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Polymorphism of the CYP2D6 gene in women with breast cancer treated with tamoxifen

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Summary

Objective: To evaluate polymorphism frequency of the CYP2D6*4, *10, and *17 alleles in women with breast cancer treated with tamoxifen. *Materials and Methods:* Ninety-five women with estrogen and progesterone receptor-positive breast carcinoma were investigated from September to December 2013. A three-ml sample of peripheral blood was collected from each patient to analyze the presence of CYP2D6 *4, *10, and *17 allele polymorphism by specific polymerase chain reaction technique (PCR) for analysis of haplotypes *1, *4, *10, and *17, determined by studies of different single-nucleotide polymorphism (SNP). The data obtained were compiled and analyzed with the aid of Excel software 2010. *Results:* The frequency of CYP2D6 alleles *4, *10, and *17 was 16%, 29%, and 2%, respectively, and haplotype *1/*10 was shown in 22% of the women. The phenotype of intermediate metabolism occurred in 8% of women. *Conclusions:* The present study showed a deficiency in tamoxifen metabolism, characterized by intermediate metabolism in 8% of Brazilian women.

Key words: Breast cancer; Tamoxifen; CYP2D6; Genetic polymorphism.

Introduction

Breast cancer is the most common form of cancer in women in Western countries [1]. In Brazil, it is a major cause of cancer-related death and approximately 57,120 new cases are seen each year [2]. Approximately 60-70% of women with newly diagnosed breast cancer have tumors expressing estrogen or progesterone receptors [3]. Tamoxifen is the standard endocrine treatment for estrogen receptor-positive breast cancer which offers clear benefits, such as a considerable reduction in recurrence and mortality rates [4, 5]. Nevertheless, 30% to 50% of patients with adjuvant tamoxifen therapy may still experience relapse and will eventually die of the disease [6, 7].

Tamoxifen is a prodrug that requires metabolic activation to exert its pharmacologic action. CYP2D6 is a key enzyme for the formation of two active tamoxifen metabolites, 4-hydroxytamoxifen and 4-hyroxy-N-desmethyl-tamoxifen (endoxifen) [6]. The CYP2D6 gene that codes this enzyme is highly polymorphic and varies according to ethnic group. It has been established that many of its genetic variants do not conduct any enzyme activity, resulting in low levels of endoxifen, the major metabolite, and according to some authors, a lack of response to tamoxifen and a poor prognosis [8, 9]. Nevertheless, some authors have shown no significant effect of CYP2D6 genotype on risk of recurrence in breast cancer patients who have received adjuvant tamoxifen therapy [10, 11]. Currently, more than 100 different alleles have been described for the CYP2D6 gene (www.cypalleles.ki.se), that are divided into alleles producing abolished, decreased, normal, and ultrarapid enzyme activity. The most important null allele responsible for abolishing enzyme activity is CYP2D6*4, whereas the common alleles which severely reduce activity are mainly represented by CYP2D6*10 and CYP2D6*17 [12].

The most important polymorphic variants are CYP2D6*4, CYP2D6*10, and CYP2D6*17 found in 25%, 38-70%, and 35% of Caucasians, Asians, and Africans, respectively [13]. There is widespread racial miscegenation in Brazil and its national healthcare system offers tamoxifen freely to all women with hormone-sensitive breast cancer even without knowing the genetic profile of these patients and its results in recurrence of patients with polymorphism; since in a search of the last 30 years conducted in PubMed, there is scarcity of study on polymorphism frequencies of CYP2D6 in Brazilian women with breast cancer [11]. This led the authors to conceive the present study.

Materials and Methods

Patients

This study was approved by the Internal Review Board of the Federal University of Piauí and all patients signed an informed consent term before study entry. Ninety-five women receiving adjuvant treatment with tamoxifen at the Oncology Division of the

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Table 1. — *Variant genotype frequencies of the CYP2D6 gene in the studied population.*

Variant genotype	Patient nº	%
*1/*1	56	59
* 1/*10	21	22
* 1/*4	10	11
* 4/*10	4	4
*10/*10	2	2
*10/*17	1	1
* 4/*17	1	1
Total	95	100%

Table 2. — *Phenotype frequencies of CYP2D6 metabolism in the studied population.*

Phenotypes	Patient nº	%
Normal (homozygous)	56	59
Normal (heterozygous)	31	33
Intermediate	8	8%
Poor	0	0%
Total	95	100%

São Marcos Hospital, from September to December 2013, who had invasive ductal, estrogen-receptor-positive Her2-negative breast carcinoma were included in the study. A three-ml sample of peripheral blood was collected from each patient and sent to the oncogenetic laboratory for polymorphism investigation, using allele-specific polymerase chain reaction (AS-PCR) with analysis of haplotypes* *1, *4, *10,* and **17* of the CYP2D6 gene, through the study of different single nucleotide polymorphisms (SNPs) and their respective initiators.

DNA extraction

For DNA extraction, 100 µl of blood from each sample were used. After storage in a 1.7-ml microtube and addition of 1.5 ml of red cell lysis buffer LISE I (Tris/HCL 0.01M pH 7.6; sucrose 0.32M; $MgCl_2$ 5mM; Triton X-100 1%) and homogenization, the material was centrifuged to 6,000rpm for two minutes and the supernatant was discarded. The procedure was repeated three times. Added to the pellet were 300 µl of white cell lysis buffer LISE II (Tris/HCL 0.01 MpH 8.5; KCl50 mM; $MgCl_2$2.5 mM. NP-40 0.45%; Tween 20 0.45%) and five µl of proteinase K (10'mg/ml) which were left in a warm bath at 55°C for five hours and at 95ºC for one hour for complete inactivation of proteinase K. DNA precipitation was performed by 1.5 ml of alcohol and centrifugation at 14,000 rpm for ten minutes. The material was left to dry in a sterilizer at 60°C. After drying, DNA was resuspended in 250 µl of mili-Q water.

AS-PCR

AS-PCR technique was standardized for analysis of haplotypes **1, *4, *10,* and **17* of the CYP2D6 gene by studies of different SNPs, with their respective initiators (Table 1). PCR reaction was performed in a final volume of 50 µl, containing 200 µM of desoxynucleotide triphosphate (dNTPs), 2.0 mM magnesium chloride ($MgCl_2$), 50 ng DNA, 200 pM of each oligonucleotide (primer) and 0.5U AmpliTaq GOLD, and PCR conditions for amplification of polymorphisms were established in initial denaturation for two minutes at 94ºC and 35 cycles at 94°C for 40 seconds, 52-60°C for one minute, and 72°C for 40 seconds, with a final extension of five minutes at 72°C.

PCR analysis

A polyacrylamide gel was standardized (system 29:1 to 8%; 10% of glycerol) for AS-PCR analysis. Electrophoresis was performed in TBE 1X buffer at 150 V for 30 minutes. Gel staining was performed by the 1% silver nitrate technique, with the gel fixed in 100 ml of fixing solution (proportion of 33 ethanol: 1 acetic acid: 119 distilled water) and two ml of 1% silver nitrate solution for five minutes. Excess silver nitrate from the gel was removed with distilled water and revealing occurred with 100 ml of developer solution (0.6M NaOH) and one ml of formaldehyde at 37%. The reaction was interrupted with fixing solution for visual analysis. The data obtained were compiled and analyzed with the help of Excel software 2010.

Results

Analysis of the results showed detection of polymorphism in heterozygosis: CYP2D6*4, *10, and *17 in 15 (16%), 26 (27%), and two (2%) women studied, respectively. Nevertheless, the presence of polymorphism in homozygosis was shown in only two (2%) women for the CYP2D6 *10 variant (Table 1).

The frequency of allele *4 was demonstrated in 15 (16%) women. Allele*10 was shown in 28 patients (30%) and allele *17 of the CYP2D6 gene appeared in two (2%) women. The frequency of CYP2D6*10 was the highest among variant alleles.

According to the metabolism phenotypes previously described, 91.5% of women studied had normal tamoxifen metabolism and 8.5% were intermediate metabolizers. Determination of the presence of an ultra metabolizer phenotype was not the aim of this study (Table 2).

Discussion

Tamoxifen is a drug commonly used in endocrine therapy of breast cancer, has weak binding to estrogen receptor, and is considered a prodrug [14]. It only becomes active after liver metabolism by the CYP2D6 enzyme which is coded by a highly polymorphic gene. Patients with genetic variants (alleles) that inhibit or reduce enzyme function do not benefit from the use of tamoxifen and show a significantly shorter recurrence-free survival period [6, 8]. Polymorphism of this gene varies according to ethnic group. Study of gene polymorphism is of great importance for metabolism and effects of many drugs, particularly anticancer drugs [12], and of interest in Brazil due to widespread miscegenation of its population.

In the present study, the presence of alleles *4, *10, and *17 of CYP2D6 gene was observed in 15 (16%), 28 (30%), and two (2%) of the patients studied, which are commonly found in Caucasians, Asians, and Africans, respectively [12]. This finding is in agreement with miscegenation existing in Brazil, where there is participation of these three

ethnic groups. However, frequency of the allele *10 was the highest (30%) among variant alleles, which is an unexpected finding, since this allele is commonly found in Asians [6, 12]. On the other hand, allele *17, commonly found in Africans, who have actively participated in Brazilian miscegenation, was less represented in the present sample (only 2%).

The association of two different alleles was observed in eight (8%) patients. To the best of the present authors' knowledge, the association is quite rare and this is the first time it has been reported in women suffering from breast cancer, explaining the miscegenation present in the Brazilian population.

In a similar manner, regarding metabolism phenotype, 8% of the women studied had intermediate metabolism. According to previous studies, this may cause a reduction in endoxifen levels and negatively modify the clinical course of these patients [7, 8, 15].

However, further studies with a larger sample of Brazilian women with breast cancer treated with tamoxifen, evaluating not only the frequency of CYP2D6 polymorphism, as also the prognosis of these patients, will be necessary.

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