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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 *CYP2D6**4 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; Sulfotransferase

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 sulfotransferase 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 sulfotransferase responsible for the sulfation of 4-OH-tamoxifen [3,4].

Genetic factors play a role in drug metabolism and can account 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 suspended in 70% ethanol in order to remove the salts. The DNA was then dissolved in TE buffer and stored at 4°C Cell lysis, protein precipitation. DNA precipitation and DNA hydration were carried out in the experiment.

Genotyping of *CYP2D6**4 Polymorphisms

Genotyping of *CYP2D6**4 gene polymorphism, polymerase chain reaction (PCR) was performed, with specific primers synthesized from Bioserve Biotechnologies Ltd. (Hyderabad, India): 5'-GCCTTCGCCAACCACTCCG-3' (forward) and 5'-AAATCCTGCTCTTCCGAGGC-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 60°C for 30 s, and extension at 72°C for 45s. 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 BstN1(Fermentas) restriction enzyme for 37°C overnight PCR products subjected to enzyme digestion was visualized on 3% agarose gel stained with ethidium bromide (Figures 1 and 2).

Genotyping of *SULT1A1**2 Polymorphisms

Genotyping of *SULT1A1**2 gene polymorphism, polymerase chain reaction (PCR) was performed, with specific primers synthesized from Bioserve Biotechnologies Ltd. (Hyderabad, India): 5'-TCCAGAATCTGTTCCAGAGCGTGC-3'(forward) and5'-CTTGGGGAGAACCATCCTCA-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 *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).

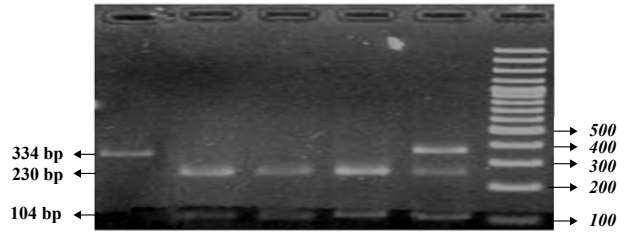


Figure 1: *CYP2D6* PCR products after restriction digestion with BstN1 on 2% agarose gel.
Lane 1, 2 3, &7 = GG Genotype, Lane 4 = GA Genotype, Lane 5 = 100bp DNA Ladder and Lane 6 =AA genotype.

Statistical analysis

Genotyping experiments were presented as allelic frequencies and Genotype distribution with those expected from Hardy-Weinberg Equilibrium (HWE) were made using chi square test, and Values of P (two - tailed) less than 0.005 were considered statistically significant. Odds ratio, were calculated using MedCalc for Windows, version 7.4.1.0 (*MedCalc Software, Mariakerke, Belgium*).

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 II and Grade III types.

CYP2D6 G 1934 A & *SULT1A1* G 638 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 *CYP2D6* G1934A and *SULT1A1* G638A polymorphisms.

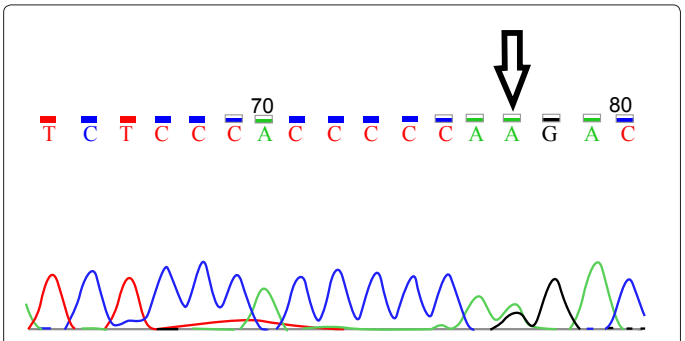


Figure 2: G-A alteration at nucleotide position 1934 in intron 3/Exon 4 Junction.

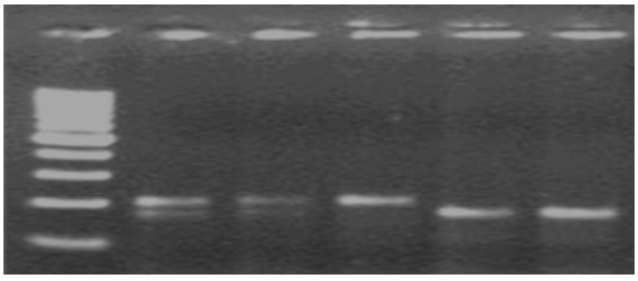


Figure 3: *SULT1A1* PCR products after restriction digestion with HhaI on 2% agarose gel.
Lane 1 = 100bp DNA Ladder, Lane 2&3 = GA genotype, Lane 4 = AA genotype and Lane 5 =GG genotype.

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 21.62% (n=8) 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 had shown GA polymorphism for *CYP2D6* 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 *SULT1A1* gene. Among these 32 patients, the average survival period observed was 36 months. 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 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 homozygous 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

CYP2D6 Genotyping	Tamoxifen Treated Cases(n=140)	Tamoxifen –Non Treated Cases(n=140)	Odds Ratio	95% CI	Chi Square	P-Value
EM	98(70%)	118(85%)	0.23	0.11 – 0.47	7.31	0.0001
IM	42(30%)	22(15%)	2.29	1.28 – 4.11	7.31	0.005

Table 1: Distribution of *CYP2D6* gene G1934A polymorphism in Tamoxifen treated and Non Tamoxifen treated cases.

SULT1A1 Genotyping	Tamoxifen Treated Cases(n=140)	Tamoxifen –Non Treated Cases(n=140)	Odds Ratio	95% CI	Chi Square	P-Value
EM	108(77.14%)	114(81.42%)	0.76	0.43 – 1.37	0.76	0.37
IM	32(22.85%)	26(18.57%)	1.29	0.72 -2.32	0.54	0.37

Table 2: Distribution of *SULT1A1* gene G638A polymorphism in Tamoxifen treated and Non Tamoxifen treated cases.

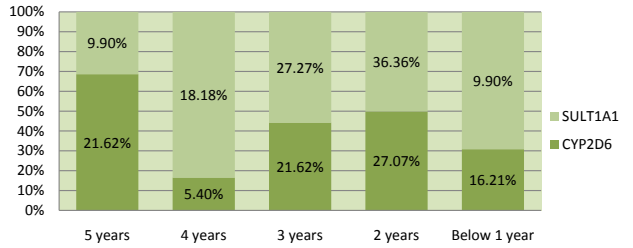
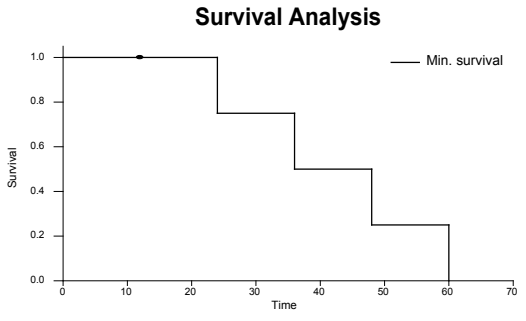
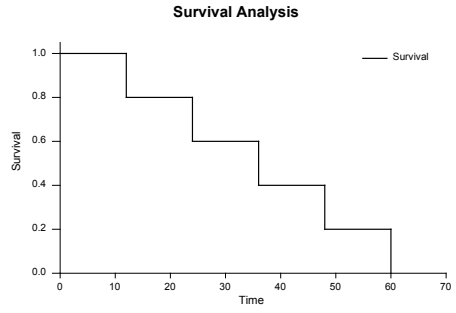


Figure 4: Graphical Representation of Yearly Interval of follow up (up to Jan 2008).



Event Time	No. of Events	No. at Risk	Probability	Std. Error
24.000	1	4	0.750	0.217
36.000	1	3	0.500	0.250
48.000	1	2	0.250	0.217
60.000	1	1	0.000	0.000

Figure 5: Mean survival time 42 months. Graphical Representation of *CYP2D6* Genotype Mean Survival Curve.



Event Time	No. of Events	No. at Risk	Probability	Std. Error
12.000	1	5	0.800	0.179
24.000	1	4	0.600	0.219
36.000	1	3	0.400	0.219
48.000	1	2	0.200	0.179
60.000	1	1	0.000	0.000

Figure 6: Mean survival time 36 months. Graphical Representation of *SULT1A1* Genotype Mean Survival Curve.

better disease-free survival in patients homozygous for *CYP2D6**4. For *CYP3A5*, *SULT1A1* and *UGT2B15* no prognostic significance was observed. Data from the correlation analysis of *CYP2D6* vs. Grades of the disease showed that, the percentage of IM was increased from Grade I to Grade III, where as in *SULT1A1* gene, we observed that there is Slight increase of IM genotype from Grade I to Grade III; 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.

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