subtypes are based on the expression of estrogen receptor (ER) alpha (ERα), the progesterone receptor (PR), and the human epidermal growth factor receptor-2 (HER2)/neu. Molecular analysis through gene expression profiling of tumors revealed four intrinsic BC subtypes: luminal ERα positive (ERα+; luminal A and luminal B), HER2 enriched, and basal-like [triple-negative BC (TNBC)] [4, 5]. TNBC lacks ERα, PR, and HER2 [6].The steroid hormone, estrogen [17β-estradiol (E2)], plays an integral role in the development of normal breast tissue. E2 can also function as a driver in the initiation and progression of BC. The majority of BC starts as hormone-dependent; approximately 70-80% of BC diagnoses are ERα+, and 55-65% are PR positive (PR+) at the time of initial diagnosis. Patients with HER2 overexpressing BC comprise approximately 15% of all BC diagnoses [7, 8, and 9]. Various lines of treatment are used including: surgery, radiation therapy, chemotherapy, hormonal therapy and targeted therapy. In those with distant metastasis, treatments are mostly aimed at improving quality of life and survival rate [7].

Tamoxifen (TAM) has been used for the treatment of estrogen-receptor-positive breast cancer for three decades and still has its place in the treatment of both early and metastatic breast cancer. Tamoxifen is the preferred endocrine therapy in premenopausal women and an acceptable option in postmenopausal women, especially in the group with low risk of relapse [10]. In early stage breast cancer, TAM reduces the 15-year risks of breast cancer recurrence and death by about a third [11].Even though the benefit of adjuvant TAM persists for years, some patients will eventually relapse and die of breast cancer [11]. In addition to causing hot flushes TAM increases the risk of endometrial cancer and thromboembolic complications [12, 13].

Tamoxifen is a prodrug and extensively metabolized in the liver to more potent metabolites including; 4-hydroxy-tamoxifen and 4-hydroxy-N-desmethyl-tamoxifen (endoxifen), to elicit its pharmacological activity [14]. Heterogeneity in patients' responses to tamoxifen among breast cancer patients is consistently observed across patient populations where administration of the same dose of this drug results in a range of outcomes which include adverse events or therapeutic failure [15, 16]. The complex metabolism of tamoxifen is primarily catalyzed by cytochrome P450 (CYP) enzymes, amongst which CYP2D6, CYP2C19, CYP3A4, CYP2B6 and CYP2C9 are presumed to be the most important isoenzymes [17]. For example, CYP2D6 plays a pivotal role in converting tamoxifen to 4-hydroxytamoxifen, or converting N-desmethyl tamoxifen, the major metabolite in patients' plasma, to endoxifen. Other isoforms of CYP, including CYP3A4, CYP3A5, CYP2C9, CYP2C19 and CYP2B6, are also involved in tamoxifen metabolism [18, 19, 20].

The term polymorphism defines monogenetic trait, caused by the presence of more than one allele at the same locus and more than one phenotype in the same population in regard to drug interaction with the organism, with the frequency of the rarest allele of more than 1%. DNA sequence variations may occur as insertions or deletions of nucleotides, differences in the copy number of repeated sequences or SNPs -Single Nucleotid Polymorphisms [21].If the mutations occur in proteins that are drug targets or drug-metabolizing enzymes, or in proteins that are involved in drug transport mechanisms, they can affect drug efficacy and safety [22]. For the CYP2C19 gene (cytochrome P450, family2, subfamily C, polypeptide 19).The CYP2C19 gene which is localized on chromosome 10. More than 20 different alleles have been described for CYP2C19, located in both the 5′-flanking region as well as in coding and noncoding parts of the gene, the CYP2C19 gene is highly polymorphic [23]. The CYP2C19 play important role in the formation of a proportion of tamoxifen metabolites, including the conversion of 4-OH-TAM to endoxifen [24].Loss of enzyme activity results from CYP2C19*3(G > A, rs4986893 alleles [25]; the *3 allele occurs in 5–10% of Asians [26].

While;CYP2C19*17 (C > T, rs11188072) has been implicated in enhanced gene transcription [27, 28]. The *17 allele is found in about 4% of Asians and 18–24% of Caucasians and Asian [29, 30, 31].

The aim of the study

To investigate the distribution of CYP2C19*3 (G > A, rs4986893) allele and CYP2C19*17 (C > T, rs11188072) allele polymorphisms in Iraq breast cancer woman taking tamoxifen therapy and their effect on tamoxifen response.

Patients and methods

Study population

This study was across-sectional observational study carried out at Imam AL-Hussein Medical City/Oncology center in kerbala and laboratories of College of Pharmacy / University of Kerbela, during the period between November(2021) and May (2022). The protocol of the study was approved by the Scientific and Ethical Committee of Pharmacy College / Kerbala University. The study was conducted on total 100 females Iraqi postmenopausal women aged 45≥ with (ER and /or PR) positive breast cancer who are taken tamoxifen tablet 20 mg per day orally were included without any diseases. All precautions have been taken in clinical settings to prevent infection of covid19. Patients excluded if they had started tamoxifen therapy simultaneously with either adjuvant chemotherapy or adjuvant radiation therapy (or both) and women who taking drugs that affect the activity of CYP2C19 enzyme (inducer or inhibitors) were excluded.

Clinical data collection

The clinical data was obtained from the medical records of consenting patients and from the patients themselves and these includes: age, weight, height, education , workplace, marital status, breast feeding, date of first menarche and last menopause, family history of breast cancer and number, date of breast cancer diagnosis, site (left ,right, bilateral), type of breast cancer ,stage and grading, immune histochemically status (ER,PR,HER2),surgery, chemotherapy, radiation, presence of osteoporosis, liver disease or any other diseases ,time on tamoxifen therapy and

duration, other drugs used.

Sample collection

Blood samples were taken from eligible females who had signed informed consent. Two ml of blood was placed in EDTA- tube for molecular analysis.

DNA Extraction

Genomic DNA was extracted from each blood sample by using the protocol andmanufacturers instruction of Favorgen Bio Tech /China for blood genomic DNA extraction kit. The DNA was collected and long term stored at -20 °C (deep freezing).Based on NCBI database, all gene information, sequence and SNP details were collected. Using specific software, primers were designed. The SNPs of CYP2C19*3(G > A, rs4986893) allele and CYP2C19*17 (C > T, rs11188072) allele polymorphisms on Iraq breast cancer woman taking tamoxifen therapy was genotyped using conventional genotyping assays by (PCR Master Mix -Promega / USA kit). There are several polymerase chain reactions (PCR) techniques differ in the principle, in this study amplification refractory mutation system (ARMS-PCR) technique use: refers to mutation detection method based on specific PCR primers. Tables 1-4 show primer sequences and PCR program for each SNPs.

Table(1):Primers sequences of CYP2C19*17 (C>T) (rs11188072) genetic polymorphism

Primers	Primer sequence (5 '->3 ')	Product size	Reference
O-F	GGCACAATCCATGAAATAAAGAAT	408 bp	Current study
O-R	AATAGTTCTCCTTGCTGCATATCC	408 bp	Current study
I-F allele T	AACGGGTCTGAACAGACCCT	200 bp	Current study
I-R allele C	TTTGGTATCTGTATGTCTTCTTGGTTAG	256 bp	Current study
O-F; Outer Forward, O-R, Outer Reverse, I-F; Inner Forward, I-R; Inner Reverse			

Table (2): Primers sequences of CYP2C19*3(G > A) (rs4986893) genetic polymorphism.

Primers	Primer sequence (5 '->3 ')	Product size	Reference
O-F	CTCCATTATTTTCCAGAAACGTTTCGAT	258 bp	Current study
O-R	TGCCATCTTTTCCAGATATTCACCC	258 bp	Current study
I-F allele A	AGGATTGTAAGCACCCCCGGA	180 bp	Current study
I-R allele G	AAAAAACTTGGCCTTACCTGGCTCC	124 bp	Current study

Table (3): PCR program for CYP2C19*17 (C>T) (rs11188072) polymorphism.

Steps	Temperature (°C)	Minute: second	Cycle
Initial denaturation	95°	05:00	1
Denaturation	95	00:30	35
Annealing	57°	00:35	
Extension	72°	00:55	
Final extention	72°	05:00	1

Table (4): PCR program for CYP2C19*3(G>A) (rs4986893) polymorphism.

Steps	Temperature (°C)	Minute: second	Cycles
Initial denaturation	95°	05:00	1
Denaturation	95°	00:30	35
Annealing	65°	00:35	
Extension	72°	00:55	
Final extension	72°	05:00	1

Statistical Analysis

The data of participants were analysed by using the statistical package for social sciences (SPSS) version 28, IBM, US. Scale variables presented in mean, standard deviation (SD), while descriptive statistics for nominal (categorical) variables represented as frequency (number of participants) and proportion (percentage). Chi-Square test was used to measure the association between categorical variables. Fisher's Exact test was used as an alternative when the Chi-Square was inapplicable.

Results

Demographic Characteristic of patients:

A total of 100 women participated in this study, which was divided into subgroups based on: Age, BMI and duration of tamoxifen treatment. The clinical demographic characteristics of patients group were summarized in table (5), the mean age of participants which was within a mean age of (51.08) years old. The descriptive table also shown an adjustment of other characteristics and risk factors which were collected through the self-reported technique, these factors included:, BMI, marital status, family history, lymph node involvement, , duration of disease and diagnosis, side effect of tamoxifen and recurrence of disease.

Table 5: Description of the demographic characteristics of the breast cancer patients taking tamoxifen therapy

Variables			Percentage %
Age (Years)			51.08 ± 4.85 mean ±SD
BMI (Kg/m^2)			28.30 ± 5.57 mean ±SD
Duration of tamoxifen (Years)			3.41 ± 2.36 mean ±SD
Duration of disease (Years)			4.18 ± 2.50 mean ±SD
Marital status	Married		93
	Single		7
Family history	Yes		44
	No		56
Breast cancer Side	Left breast		45
	Right breast		55
Lymph node involvement	Yes		64
	No		36
Surgery	Yes		94
	No		6
Chemotherapy	Yes		91
	No		9
Radiotherapy	Yes		79
	No		21
Immunohistochemical tests	HER2	Negative	67
		Positive	33
	Positive for both ER/PR		98
	ER positive /PR negative		2
Side effects	Hot flashes		13
	Joint pain		76
	Both (Hot flashes & Joint pain)		9
	Endometrial hyperplasia		2
Recurrence	Yes		7
	No		93

Genetic Analysis

Results of CYP2C19 Genotype Reactions

Genotyping of CYP2C19 *17 (C > T, rs 11188072) allele

Genotyping of CYP2C19 *17 (C > T, rs 11188072) alleles were classified into 3 genotypes:

1. The major genotype group (CC) homozygous for the allele C.
2. The minor genotype group (TT) homozygous for the allele T.
3. Heterozygous (CT).

Table (6) summarizes the distribution of genotyping groups of CYP2C19 *17 genotype

(C > T , rs 11188072).

Table 6: Distribution of CYP2C19 *17 (C > T, rs 11188072) genotype in breast cancer patients taking tamoxifen

Variable	Group	Frequency	Percentage
Genotype	**CC (wild)**	71	71%
	TT (homo)	12	12%
	CT (hetero)	17	17%
Data Presented by numbers and percentage			

Genotyping of CYP2C19*3 (G > A, rs4986893) allele

Genotyping of CYP2C19*3 (G > A, rs4986893) alleles were classified into three genotypes:

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

Table (7) summarizes the distribution of genotyping groups of for rs 4986893 in patients with breast cancer

Table (7): Distribution of CYP2C19*3(G > A, rs4986893) genotype in breast cancer patients taking tamoxifen therapy

Variable	Group	Frequency	Percentage
Genotype	GG (wild)	20	20%
	GA (hetero)	55	55%
	AA (homo)	25	25%
Data Presented by numbers and percentage			

- **Rs 11188072**

The result of comparison between observed and anticipated values for rs 11188072. Gene in the tested population was shown in figure (1), and table (8). The distribution and percentage of individuals having rs 11188072 differ from those expected under Hardy–Weinberg equilibrium {number of observed vs expected were: CC (71, 63.2); TT (12, 4.2); CT (17, 32.6) (goodness-of-fit $\chi 2$ for rs 11188072; 22.891, $P < 0.001$} and therefore it was statistically significant.

Table (8): Hardy–Weinberg equilibrium for(C > T, rs11188072) genotype in breast cancer patients taking tamoxifen therapy

Genotypes			Alleles		Hardy–Weinberg equilibrium X^2 test
			T	C	
Genotype N= 100	Frequency	%	0.795	0.205	22.891

CC (Wild Type)	71	71%			**P < 0.001 [S]**
CT (heterozygous mutant type)	17	17%			
TT (homozygous mutant type)	12	12%			

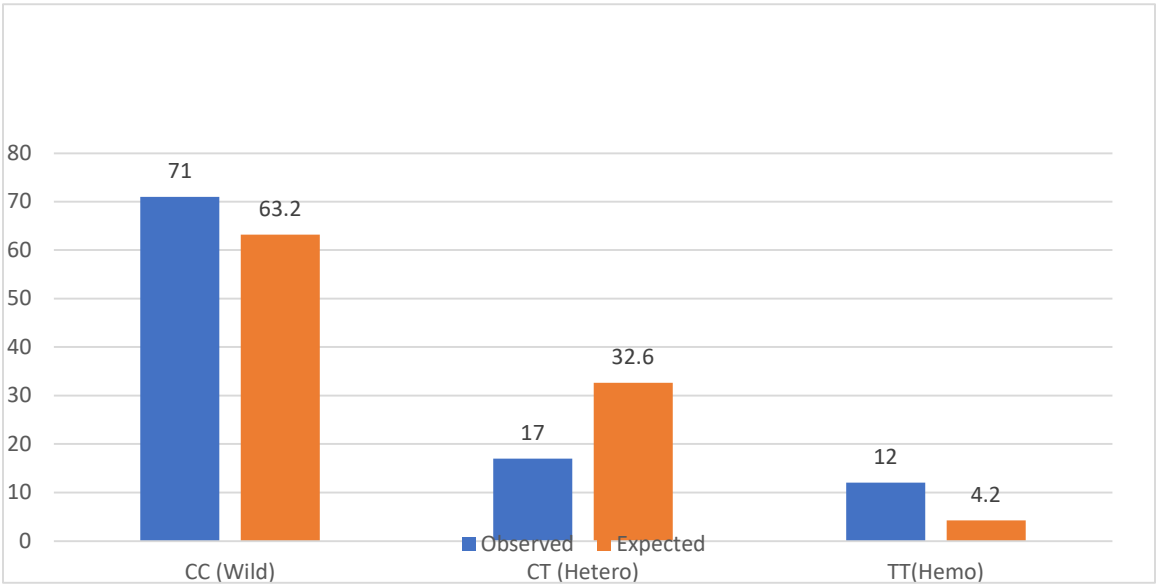

Figure (1): observed (Obs.) vs. expected (Exp.) genotype frequencies % of (C > T, rs11188072) breast cancer patients taking tamoxifen therapy

- **Rs 4986893**

The result of comparison between observed and anticipated values for SNP with rs 4986893 in the tested population were shown in figure (2) and table (9). The distribution and percentage of individuals having **rs 4986893** differ from those expected under Hardy–Weinberg equilibrium {number of observed vs expected were: GG (20, 10.6); AA (25, 45.6); GA (25, 43.9) (goodness-of-fit $\chi 2$ for rs 93 18.507, $P < 0.001$} and therefore it was statistically significant.

Table (9): Hardy–Weinberg equilibrium for (G > A, rs4986893rs) in breast cancer patients taking tamoxifene therapy

Genotypes			Alleles		Hardy–Weinberg equilibrium X^2 test
			T	C	
Genotype N= 100	Frequency	%			
GG (Wild Type)	20	20	0.325	0.675	18.507 **P < 0.001 [S]**
GA(heterozygous mutant type)	25	25			
AA (homozygous mutant type)	55	55			

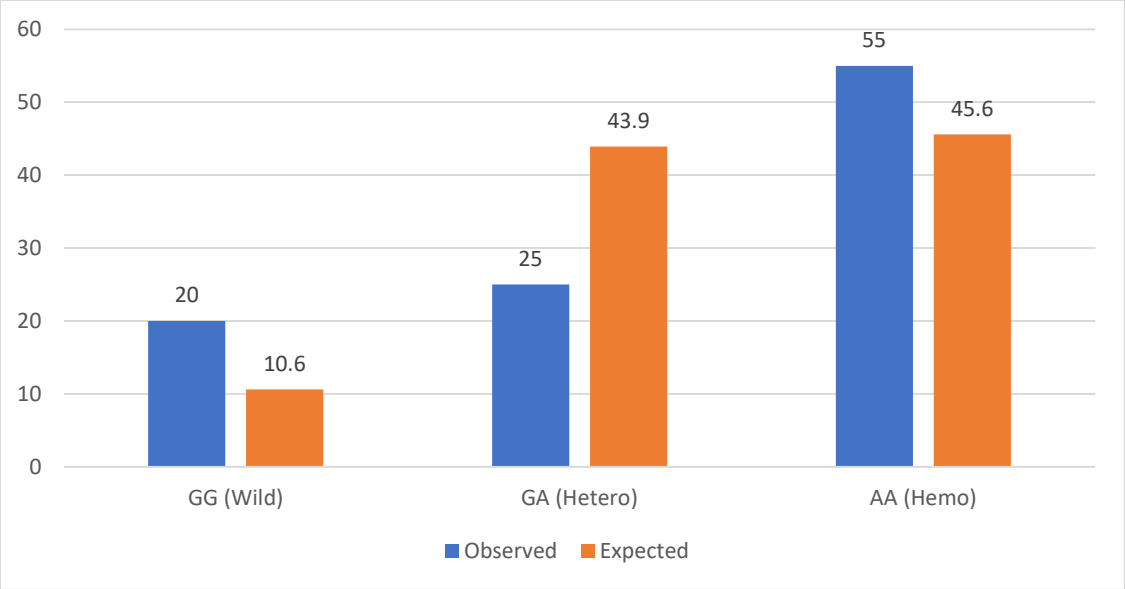

Figure (2): Observed (Obs.) vs expected (Exp.) genotype frequencies % of (G > A, rs4986893)) in breast cancer patients taking tamoxifene therapy

Table (10): Association of side effects with demographic data

Demographic parameters		Patient Genotype (N=100)				P value
		Hot flashes	Joint pain	Both	Endometrial hyperplasia	
Age group	44 - 49 Years	5	33	7	0	0.252
	50 - 55 Years	6	29	1	2	
	56 - 65 Years	2	14	1	0	
BMI group	Obese	7	48	5	1	0.887
	No Obese	6	28	4	1	
Treatment duration	Less than 5 Years	10	62	7	2	0.876
	More than 5Years	3	14	2	0	
Diagnosis duration	Less than 5 Years	8	53	7	2	0.668
	More than 5Years	5	33	2	0	
Results are presented as mean ± SD, or n= number of subjects and percentage, p<0.05 considered, significantly different, [S]= Significant, [NS]= Non significant						

Table 11: Association of recurrence with demographic data

Group		Recurrence		P Value
		No	Yes	
Age group	44 - 49 Years	44	1	0.025*

	50 - 55 Years	32	6	
	56 - 65 Years	17	0	
BMI group	Obese	57	4	0.828
	No Obese	36	3	
Treatment duration	Less than 5 Years	75	6	0.742
	More than 5 Years	18	1	
Diagnosis duration	Less than 5 Years	66	4	0.441
	More than 5 Years	27	3	
Results are presented as mean ± SD, or n= number of subjects and percentage, p<0.05 considered ,significantly different, [S]= Significant, [NS]= Non significant				

Table (12): Difference between side effect and recurrence in rs 11188072 SNP

Demographic parameters		**Genotype n=100**			**P value**
		CC (N=71)	CT (N=17)	TT (N=12)	
Side effect	Hot flashes	9	2	2	0.21
	Joint pain	54	13	9	
	Both (Hot flashes & Joint pain)	7	2	0	
	Endometrial hyperplasia	1	0	1	
Recurrence	Yes	65	17	11	0.70
	No	6	0	1	

Table (13): Difference between side effect and recurrence in rs rs4986893SNP

Demographic parameters		**Genotype n=100**			**P value**
		GG (N=20)	GA (N=25)	AA (N=55)	
Side effect	Hot flashes	1	4	8	0.19
	Joint pain	18	21	37	
	Both (Hot flashes & Joint pain)	1	0	8	
	Endometrial hyperplasia	0	0	2	
Recurrence	Yes	3	1	3	0.32
	No	17	24	52	

Discussion

Adjuvant therapy for breast cancer is used after considering: the patient age, tumor staging,

biological factors, and tumor volume these therapies include: surgery, hormonal therapy (such as tamoxifen [TMX], toremifene), anti-HER2 drugs, and chemotherapy [32, 33]. If hormone receptor-positive breast neoplasms, hormonal therapy is mandatory [33,34,35]. TMX is the main adjuvant hormonal therapy used in pre and postmenopausal patients. Considering that 70% of the breast neoplasms are estrogen receptor-positive (ER), the use of TMX is commonly recommended as the first choice hormonal therapy for breast cancer [36, 32, 34].

Breast cancer is complex disease and is associated with many different causes. Apart from genetic predisposition; many other factors could have an impact on developing breast cancer among women including: demographic characteristics, clinical, reproductive, and environmental features [37]. Beside female gender, age considers strongest risk factors for breast cancer. In this study mean age is (51.08) with more frequency occur between age group of (44-49), and less frequency occur when increase age (56-65) group show in table (5) this is disagreement with previous study [38]. Body mass index (BMI) is important probability factor for increase breast cancer, in this study mean for BMI is (28.30) previous study showing that female with greater (BMI) are at a greater risk for developed breast cancer compared to those with low BMI [39]. The researchers' also observed that greater BMI is associated with more aggressive biological features of tumor including a higher percentage of lymph node metastasis and greater size. Increased body fat will enhances the inflammatory state and affects the levels of circulating hormones facilitating pro-carcinogenic events [40]. Marital status is another risk factor that descriptive in this study ,we found 93% of breast cancer woman married and 7% unmarried so that some studies have reported that no significant correlation between marriage and breast cancer risk while other studies have shown that marriage is a protective factor for disease outcomes, also there are studies showed that married women have increased breast CA risk compared with the unmarried [41], as in this study ,the explanation of these result may be due to genetic factors that contributed in breast cancer development. In this study 56% of cases not associated with family history of BC, Several studies showed that breast CA risk increased by 67% among women with a first – degree relative diagnosed with same disease and twofold in female with more than one relative affected, this association may be due to that both patients share inheritable genetic susceptibility (BRCA mutation) [42]. While in this study most of females had no family history of breast cancer. CYP2C19 was predicted to be an important biomarker for response to tamoxifen because it has similar in vitro activities to CYP2D6 and can also catalyze the conversion of tamoxifen to endoxifen [43]. The expression of estrogen receptor upon binding to tamoxifen shows how the resistance to tamoxifen will develop in the tumor microenvironment. The alteration in the expression of ERα or ERβ, change in co-regulatory proteins, abnormal expression of microRNA and genetic polymorphisms play a role in tamoxifen metabolic activity [44, 45]. Because CYP2C19 play important role in tamoxifene metabolism, in this cross section study will focus on CYP2C19*17 (rs11188072) and CYP2C19*3 (rs4986893) on Iraqi woman with BC and their effect on tamoxifen response.

Hardy-Weinberg equilibrium for CYP2C19*17 (rs11188072) in breast cancer female treated with tamoxifen. Table(8) displayed the genotype and allele frequencies for rs11188072. For both the

recessive and the dominant models, the allele and genotype frequency distributions were with agreement with Hardy-Weinberg equilibrium ($p < 0.05$). A significant difference was discovered for these polymorphisms of the study participants. In the study, wild genotypes (CC) of the rs11188072 polymorphism had a genotype frequency of 71 percent, heterozygous mutant genotype (CT) had a frequency of 17 percent, and homozygous mutant(TT) had a genotype frequency of about 12 percent. No explanation was provided. Additional research is needed to examine the other genotypes and to compare the plasma medication concentrations in patients receiving tamoxifen therapy.

Hardy-Weinberg equilibrium for CYP2C19*3 (rs4986893) in breast cancer female treated with tamoxifen, Table (9) displayed the genotype and allele frequencies for rs4986893. For both the recessive and the dominant models, the allele and genotype frequency distributions were in agreement with Hardy-Weinberg equilibrium ($p < 0.05$). A significant difference was discovered for these polymorphisms of the study participants. In the study, wild genotypes (GG) of rs4986893 had a frequency of 20 percent, heterozygous (GA) type BC female had a frequency of 25 percent, and homozygous mutants (AA) type had a genotype frequency of about 55 percent. No explanation was provided. Additional research is needed to examine the other genotypes and to compare the plasma medication concentrations in patients receiving tamoxifene therapy. From this study we provided Wild type (CC) of (rs11188072) and homozygous mutants (AA) of rs4986893 highest frequency between Iraqi population with breast cancer woman. This study was first study for two presented SNPs.

The variant CYP2C19*17 has been associated with a fast metabolizer phenotype of CYP2C19 due to an increased expression of CYP2C19 [46, 47].So that theoretically and according to previous study breast cancer woman who used tamoxifen therapy and carrier CYP2C19*17 (rs11188072), will increase tamoxifen metabolism and lead to an augmented production of 4-OH-tamoxifen (active metabolite of tamoxifen), which may improve the treatment outcome as reported by Schroth et al [48], that was inversely with CYP2C19*3(G > A, rs4986893) alleles cause loss of enzyme activity [49].In this cross section study didn't find significant effect of CYP2C19 on tamoxifen response this results is agree with previous study, Okishiro et al. did not find an association between CYP2C19*3 genetic polymorphisms leading to absent enzyme activity, and recurrence rate of breast cancer in users of tamoxifen [50], nor did others find any correlation between CYP2C19 and tamoxifen efficacy [51].

Adverse effect of tamoxifen include; menopausal symptoms (hot flashes, atrophic vaginitis, irregular menses), ocular toxicity, thrombocytopenia or leukopenia and gynecologic complications (endometrial cancer, endometrial hyperplasia and polysps Ovarian) [52, 53].

Association of side effect with demographic data in table (10) highest frequency of side effect is pain joint occurring in related with duration of treatment but it is non- significant ,this results explain by in several retrospective or cross-sectional trials that specifically surveyed treatment related musculoskeletal adverse effects, the incidence of joint-related adverse effects was reported to be between 30% and 66%. Moderate to high-grade joint-related symptoms are also associated with poor adherence to therapy or treatment discontinuation, besides interfering with the daily

activities of the survivors [54,55,56] and low 25(OH)D concentrations are strongly linked with increased bone fracture rates and lower physical performance, especially in postmenopausal women[57,58].

Association of recurrence with demographic data further detected between demographic factors and recurrence of breast cancer patient taking tamoxifene, If we compered between these group such as aging group high recurrence value in range of (50-55) years old and high no recurrence value in age group of (44-49) years old, these difference led to age group statistically significant association with the recurrence of breast cancer. Obese woman have high recurrence value than non- obese , this results agree with previous study [59] that obesity might be a reason for greater mortality rates and a higher probability of cancer relapse ,but this result is statistically non-significant. According to duration of treatment the results when duration of treatment less than 5 years recurrence is more than treatment when longer 5 years this results is agree with previous study ,after surgery the use of tamoxifen for (5-10 years) in ER-positive breast cancer diagnostic female can reduce the recurrence rate by 41% and mortality rate by 34% [60] but this results statistically non-significant.
The results above are non -satisfactory because we need further investigation such as need to measure concentration of endoxifene (active metabolite of tamoxifene) and other metabolite and study genetic variant in other enzymes such as CYP2C6 that play main role in metabolism of tamoxifen could contribute to individual variability in tamoxifen response and may warrant further investigation and need large population.

Conclusions

This cross sectional observational study may be the first one done to demonstrate and assessing the role of CYP2C19 genetic polymorphism in tamoxifen therapy response in Iraqi breast cancer female .For this study genotypes frequencies of CYP2C19*3 (G > A, rs4986893) and CYP2C19 *17 (C > T, rs 11188072) found to be consistent with Hardy–Weinberg equilibrium and the results were statistically significant. From this study there is no significant associated between CYP2C19*3 (G > A, rs4986893) and CYP2C19 *17 (C > T, rs 11188072) genetic polymorphism and tamoxifen response in Iraqi BC women , although it is non- significant statistically results CYP2C19 genotype may possibly be concidered a predictive factor for survival in breast cancer patients using tamoxifen with further investigation need.

Reference

1. Sung, H.; Ferlay, J.; Siegel, R.L.; Laversanne, M.; Soerjomataram, I.; Jemal A.; Bray, F. Global Cancer Statistics 2020: GLOBOCAN Estimates ofIncidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J. Clin. 2021, 71, 209–249
2. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: globocan estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer JClin. 2021 ; 71:209-49.

3. Polyak K. Breast cancer: origins and evolution. J Clin Invest. 2007;117:3155-63.
4. Fragomeni SM, Sciallis A, Jeruss JS. Molecular subtypes and local-regional control of breast cancer. Surg Oncol Clin N Am. 2018;27:95-120.
5. Calle EE, Kaaks R. Overweight, obesity and cancer: epidemiological evidence and proposed mechanisms. Nat Rev Cancer. 2004;4:579-91.
6. Lehmann BD, Bauer JA, Chen X, Sanders ME, Chakravarthy AB, Shyr Y, et al. Identification of human triple-negative breast cancer subtypes and preclinical models for selection of targeted therapies. J Clin Invest. 2011;121:2750-67.
7. Reeder JG, Vogel VG, 2008. Breast cancer prevention. Cancer treatment and research 141: 149–64.
8. Masuda H, Baggerly KA, Wang Y, Zhang Y, Gonzalez-Angulo AM, Meric-Bernstam F, et al. Differential response to neoadjuvant chemotherapy among 7 triple-negative breast cancer molecular subtypes. ClinCancer Res. 2013;19:5533-40.
9. Bianchini G, Balko JM, Mayer IA, Sanders ME, Gianni L. Triple-negative breast cancer: challenges and opportunities of a heterogeneous disease. Nat Rev Clin Oncol. 2016;13:674-90
10. Goldhirsch A, Ingle JN, Gelber RD, Coates AS, Thurlimann B, Senn HJ, Panel members: Thresholds for therapies: highlights of the St Gallen International Expert Consensus on the primary therapy of early breastcancer 2009. Ann Oncol 2009, 20(8):1319-1329.
11. Early Breast Cancer Trialists' Collaborative Group (EBCTCG), Davies C,Godwin J, Gray R, Clarke M, Cutter D, Darby S, McGale P, Pan HC, Taylor C,Wang YC, Dowsett M, Ingle J, Peto R: Relevance of breast cancerhormone receptors and other factors to the efficacy of adjuvanttamoxifen: patient-level meta-analysis of randomised trials. Lancet 2011,378(9793):771-784.
12. Early Breast Cancer Trialists' Collaborative Group (EBCTCG): Tamoxifen for early breast cancer: an overview of the randomised trials. Early Breas Cancer Trialists' Collaborative Group. Lancet 1998, 351(9114):1451-1467.
13. Deitcher SR, Gomes MP: The risk of venous thromboembolic disease associated with adjuvant hormone therapy for breast carcinoma: asystematic review. Cancer 2004, 101(3):439-449.
14. Desta Z et al. (2004) Comprehensive evaluation of tamoxifen sequentialbiotransformation by the human cytochrome P450 system in vitro: prominent roles for CYP3A and CYP2D6. Journal of Pharmacology andExperimental Therapeutics 310, 1062–1075.
15. Saladores P et al. (2015) Tamoxifen metabolism predicts drug concentrationsand outcome in premenopausal patients with early breast cancer.Pharmacogenomics Journal 15, 84–94.
16. Crewe HK et al. (2002) Metabolism of tamoxifen by recombinant humancytochrome P450 enzymes: formation of the 4-hydroxy, 4'-hydroxy andN-desmethyl metabolites and isomerization of trans-4-hydroxytamoxifen.Drug Metabolism & Disposition 30, 869–874.
17. Kiyotani K et al. (2012) Pharmacogenomics of tamoxifen: roles of drugmetabolizing enzymes and transporters. Drug Metabolism andPharmacokinetics 27, 122–131.
18. Lim JS et al. (2011) Impact of CYP2D6, CYP3A5, CYP2C9 and CYP2C19 polymorphisms on tamoxifen pharmacokinetics in Asian breast cancerpatients. British Journal of Clinical

Pharmacology 71, 737–750.
19. Lim JS et al. (2016) Association of CYP2C19*2 and associated haplotypes with lower norendoxifen concentrations in tamoxifen-treated Asian breastcancer patients. British Journal of Clinical Pharmacology 81, 1142–1152.
20. Murdter TE et al. (2011) Activity levels of tamoxifen metabolites at theestrogen receptor and the impact of genetic polymorphisms of Phase Iand II enzymes on their concentration levels in plasma. ClinicalPharmacology & Therapeutics 89, 708–717.
21. Relling MV, Giacomini KM (2006) Pharmacogenetics. In: Brunton LL, (Ed.), Goodman & Gilman's The Pharmacological Basis of Therapeutics. (11th edn), McGraw-Hill, New York, USA, 1: 93-115.
22. Scott SA, Sangkuhl K, Gardner EE, Stein CM, Hulot JS, et al. (2011) Clinical Pharmacogenetics Implementation Consortium Guidelines for cytochrome P450-2C19 (CYP2C19) genotype and clopidogrel therapy. Clin Pharmacol Ther 90(2): 32.
23. Sim SC and Ingelman-Sundberg M (2006) The human cytochrome P450 Allele Nomenclature Committee Web site: submission criteria, procedures, and objectives.Methods Mol Biol 320:183-191.
24. Kiyotani, K., Mushiroda, T., Nakamura, Y. & Zembutsu, H. Pharmacogenomics of tamoxifen: roles of drug metabolizing enzymesand transporters. Drug Metab Pharmacokinet 27, 122–31 (2012).
25. De Morais, S. M. et al. Identification of a new genetic defect responsible for the polymorphism of (S)-mephenytoin metabolism inJapanese. Mol Pharmacol 46, 594–8 (1994).
26. Brauch, H. et al. Tamoxifen use in postmenopausal breast cancer: CYP2D6 matters. J Clin Oncol 31, 176–80 (2013(
27. Sim, S. C. et al. A common novel CYP2C19 gene variant causes ultrarapid drug metabolism relevant for the drug response to proton pump inhibitors and antidepressants. Clin Pharmacol Ther 79, 103–13 (2006).
28. Powers, J. L. et al. Multi-gene and Drug Interaction Approach for Tamoxifen Metabolite Patterns Reveals Possible Involvement of CYP2C9, CYP2C19 and ABCB1. J Clin Pharmacol. doi:10.1002/jcph.771 (2016).
29. Scott, S. A. et al. Clinical Pharmacogenetics Implementation Consortium guidelines for cytochrome P450-2C19 (CYP2C19) genotype and clopidogrel therapy. Clin Pharmacol Ther 90, 328–32 (2011).
30. Li-Wan-Po, A., Girard, T., Farndon, P., Cooley, C. & Lithgow, J. Pharmacogenetics of CYP2C19: functional and clinical implicationsof a new variant CYP2C19*17. Br J Clin Pharmacol 69, 222–230 (2010).
31. Schroth, W. et al. Breast cancer treatment outcome with adjuvant tamoxifen relative to patient CYP2D6 and CYP2C19 genotypes. JClin Oncol 25, 5187–93 (2007)
32. Souza RDM, Martins DMF, Chein MBC, Brito LMO. Importância do CYP2D6 em usuárias de tamoxifeno no câncer demama.Femina2011;39:267–274
33. Drăgănescu M, Carmocan C. Hormone therapy in breast cancer.Chirurgia (Bucur)

2017;112(04):413–417 Doi: 10.21614/chirurgia.112.4.413
34. Abdel-Hafiz HA. Epigenetic mechanisms of tamoxifen resistancein luminal breast cancer. Diseases 2017;5(03):E16 Doi: 10.3390/diseases5030016
35. Fleeman N, Payne K, Newman WG, et al. Are health technology assessments of pharmacogenetic tests feasible? A case study ofCYP2D6 testing in the treatment of breast cancer with tamoxifen.Per Med 2013;10(06):601–611 Doi: 10.2217/pme.13.60
36. Jahangiri R, Mosaffa F, Emami Razavi A, Teimoori-Toolabi L, Jamialahmadi K. Altered DNA methyltransferases promoter methylation and mRNA expression are associated with tamoxifen response in breast tumors. J Cell Physiol 2018;233(09):7305–7319 Doi: 10.1002/jcp.26562
37. Brinton LA, Gaudet MM, Gierach GL. Breast cancer. In: Thun MJ, Linet MS,Cerhan JR, Haiman CA, Schottenfeld D, Eds. Cancer Epidemiology andrevention. 4th ed. New York, NY: Oxford University Press, 2018; pp.861–888.
38. Thakur P; Seam RK; Gupta MK; Gupta M; Sharma M and Fotedar V. Breastcancer risk factor evaluation in a Western Himalayan state: A case–controlstudy and comparison with the Western World. South Asian Journal ofCancer. 2017;6(3):106.
39. Wang, X.; Hui, T.-L.; Wang, M.-Q.; Liu, H.; Li, R.-Y.; Song, Z.-C.Body Mass Index at Diagnosis as a Prognostic Factor forEarly-Stage InvasiveBreast Cancer after Surgical Resection. Oncol. Res. Treat. 2019,42, 195–201. [CrossRef]
40. James, F.; Wootton, S.; Jackson, A.; Wiseman, M.; Copson, E.;Cutress,R. Obesity in breast cancer—What is the risk factor? Eur. J.Cancer 2015, 51, 705–720. [CrossRef]
41. Balekouzou A; Yin P; Pamatika CM; Bekolo CE; Nambei SW; Djeintote M;et al. Reproductive risk factors associated with breast cancer in women inBangui: a case–control study. BMC Women's Health. 2017;17(1):14.
42. Bravi F; Decarli A and Russo AG. Risk factors for breast cancer in a cohortof mammographic screening program: a nested case–control study within the FR iCaM study. Cancer Medicine. 2018;7(5):2145-52CYP2C19
43. Crewe HK, Notley LM, Wunsch RM, Lennard MS, Gillam EM. Metabolism of tamoxifen by recombinant human cytochrome p450 enzymes: formation of the 4-hydroxy, 4′-hydroxy and N-desmethyl metabolites and isomerization of trans-4-hydroxytamoxifen. Drug Metab Dispos. 2002;30:869–874. [PubMed] [Google Scholar]
44. Goetz MP et al.J Clin Oncol..2005 23:9312 [PMID: 16361630].
45. Garcia-Becerra Ret al.Int J Mol Sci. 2012 14:108 [PMID:23344024]
46. Baldwin RM, Ohlsson S, Pedersen RS, Mwinyi J, IngelmanSundberg M, Eliasson E, Bertilsson L (2008) Increased omeprazole metabolism in carriers of the CYP2C19*17 allele; a pharmacokinetic study in healthy volunteers. Br J Clin Pharmacol65:767–774
47. Sim SC, Risinger C, Dahl ML, Aklillu E, Christensen M, Bertilsson L, Ingelman-Sundberg M (2006) A common novel CYP2C19 gene variant causes ultrarapid drug metabolism relevant for the drug response to proton pump inhibitors and antidepressants. Clin Pharmacol Ther 79:103–113
48. Schroth W, Antoniadou L, Fritz P, Schwab M, Muerdter T, ZangerUM, Simon W, Eichelbaum

M, Brauch H (2007) Breast cancertreatment outcome with adjuvant tamoxifen relative to patientCYP2D6 and CYP2C19 genotypes. J Clin Oncol 25:5187–5193
49. Brauch, H. et al. Tamoxifen use in postmenopausal breast cancer: CYP2D6 matters. J Clin Oncol 31, 176–80 (2013)
50. Okishiro M, Taguchi T, Jin Kim S,Shimazu K, Tamaki Y, Noguchi S: Geneticpolymorphisms of CYP2D6*10 andCYP2C19*2,*3 are not associated withprognosis, endometrial thickness, or bonemineral density in Japanese breast cancerpatients treated with adjuvant tamoxifen.Cancer, 115(5), 952–961 (2009
51. Serrano D, Lazzeroni M, Zambon CF et al.:Efficacy of tamoxifen based on cytochromep450 2D6, CYP2C19 and SULT1A1genotype in the italian tamoxifenprevention trial. Pharmacogenomics J. (2010)(Epub ahead of print)
52. Buzdar, A. U. (Ed.). (2007). Endocrine Therapies in Breast Cancer . Oxford University Press.
53.Golan, D. E., Tashjian, A. H., & Armstrong, E. J. (Eds.). (2011). Principles of pharmacology: the pathophysiologic basis of drug therapy. Lippincott Williams & Wilkins .
54. Nyrop KA, Callahan LF, Rini C, et al. Adaptation of an evidence-basedarthritis program for breast cancer survivors on aromatase inhibitortherapy who experience joint pain. Prev Chronic Dis. 2015;12:E91.
55. Crew KD, Greenlee H, Capodice J, et al. Prevalence of joint symptomsin postmenopausal women taking aromatase inhibitors for early-stagebreast cancer. J Clin Oncol. 2007;25(25):3877–3883.
56. Hershman DL, Shao T, Kushi LH, et al. Early discontinuation and nonadherence to adjuvant hormonal therapy are associated with increasedmortality in women with breast cancer. Breast Cancer Res Treat. 2011;126(2):529–537
57. Kuchuk NO, Pluijm SM, van Schoor NM, Looman CW, Smit JH, Lips P.Relationships of serum 25-hydroxyvitamin D to bone mineral density and serum parathyroid hormone and markers of bone turnover in olderpersons. J Clin Endocrinol Metab. 2009;94(4):1244–1250.
58. Martin-Herranz A, Salinas-Hernandez P. Vitamin Dsupplementationreview and recommendations for women diagnosed with breast or ovary cancer in the context of bone health and cancer prognosis/risk. Crit RevOncol Hematol. 2015;96(1):91–99.
59. Sun, L.; Zhu, Y.; Qian, Q.; Tang, L. Body mass index and prognosis ofbreast cancer. Medicine 2018, 97, e11220. [CrossRef]
60. Mirzabeigi MN; Nelson JA; Fischer JP; Kovach S;, Serletti JM; Wu LC andet al. Tamoxifen (selective estrogen-receptor modulators) and aromataseinhibitors as potential perioperative thrombotic risk factors in free flap breast reconstruction. Plastic and Reconstructive Surgery. 2015;135(4):670e-9e.