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Evaluation of Endometrial Thickness and Bone Mineral Density Based on CYP2D6 Polymorphisms in Turkish Breast Cancer Patients Receiving Tamoxifen Treatment

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Key Words

Breast cancer · CYP2D6 · Endometrial thickness · Bone mineral density · Hepatosteatosis · Tamoxifen

Abstract

Background: Several previous studies have examined the effect of CYP2D6 gene polymorphism on the efficacy and metabolism of tamoxifen (Tamoxifen Teva, Nolvadex) in the treatment of breast cancer. In the present study, the metabolic profiles associated with various CYP2D6 genotypes were evaluated. ***Method:*** In the present study 92 Turkish breast cancer patients with early-stage hormone receptor-positive tumors treated with adjuvant tamoxifen (20 mg) were evaluated for CYP2D6 genotype and metabolic profiles. Known side effects of tamoxifen treatment, including endometrial thickening, changes in serum lipid levels and bone density, and hepatosteatosis, were evaluated according to the CYP2D6 polymorphism. ***Result:*** The distribution of metabolic characteristics in the Turkish population was as follows: 77.1% normal metabolism, 11.5% intermediate metabolism, 5.2% ultrarapid metabolism, and 2.1% poor metabolism. The CYP2D6 genotypes associated with rapid metabolism were CYP2D6 3X*1/*1 duplication (DUP) and CYP2D6 2X*1/*2, while poor metabolism was associated with the genotypes CYP2D6 *3/*4 and CYP2D6 *6/*6. There was no statistically significant relationship between metabolic characteristics and bone density or hepatosteatosis. A statistically significant difference in total cholesterol and triglycerides was detected in lipid profile analysis ($p = 0.003$, $p = 0.02$). Assessment of endometrial thickness revealed a significant association of hyperplasia and poor metabolism, and an association between atrophy and ultrarapid metabolism ($p = 0.01$). ***Conclusion:*** Significant development of endometrial hyperplasia was identified among individuals with poor tamoxifen metabolism. As a result, tamoxifen may be a significant predictor of endometrial thickening among individuals with poor metabolic characteristics.

Introduction

Tamoxifen, a selective estrogen receptor modulator, is an effective chemotherapeutic in women with high-risk breast cancer and in the prevention of recurrence in cases of estrogen receptor-positive breast cancer [1]. Tamoxifen is effective in the target tissue as both an estrogenic and antiestrogenic agent [2, 3].

Tamoxifen is taken in the form of a pro-drug and is metabolized into the potent metabolite endoxifen. The biotransformation of tamoxifen to endoxifen is catalyzed

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by the cytochrome P450 enzymes, including CYP2D6, CYP2C9, CYP3A4, CYP3A5, and CYP2C19. CYP3A4 and CYP3A5 catalyze the demethylation reaction, and CYP2D6 facilitates hydroxylation reactions [4, 5]. The CYP2D6 enzyme plays a major role in the conversion of tamoxifen to endoxifen and is encoded by the CYP2D6 gene. Previous reports suggest that as many as 46 unique CYP2D6 alleles can result in the loss of enzyme function [6, 7].

The use of tamoxifen may also result in changes to the lipid profile [8–10], bone mineral density (BMD) [11], and endometrial thickness (ET) [12, 13].

Tamoxifen is associated with positive changes in lipid profile, decreasing total cholesterol and low-density lipoprotein (LDL) and increasing high-density lipoprotein (HDL). Nevertheless, the specific effects of tamoxifen on lipid profile vary between patients. Single nucleotide polymorphisms in the gene encoding the estrogen receptor may explain variation in response to tamoxifen [8]. Although the pathophysiology of nonalcoholic steatohepatitis (NASH) is not fully understood, several studies have reported an association between tamoxifen and hepatosteatosis incidence [9, 10].

Tamoxifen has differential effects on BMD, such that among premenopausal women with breast cancer, tamoxifen caused significant bone loss, while the same drug showed partially protective effects in patients who went into menopause early due to chemotherapy [11].

A significant increase in ET was observed at 1 year following tamoxifen treatment and was attributed to the partial estrogenic effects of the drug [12, 13]. Previous studies have investigated the relationship between CYP2D6 polymorphisms and the risk of side effects during tamoxifen treatment [14–16]. The CYP2D6 *10/*10 and CYP2C19 genotypes were not associated with changes in prognosis, ET, BMD, or total cholesterol levels among a cohort of Japanese patients treated with tamoxifen [14]. In addition, patients with the CYP2D6 *4/*4 polymorphism exhibit typical ET [15], however it has been reported that the CYP2D6 variant rs1800716 is associated with increased ET [16].

Tamoxifen treatment significantly prolongs survival among breast cancer patients. CYP2D6 polymorphisms fundamentally alter tamoxifen metabolism and may result in changes in side effect profile and treatment effectiveness. The frequency of gene polymorphisms varies significantly between populations. In the present study, we evaluated the relationship between CYP2D6 genotype and ET, BMD, blood lipid profiles, and hepatosteatosis in a Turkish population undergoing tamoxifen treatment for breast cancer. We identified a statistically significant increase in ET among individuals carrying the CYP2D6 *3/*4 and CYP2D6 *6/*6 polymorphisms associated with poor tamoxifen metabolism.

Materials and Methods

Study Population

This study included a total of 115 patients diagnosed with breast cancer and admitted to the Çukurova University Faculty of Medicine, Department of Medical Oncology between 2007 and 2012. Patients included in the study were early-stage breast cancer patients receiving 20 mg/day tamoxifen adjuvant. All of the patients received cytotoxic chemotherapy prior to the administration of tamoxifen. Patients diagnosed with diabetes mellitus, hypertension, hyperlipidemia, and patients using any medications including antidepressants were not included in the study. Certain drugs and other xenobiotic agents can act as powerful inhibitors of the CYP-P450 enzyme system and therefore patients receiving drugs known to affect tamoxifen metabolism were excluded from the study.

Sample Collection and Genotype Analysis

Genomic DNA was extracted using the QIAamp DNA Blood Midi Kit (Qiagen, Valencia, Calif., USA) and eluted materials were normalized to a concentration of 10 ng/μl using a NanoDrop spectrophotometer. Patient DNA samples were analyzed using the xTAG™ CYP2D6 v2 kit to determine the CYP2D6 genotype.

Polymerase chain reaction (PCR) was performed using a 9700 thermal cycler (PE Applied Biosystems) according to the following conditions: initial denaturation at 98°C for 3 min, followed by 35 cycles of 95°C for 60 s, 66°C for 30 s, 72°C for 2 min 30 s, and a final elongation step of 72°C for 5 min. PCR products and amplicons were hybridized to the microarray and stained with a streptavidin-phycoerythrin conjugate. Fluorescence associated with hybridization to specific probe features was detected by a laser-illuminated, confocal scanner and luminescence was detected by using a Luminex® 200 xMAP device.

Data analysis software interpreted the hybridization pattern of probes that were specifically complementary to either wild-type or mutant sequences for each polymorphic site. The assay also detected the presence of gene duplications and gene deletions. A total of 14 alleles including CYP2D6 *1, *2, *3, *4, *5 (deletions) and *6, *7, *8, *9, *10, *11, *15, *17, *41 were evaluated (http://www.imm.ki.se/CYPalleles/cyp2d6.htm). The alleles were divided into four subgroups based on their activity: (I) functional or wild-type alleles CYP2D6 *1, *2, *35, (II) partially active alleles CYP2D6 *9, *10, *17, *41, (III) inactive alleles CYP2D6 *3, *4, *5, *6, *7, *8, and (IV) duplicated genes CYP2D6 *1 × N, *2 × N, *35 × N, *4 × N, *10 × N, *41 × N. The predicted CYP2D6 phenotypes were classified based on the CYP2D6 allele genotypes. CYP2D6 alleles were classified into four catagories based on the metabolic phenotype associated with each: normal or extensive metabolizers (EM), intermediate metabolizers (IM), ultrarapid metabolizers (UM), and poor metabolizers (PM). The EM group included two active CYP2D6 alleles or one active allele and one partially active allele. The IM group had one active and one inactive allele or one partially active and one inactive CYP2D6 alleles. There were no active CYP2D6 alleles in the PM group. The UM group had three or more active CYP2D6 alleles due to duplication of one active allele.

Table 1. Patient characteristics (n = 96)

Characteristics of patients		Patients	
		n	%
Age, years	min	32	
	median	45	
	max	62	
Menopausal status	premenopausal	89	92.71
	postmenopausal	7	7.29
Histology	ductal	78	81
	lobular	11	11.45
	other	2	2
Tumor size, cm	Tis/T1	25	26.86
	T2	51	52.81
	T3/T4	20	20.22
Nodal status	N0	42	43.48
	N1	27	28.26
	N2	27	28.26
Grade	I	7	7.3
	II	60	62.5
	III	21	21.9
Estrogen receptor status	<50	25	26
	50–80	27	28
	81–90	23	24
	>90	21	21
Progesterone receptor status	<10	24	25.0
	10–50	27	28.1
	51–80	27	28.1
	>80	18	18.8
HER2	negative	27	28.1
	positive	67	69.8
Stage	I	17	17.7
	II	43	43.8
	III	35	36.9
Metastasis/recurrence	yes	5	5.2
	no	90	93.8
Follow-up, months	minimum	6	
	median	32	
	maximum	71	

BMD and ET

The effects of tamoxifen treatment on BMD and ET were evaluated according to metabolic type among postmenopausal breast cancer patients. The lumbar spine (lumbar segments 2–4, L2–L4) was measured using a dual-energy X-ray absorptiometer (Lunar DPX Medical Systems), while the ET was measured by transvaginal ultrasonography (Sono-Vis MSC, Siemens AG/Bayer, Munich, Germany).

Statistical Analysis

The SPSS 19.0 software package was used for statistical analysis of the compiled dataset. Categorical measurements are reported as number and percentage, while quantitative measurements are reported as mean and standard deviation. The statistical significance of changes in BMD and ET after the initiation of tamoxifen treatment was evaluated using the χ^2 test. The χ^2 test was used for comparison of categorical measurements between patient groups; a p value of <0.05 was considered statistically significant. Kaplan-Meier analysis was used to evaluate the relationship between disease metastasis and CYP2D6 genotype.

Table 2. Incidence of CYP2D6 polymorphisms

Allele genotype	n	%
1*1*	11	11.95
1*10*	8	8.69
1*17*	1	1.08
1*2*	19	20.65
1*3*	1	1.08
1*4*	9	9.78
1*41*	14	15.21
1*5*	1	1.08
1*7*	2	2.17
10*10*	1	1.08
10*41*	1	1.08
2*1*	1	1.08
2*2*	3	3.26
2*4*	6	6.52
2*41*	3	3.26
2*6*	1	1.08
3*4*	1	1.08
3*7*	1	1.08
4*41*	3	3.26
41*41*	3	3.26
5*41*	1	1.08
6*6*	1	1.08
Total	92	100.00

Results

A total of 115 breast cancer patients treated with adjuvant tamoxifen were enrolled in the study, however DNA isolation could not be performed in 13 blood samples because of hemolysis. Therefore, data was obtained from a total of 92 patients (table 1). The mean (±SD) age of patients in the study group was 44.81 ± 6.44 years. The total follow-up time ranged from 6 to 71 months (mean: 32 months).

The most frequent CYP2D6 gene polymorphism was *1/*2, which occurred in 20.6% (n = 19) of the study group. CYP2D6 polymorphisms *1/*41, *1/*1, *1/*4, *1/*10 occurred at a frequency of 15.21% (n = 14), 11% (n = 11), 9.78% (n = 9), and 8.69% (n = 8.69), respectively (table 2). CYP2D6 gene polymorphisms were classified according to the known metabolic phenotype associated with each: EM (n = 74), 77.1%, IM (n = 11), 11.5%, UM

Table 3. Incidence of metabolizer groups

	n	%
UM	5	5.43
EM	74	80.43
IM	11	11.95
PM	2	2.17
Total	92	99.98

Table 4. The evaluation of changes in blood lipid levels and the metabolizer groups

	CYP2D6 groups, %				p
	UM	EM	IM	PM	
Cholesterol					
Normal	4.2	87.5	5.6	2.8	*0.003*
High	6.3	56.3	37.5	0.0	*0.003*
LDL					
Normal	3.6	84.3	9.6	2.4	0.118
High	16.7	50.0	33.3	0.0	0.118
HDL					
Normal	6.1	78.8	9.1	6.1	0.295
High	5.3	82.5	12.3	0.0	0.295
Tryglycerides					
Normal	1.7	88.3	6.7	3.3	*0.029*
High	10.7	67.9	21.4	0.0	*0.029*

Table 5. Evaluation of the effects of metabolizer groups on liver

	CYP2D6 groups, %				p
	UM	EM	IM	PM	
Liver enzymes					
Normal	5.8	80.2	11.6	2.3	0.807
High	0.0	100.0	0.0	0.0	0.807
Hepatosteatosis					
Existent	8.7	82.6	8.7	0.0	0.665
None	4.5	79.1	13.4	3.0	0.665
Hepatosteatosis levels					
0.0	8.7	82.6	8.7	0.0	0.168
1	8.0	80.0	12.0	0.0	0.168
2	0.0	90.0	6.7	3.3	0.168
3	8.3	50.0	33.3	8.3	0.168

(n = 5), 5.2% and PM (n = 2), 2.1% (table 3). The CYP2D6 genotypes included in the PM group were CYP2D6 *3/*4 and CYP2D6 *6/*6, while the CYP2D6 genotypes classified as UM were CYP2D6 3X*1/*1 duplication (DUP), and CYP2D6 2X*1/*2.

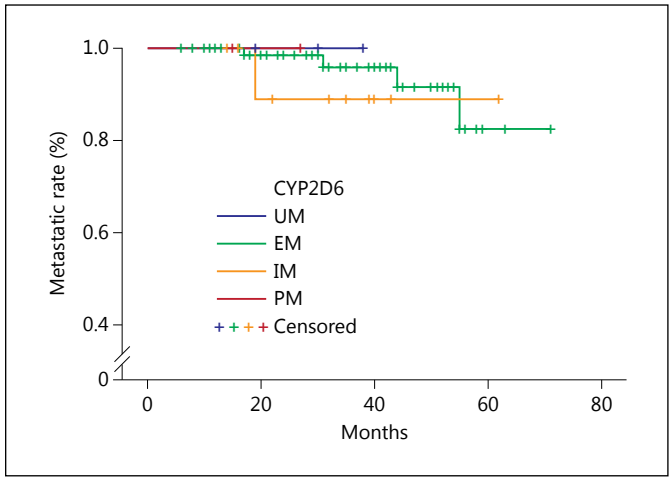

Fig. 1. Metastasis rates were calculated with the Kaplan-Meier method according to metabolizer groups.

Serum lipids, cholesterol, LDL, HDL and triglycerides were evaluated in all patients. Normal cholesterol levels were found in 79.2% (n = 76) of the study patients and high cholesterol levels were found in 16.7% (n = 16) of the study group. LDL levels were normal in 90.6% (n = 90) of patients and high in 6.3% (n = 6) of patients. HDL levels were normal in 34.4% (n = 33) and high in 63.5% (n = 61) of the study patients, while triglyceride levels were normal in 63.5% (n = 61) and high in 32.3% (n = 31) of the patient group. There was a significant difference in total cholesterol (p = 0.03) and triglyceride (p = 0.029) between metabolic phenotype groups (table 4).

Liver function tests demonstrated liver deterioration in 4.2% (n = 4) of patients, while 93.8% (n = 90) exhibited no signs of liver deterioration. Abdominal ultrasound identified hepatosteatosis in 72.9% (n = 70) of patients, while no hepatosteatosis was detected in 24% (n = 23) of the patients. Steatosis grading indicated that 28.1% (n = 27) of patients were grade I, 31.3% (n = 30) were grade II, and 13.5% (n = 13) were determined to be grade III. There was no statistically significant relationship between CYP2D6 polymorphism and liver enzyme levels, presence of hepatosteatosis, or degree of hepatosteatosis (p = 0.8, p = 0.6, p = 0.1; table 5).

Kaplan-Meier analysis with log-rank test was performed in order to assess the development of metastases according to metabolic phenotype; no statistically significant relationship was identified (p = 0.87; fig. 1).

BMD measurements revealed the presence of osteoporosis in 12.5% (n = 12) of patients, osteopenia in 44.8% (n = 43), and normal bone density in 32.3% (n = 31) of

Table 6. Effect of the metabolizer groups on BMD and ET

	CYP2D6 groups, %				p
	UM	EM	IM	PM	
Bone densitometer					
Normal	6.7	86.7	3.3	3.3	0.624
Osteopenia	4.8	76.2	16.7	2.4	0.624
Osteoporosis	0.0	80.0	20.0	0.0	0.624
ET					
<5, atrophic	4.3	83.0	12.8	0.0	*0.019*
5–10, normal	7.7	76.9	12.8	2.6	*0.019*
>10, hyperplastic	0.0	66.7	0.0	33.3	*0.019*

patients. There was no statistically significant relationship between metabolic phenotype and BMD (p = 0.624; table 6).

ET analysis determined that 51% (n = 49) of patients had a measurement of 1, 41.7% (n = 40) were measured at 2, and 3.1% (n = 3) had an observed measurement of 3. There was a statistically significant difference in ET among the metabolic phenotypes. While the PM group exhibited marked hyperplasia in the endometrium, the UM group exhibited an atrophic endometrium (p = 0.019; table 6).

Discussion

Tamoxifen is administered as a pro-drug and is metabolized into the highly potent, active drugs 4-hydroxytamoxifen and endoxifen, the most potent of which is 4-hydroxy-tamoxifen [6]. Differences in the concentration of active metabolites may contribute to differences in drug response among individuals [4]. Numerous studies have investigated CYP2D6 polymorphism, demonstrating the role of tamoxifen metabolism in determining the risk of breast cancer recurrence. Specific side effects of tamoxifen have been associated with CYP2D6 mutations [1–14, 17–19].

In the present study we investigated the distribution of CYP2D6 polymorphisms among breast cancer patients receiving adjuvant tamoxifen treatment. The most common polymorphism was the *1 allele and the most frequently identified allele pair was CYP2D6 *1*2. In addition, we compared histological grade, estrogen receptor expression, progesterone receptor expression, HER2 expression, and lymph node involvement among PM, IM, EM and UM groups, but did not identify any significant differences associated with CYP2D6 phenotype. Similarly, there was no significant difference between the incidence of recurrence and CYP2D6 metabolic phenotype.

Although tamoxifen is known to influence serum lipids in some breast cancer patients, little is known regarding the specific mechanisms involved. The effect of tamoxifen of lipid parameters varies between patients. Though not completely understood, variation may arise as a result of single nucleotide polymorphisms in the gene encoding the estrogen receptor [8]. Insufficient data is available of the potential relationship between serum lipids and CYP2D6. One study suggested that changes in lipid profile were related to postmenopausal state, but no connection between gene polymorphism and lipid profile was identified [20]. Another study compared premenopausal and postmenopausal women using tamoxifen and reported reduced cholesterol, elevated HDL and reduced triglyceride levels among premenopausal women. The same study reported a significant decrease in total cholesterol and LDL levels and an increase in triglyceride levels among individuals undergoing tamoxifen treatment [8]. Treatment with tamoxifen for 1 year was associated with a significant reduction in total cholesterol [14]. In the present study, a significant relationship between CYP2D6 phenotype and total cholesterol and triglyceride levels was identified. Total cholesterol and triglyceride levels were elevated in the UM group relative to all other CYP2D6 phenotypes. There was no significant difference in HDL and LDL among CYP2D6 phenotypes.

Although NASH is primarily associated with obesity and diabetes, it may also develop as a result of the use of certain drugs, including tamoxifen. Tamoxifen-induced hepatosteatosis was first identified in case reports [20, 21]. Imaging studies have confirmed that hepatosteatosis occurs in as many as 33% of tamoxifen-treated patients [22–24]. A retrospective study detected 24 cases of NASH among 1,105 breast cancer patients screened by liver biopsy. Among those 24 patients, 13 had impaired liver function and 17 had developed NASH subsequent to tamoxifen use. Follow-up studies of these patients determined that they were recovering following discontinuation of tamoxifen. The mechanisms that facilitate NASH development in the presence of tamoxifen are not fully understood. The accumulation of lipids in the liver, cytokine activation, mitochondrial dysfunction, and oxidative stress have all been proposed as potential mechanisms of NASH [9]. The side effects of tamoxifen, including weight gain and hypertriglyceridemia, may contribute to hepatosteatosis. In one retrospective study, 36% of the patients experienced weight gain [9]. In the present study,

weight gain occurred in 62.5% of patients treated with tamoxifen. This weight gain likely facilitates the development of hepatosteatosis. No statistically significant association between hepatosteatosis and CYP2D6 phenotype was observed.

Tamoxifen preserves bone mass and reduces the incidence of symptomatic fractures among postmenopausal women, although no such effects are apparent among premenopausal women. Postmenopausal women treated with tamoxifen have low endogenous estrogen levels, which can contribute to decreased bone loss. However, tamoxifen may act as an antiestrogen factor in premenopausal women, who exhibit high levels of endogenous estrogen. In one study measuring bone density 3 years after the initiation of tamoxifen treatment, 4.6% bone density loss in the lumbar vertebrae was observed [11]. In the present study, there was no significant relationship between CYP2D6 polymorphism and BMD. A previous study also determined that CYP2D6 *10/*10 polymorphisms are not associated with BMD [14], and the present study validates this result.

Previous studies have examined the association between endometrial wall thickening and CYP2D6 polymorphism [14, 16]. A study conducted in Japan demonstrated no significant relationship between ET and CYP2D6 *10/*10, CYP2D6 wild-type/wild-type, CYP2D6 wild-type/*10 genotypes, polymorphisms that occur at a rate of 15–20% in the Japanese population [14]. CYP2D6 *10/*10 was identified as a partially active allele in that study, and this enzyme activity may contribute to the low risk of ET. It has recently been observed that women homozygous for the mutant variant rs1800716 are at increased risk of developing endometrial thickening [16]. In the present study, we identified a significant relationship between CYP2D6 phenotype and the presence of ET. The PM phenotype was associated with normal or hyperplastic endometrium and exhibited no signs of atrophy. The UM phenotype was associated with normal or atrophic endometrium. The genotypes CYP2D6 *3/*4 and CYP2D6 *6/*6 resulted in the PM phenotype. The relationship between the PM phenotype and the presence of increased ET in our study group was consistent with recent reports [16].

Allele frequency differs among populations, resulting in phenotypic differences including altered drug metabolism [25–27]. Thus, genetic variation is a significant determinant of the efficacy and side effect profile of tamoxifen. In this study, we evaluated the frequency of CYP2D6 polymorphisms in a population of Turkish breast cancer patients undergoing tamoxifen therapy. The majority of these patients exhibit the EM phenotype, which was not associated with changes in ET or bone density. However, the PM phenotype was associated with increased ET. Identification of patient genotype prior to treatment may accurately predict the potential post-treatment side effects of tamoxifen. According to the ATLAS study that compared 5-year adjuvant tamoxifen treatment to 10-year treatment, the 10-year treatment was shown to have higher survival rates [28]. The present study underscores the need for improved understanding of the effects of tamoxifen. In conclusion, early detection of CYP2D6 *3/*4 and CYP2D6 *6/*6 polymorphisms in women undergoing tamoxifen treatment for breast cancer may predict the development of ET.

Disclosure Statement

The authors declare that they have no conflict of interest.

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