Clinical Impact of CYP2D6 and SULT1A1 Polymorphisms and Tamoxifen with Breast Cancer

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Abstract

In the present study we determined the frequency of functional polymorphisms in cytochrome P450 CYP2D6 (*4), and sulphotransferase 1A1 (SULT1A1*2) in tamoxifen-treated patients with breast cancer. In our study, we enrolled 140 tamoxifen treated post menopausal women and 140 tamoxifen non treated cases. All cases were genotyped by using PCR with restriction fragment length polymorphism. Our results indicate that, in breast cancer cases both CYP2D6 and SULT1A1 genes showed significant association with intermediate metabolizes. We also found 9 recurrent cases with CYP2D64 and 7 recurrent cases with SULT1A1 polymorphisms. This indicates that patients with CYP2D6 and SULT1A1 gene GA polymorphisms showed shorter survival periods. However, the present data suggest that genetic variation in CYP2D6 and SULT1A1 may predict response to tamoxifen therapy. It is therefore concluded that Genetic screening of the tamoxifen metabolizing enzymes for the presence of these polymorphisms in breast cancer patients will become increasingly useful in individualizing drug therapy, especially for drugs with a narrow therapeutic index.

Keywords: Polymorphisms; Tamoxifen; Sulphotransferase

Introduction

Tamoxifen is commonly used as a hormonal therapy for patients with oestrogen-receptor (ER)-positive breast cancer. The biotransformation of tamoxifen is mediated by cytochrome P450 enzymes mainly through demethylation and hydroxylation to form several primary metabolites, principally 4-OH-tamoxifen, -OH-tamoxifen, N-desmethyl-tamoxifen and 4-OH-N-desmethyl-tamoxifen. 4-OH-tamoxifen is a more potent antioestrogen than the mother substance and is capable of binding to ER with greater affinity [1,2]. Endoxifen has 100-fold greater affinity for the estrogen receptor and is 30 - 100 fold more potent than tamoxifen in suppressing estrogen dependent cell proliferation. Endoxifen is considered an entity responsible for significant pharmacologic effect of tamoxifen. A further step in the metabolism of tamoxifen is sulfate conjugation, catalyzed by members of the sulphotransferase family (SULT) that generally increase the solubility and facilitates excretion of the drug. SULT1A1 is a major form of phenol SULT in adult human liver and has been shown to be the primary sulphotransferase responsible for the sulfation of 4-OH-tamoxifen [3,4].

Genetic factors play a role in drug metabolism and can account for the differences in response in both the efficacy and the toxicity of some drugs. Polymorphisms affecting the enzyme activity have been found in both cytochrome P450 2D6 (CYP2D6) and SULT1A1. In CYP2D6 the most common non-functional allele is CYP2D6*4. This polymorphism generates a G>A transition at the first nucleotide of exon 4 in the CYP2D6 gene, leading to a truncated non-functional gene product [5]. Further, the most frequent polymorphism in the SULT1A1 gene is a G>A transition at nucleotide 638, defining the SULT1A1*2 allele, which is correlated with a diminished capacity to sulphate SULT1A1 substrates [6]. Results from recent studies [7,8] indicate that CYP2D6 and SULT1A1 genotypes may influence outcome of tamoxifen treated patients. In the present study we investigated the genotypes of CYP2D6 and SULT1A1 genes and evaluate its clinical outcome in breast cancer patients with and without tamoxifen treatment.

Materials and Methods

Study population

Breast carcinoma patients were assessed on the basis of clinical and pathological examinations. This Study is a Hospital-based case-control study conducted in South Indian population. All incidents of breast cancer cases were newly diagnosed during the study period Ethical committee approved the study for the benefit of humans in general. The procedures followed were in accordance with the ethical standards of responsible committee of the Institutes/Hospitals.

Selection criteria

Senior pathologists confirmed all diagnoses. We interviewed and collected the data about the patient’s demographic factors; we collected the information on age, smoking, chewing, usual alcohol intake, and previous cancer diagnoses. Participants were also asked about their family history of cancer, and the clinical information for these cases was obtained from medical records like tumor size, stage, and whether they were receiving chemotherapy, and radiotherapy. Patients were recruited following certain inclusion and exclusion criteria, which were determined before the beginning of the study.

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Inclusion & exclusion criteria

All new cases of clinically confirmed breast cancer would be taken for study. Patients of confirmed breast cancer who give their consent were included. All patients who refuse to give consent were excluded.

DNA isolation

DNA was isolated from the tissue samples from breast cancer patients and blood samples from healthy volunteers by a rapid non-enzymatic method by salting out cellular proteins with saturated solution and precipitation by dehydration [9]. The red blood cells were lysed completely using RBC lyses solution. The lysate were then treated with cell lysis solution in order to lyse the cell components. The protein content is removed by protein precipitation solution. The precipitated DNA was then dissolved in 70% ethanol in order to remove the salts. The DNA was then dissolved in TE buffer and stored at 4°C Cell lysis, DNA precipitation and DNA hydration were carried out in the experiment.

Genotyping of \textit{CYP2D6} *4 Polymorphisms

Genotyping of \textit{CYP2D6} *4 gene polymorphism, polymerase chain reaction (PCR) was performed, with specific primers synthesized from Bioserve Biotechnologies Ltd. (Hyderabad, India): 5’-GGCTTCGCAAACACTCCG-3’ (forward) and 5’-AAATCCTGCTCTTCCGAGG-3’ (reverse). A three-step PCR was standardized using a takarathermocycler and carried out with initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 60°C for 30 s, and extension at 72°C for 45 s. A final extension at 72°C for 5 min was carried out. Amplification products corresponding to 334bp, respectively, were visualized after electrophoresis in an ethidium-bromide-stained 2% agarose gel. The amplified PCR products were performed RFLP using \textit{Hha1}(Fermentas) restriction enzyme for 37°C overnight PCR products subjected to enzyme digestion was visualized on 3% agarose gel stained with ethidium bromide (Figure 3).

Genotyping of \textit{SULT1A1} *2 Polymorphisms

Genotyping of \textit{SULT1A1} *2 gene polymorphism, polymerase chain reaction (PCR) was performed, with specific primers synthesized from Bioserve Biotechnologies Ltd. (Hyderabad, India): 5’TCCAGAATCTGTTCCAGACCTG-3’(forward) and 5’-CTTGGGGAGAAACATCTCCTCA-3’ (reverse). A three-step PCR was standardized using an takarathermocycler and carried out with initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 58°C for 30 s, and extension at 72°C for 45 s. A final extension at 72°C for 5 min was carried out. Amplification products corresponding to 200bp, respectively, were visualized after electrophoresis in an ethidium-bromide-stained 2% agarose gel. The amplified PCR products were performed RFLP using \textit{Hha1}(Fermentas) restriction enzyme for 37°C overnight PCR products subjected to enzyme digestion was visualized on 3% agarose gel stained with ethidium bromide (Figure 3).

Results

The present study was carried out in 140 tamoxifen treated cases and 140 tamoxifen non treated cases. The study was approved by ethical committee and informed consent was taken. Breast Cancer patients were divided into 2 groups according to age at diagnosis, these are 40-59 and 60 years above. Incidence of breast cancer was high in the age groups 40-59 (64%) years when compared to other age groups. In the present study Grade II showed the highest frequency (61.25%) when compared to Grade III (21.25%), other types of tumor grade like Grade I (17.5%) showed very low frequency when compared to Grade III (21.25%).

In the present study Grade II showed the highest frequency (61.25%) when compared to Grade III (21.25%), other types of tumor grade like Grade I (17.5%) showed very low frequency when compared to Grade II and Grade III types.

\textbf{CYP2D6} G1934 A & \textbf{SULT1A1} G638 A Genotyping Analysis in tamoxifen treated and non treated Breast cancer patients

In our study, we screened all the tamoxifen treated and non treated cases for \textit{CYP2D6} G1934A and \textit{SULT1A1} G638A polymorphisms.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2.png}
\caption{G-A alteration at nucleotide position 1934 in intron 3/Exon 4 Junction.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure3.png}
\caption{\textit{SULT1A1} PCR products after restriction digestion with \textit{Hha1} on 2% agarose gel.}
\end{figure}
Tables 1 and 2 shows the results of CYP2D6 genotypes, out of 140 tamoxifen treated cases 70% (n=98) cases were extensive metabolizers, 30% (n=42) cases were intermediate extensive metabolizers, and in tamoxifen not treated cases out of 140 cases 85% (n=118) cases were extensive metabolizers, 15% (n=22) cases were intermediate extensive metabolizers, In SULT1A1 genotypes out of 140 cases tamoxifen treated cases, 77.14% (n=108) cases were extensive metabolizers, 22.85% (n=32) cases were intermediate extensive metabolizers, and there were no poor metabolizers cases, and in tamoxifen not treated cases out of 140 cases 81.42% (n=114) cases were extensive metabolizers, 18.57% (n=26) cases were intermediate metabolizers.

Yearly Interval follow up in tamoxifen treated Postmenopausal breast cancer women with CYP2D6*4 and SULT1A1 polymorphisms

Out of total 140 tamoxifen treated cases 42 cases showed CYP2D6*4 polymorphism. In this we found 86.62% (n=81) cases received the drug for 5 years, 5.40% (n=2) cases received for 4 years, 21.62% (n=8) cases received for 3 years and 27.07% (n=10) cases were receiving from last 2 years and 16.21% (n=6) cases were receiving from below 1 year. Out of total 140 tamoxifen treated cases 32 cases had SULT1A1 polymorphism, out of these 32 cases 9.90% (n=2) cases were on the drug for 5 years, 18.18% (n=4) cases were on drug for 4 years, 27.27% (n=6) cases received for 3 years, 36.36% (n=8) cases received for 2years, and 9.90% (n=2) cases were receiving from below 1 year perhaps continuing till date. In our study we found 9 recurrence cases with CYP2D6*4 polymorphism, 7 recurrence cases with SULT1A1 polymorphisms, we found 2 were no more after 5 years of treatment, and in non tamoxifen cases 4 recurrent cases which were showing CYP2D6*4 and SULT1A1*1 polymorphisms and no deaths were reported (Figure 4).

Survival study of tamoxifen treated breast cancer cases

To evaluate the prognostic significance of tamoxifen therapy in breast cancer patients, survival study was carried out for 5 years. Out of 140 tamoxifen-treated cases, 42(30%) cases showed GA polymorphism for SULT1A1 gene. Among these 42 patients, the average survival period observed was 42 months. Out of 140 tamoxifen treated cases, 32 (22.85%) cases had shown GA polymorphism for CYP2D6 gene. Among these 32 patients, the average survival period observed was 36 months. The prognostic study indicates that patients with CYP2D6 and SULT1A1 gene polymorphisms had shown shorter survival periods than others. Survival details are presented in Figures 5 and 6.

Discussion

Tamoxifen metabolizing genes like CYP2D6 and SULT1A1 are the most important polymorphic genes, and its genotype frequency and clinical relevance have been extensively investigated in different ethnic groups. In humans there are a large number of different polymorphic sites in tamoxifen metabolizing genes. Tamoxifen has been reported to be metabolized by CYP2D6 to the more potent metabolite endoxifen [10]. Endoxifen concentrations vary widely in clinical studies which may be attributed to interindividual differences in genetic metabolism and drug interaction. The serum concentration of an active metabolite of tamoxifen, namely endoxifen, is effected by CYP2D6 status (polymorphism) in a gene dose dependent manner, with low, intermediate and high concentrations in homozygous variant, heterozygous and homoygous wild-type patients, respectively.

To date, very few studies on breast cancer patients in the south Indian population is available. Hence this is the first study analyzed the CYP2D6 and SULT1A1 gene polymorphisms in breast cancer patients who are receiving tamoxifen therapy and patients who were not treated with tamoxifen. We found that in CYP2D6 IM (intermediate extensive metabolizers) showed high frequency (P=0.005) in tamoxifen treated cases than tamoxifen non treated cases. Studies from Australia and India suggests that, CYP2D6 enzyme activity was found to be decreased in individuals carrying *4 alleles [11,12]. Previous studies [13,14] indicate endoxifen concentrations lower in patients with CYP2D6*4 polymorphism. Since endoxifen is the primary active metabolite of tamoxifen, decreased concentrations could impair pharmacological activity and clinical outcomes on breast cancer treatment. Previous study reported that, CYP2D6 has an important role in the metabolic activation of tamoxifen and suggests that women with the CYP2D6 *4/*4 genotype may be less likely to benefit from tamoxifen as a chemopreventive agent [7]. In this study we did not found any PM (poor metabolizers) in both CYP2D6 and SULT1A1 in treated and non treat breast cancer women. In SULT1A1 gene we found IM showed high frequency in tamoxifen treated cases but statistically not significant. The impact of the SULT1A1 polymorphism on sulfotransferase activity leads to drug clearance will become slow and it may leads to drug toxicity.

In our study, we also found 9 recurrent cases with CYP2D6*4 and 7 recurrent cases with SULT1A1 polymorphisms. Thus we suggest that genetic variations in these enzymes may be associated with overall recurrence of disease. The prognostic study indicates that patients with CYP2D6 and SULT1A1 gene GA polymorphisms had shown shorter survival periods than others. Survival details are presented in Figure 3. In previous studies Nowell et al. suggested that genetic changes in phase II enzymes were associated with overall survival and recurrence of disease [15]. In a previous report Wegman et al. [8] observed that the patients with breast cancer randomized to treatment with and without tamoxifen, with genetic polymorphism in CYP2D6 and SULT1A1 may predict the benefit of tamoxifen therapy with a significantly improved disease free survival in patients that were carriers of the CYP2D6*4 allele and/or were homozygous for the SULT1A1*1 allele. Following this study, Wegman et al. 2007 [16] investigated different and larger cohort, which also included additional polymorphic enzymes that participate in the biotransformation and elimination of tamoxifen. Prognostic evaluation in the total population revealed a significantly
however the difference was not significant in all the 3 grades of IM & EM. Intermediate metabolizers of CYP2D6*4 & SULT1A1 genes may be associated with advanced stages of the disease.

**Conclusion**

We conclude that genetic polymorphism in CYP2D6 & SULT1A1 may predict the benefit of tamoxifen therapy. Assessment of CYP2D6 & SULT1A1 metabolic status before initiation of therapy may help to identify patients at risk for no response to therapy or toxic drug effects and is needed to ensure optimal dosing recommendations.

**References**


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