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Full Length Research Paper

Genotyping of tamoxifen metabolizing enzyme (CYP2D6*4) and its clinical impact in breast cancer patients

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Tamoxifen is a non-steroidal anti-estrogen drug widely used in the treatment of breast cancer and metabolized by CYP2D6. In this study, we compared the patients who were receiving either tamoxifen or other chemo drugs, forming a two-arm study. We genotyped 140 tamoxifen treated postmenopausal women with breast cancer, 140 non-tamoxifen treated pre and postmenopausal women with breast cancer and 124 controls, using PCR-RFLP method. In arm-1 study CYP2D6 genotype frequencies of metabolizers were classified as extensive metabolizers (EM) 70% (n = 98), intermediate metabolizers (IM) 30% (n = 42) in tamoxifen treated cases and 85% (n = 118), 15% (n = 22) in non-tamoxifen treated cases respectively. Tamoxifen treated IM -carriers showed 24.32% (n = 9) recurrence. Recurrent cases were not found in non-tamoxifen treated group. CYP2D6*4 allele carriers were high but this allele carrier was found to reduce the risk of recurrence when treated with tamoxifen. In arm-2 study CYP2D6 genotype frequencies of EM, IM and Poor metabolizers (PM) were 79.57% (n = 113), 14.08% (n = 20), 6.33% (n = 9) in non tamoxifen treated breast cancer cases. In controls the EM, IM, and PM genotypes were 93.54% (n = 116), 5.64%) (n = 7) and 0.80% (n = 1) respectively. Statistical analysis indicated that p value of both IM (P- value 0.03) and PM (P- value 0.04) carriers were associated with the risk of breast cancer. PM showed poor therapeutic outcome, which may be due to low level of the tamoxifen metabolite-endoxifen.

Key words: Breast cancer, tamoxifen, CYP2D6*4, poor metabolizers (PM), extensive metabolizers (EM), intermediate metabolizers (IM).

INTRODUCTION

The selective estrogen receptor modulator, tamoxifen, has been widely used for more than 25 years for the endocrine treatment of all stages of hormone receptor–positive breast cancer. The United States Food and Drug Administration also approve Tamoxifen for the prevention of breast cancer in women at high risk for developing the disease. The human genome includes at least 57 genes coding for cytochrome P450 proteins and 29 pseudo-genes (Danielson 2002). Cytochrome P450 (CYP) 2D6 is clinically important since this enzyme metabolizes many drugs, such as antiarrythmic and psychiatric drugs, as well as endogenous compounds (Rendic, 2002).

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CYP2D6 is one of the most important human P450s based on the number of its drug substrates, which include more than 50 commonly used drugs (Cholerton et al., 1992). CYP2D6 is a polypeptide of 497 amino acids. The enzyme accounts for only a small percentage of all hepatic P450s, but its role in drug metabolism is extensively higher than its relative content (Zanger et al., 2004). This enzyme has a wide range of activity within human populations, with inter-individual rates of metabolism differing more than 10 000-folds (Kroemer et al., 1995; Nebert, 1997; Sachse et al., 1997; West et al., 1997).

It is highly polymorphic gene, comprising of more than 80 known polymorphisms within the coding and promoter regions. Debrisoquine-4-hydroxylase, a cytochrome P450 enzyme known as CYP2D6 enzyme, metabolizes differ-

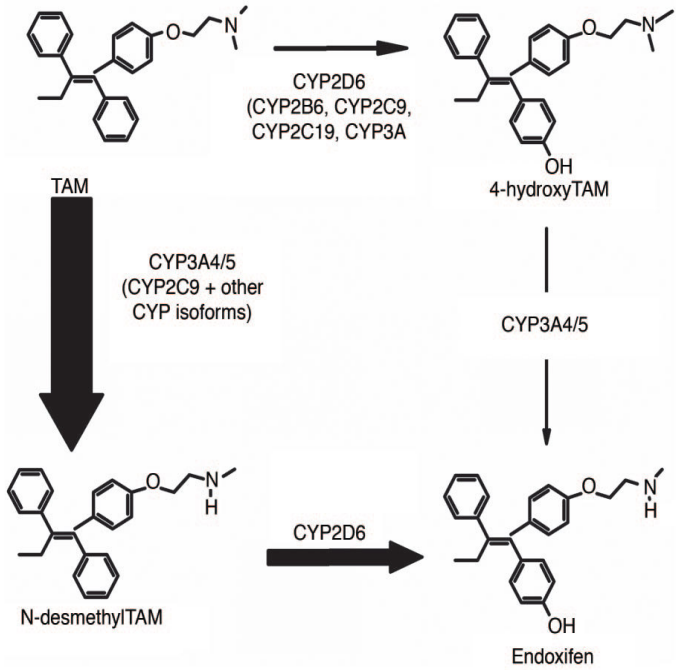

Figure 1. CYP2D6 Mediation in Biotransformation of Tamoxifen metabolites.

ent types of pharmaco-therapeutic drugs, such as tricyclic antidepressants and neuroleptics. The gene encoding cytochrome P-450 2D6 monooxygenase isoenzyme (CYP2D6) is located on chromosome 22 q13.1. It seems that its location lies within the region where structural aberrations are very common (Gough et al., 1993).

Tamoxifen and its metabolites compete with endogenous oestrogen for the ligand-binding domain of the ER. The complex formation between tamoxifen, or its active metabolites and the estrogen receptor (ER) inhibits recruitment of co-activator complexes necessary for transcription of oestrogen-responsive genes (Ali et al., 2002). The biotransformation of tamoxifen is mediated by cytochrome P450 enzymes mainly through demethylation and hydroxylation to form several primary metabolites, principally 4-OH-tamoxifen, α-OH-tamoxifen, *N* -desmethyl-tamoxifen and 4-OH- *N* -desmethyl-tamoxifen (Figure 1). 4-OH-tamoxifen is a more potent anti-oestrogen than the mother substance and is capable of binding to ER with greater affinity (Fabian et al., 1981; Coezy et al., 1982).

From experimental studies it has been shown that the transformation of tamoxifen into 4-OH-tamoxifen is mainly catalysed by the liver enzyme CYP2D6 (Dehal et al., 1997; Boocock et al., 2002). 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.

Matthew et al. (2005), strongly suggest another tamoxifen metabolite, 4-hydroxy-*N*-desmethyl tamoxifen (endoxifen), previously characterized by Lien et al. (1989, 1990 and 1991) as more important than 4-OH-tamoxifen in terms of the relative contribution to the overall anticancer effect of tamoxifen and thus to inter-individual variability in response to the drug. Endoxifen has identical properties and potency compared with 4-OH tamoxifen in terms of its binding affinity to ERs, suppression of estradiol-stimulated cell proliferation, 4 and gene expression. Furthermore, steady-state plasma endoxifen concentrations are 5- to 10-fold higher than 4-OH tamoxifen. Although multiple enzymes catalyze the metabolism of tamoxifen to 4-OH tamoxifen, endoxifen is formed predominantly by the CYP2D6 mediated oxidation of *N*-desmethyl tamoxifen, the most abundant tamoxifen metabolite. Recent clinical studies have demonstrated that women receiving tamoxifen either carry genetic variants associated with low or absent CYP2D6 activity or who receive concomitant mediations, are known to inhibit CYP2D6 activity and have significantly lower levels of endoxifen (Stearns et al., 2003; Jin et al., 2005; Desta et al., 2004).

In our earlier studies we showed a few novel polymorphisms in CYP3A4 gene in breast cancerpatients receiving chemotherapy (Suman and Jamil, 2006). Hence polymorphic studies in drug metabolizing genes forms an informative area of breast cancer research. In addition, we have also shown that DPD and MDR1 genes are involved in characterizing the breast cancer patients with respect to response of various neoplastic drugs (Kumar et al., 2006; Shaswati et al., 2007).

CYP2D6 polymorphism has been associated with various human cancer susceptibilities such as lung, breast, skin and prostate cancers. It has hypothesized that the existence of these alternatively splicing variants may impact the expression and functions of *CYP2D6* in extrahepatic tissues and the alternation of *CYP2D6* might play an important role in determining cancer risk. Polymorphisms affecting the enzyme activity have been found in cytochrome P450 2D6 (Sachse et al., 1997). Among Caucasians the most frequent inactivating polymorphism in *CYP2D6* is the *CYP2D6*4* allele, which generates a G → A transition at nucleotide 1934 leading to a disruption of the reading frame and to a truncated non-functional gene product (Hanioka et al., 1990). According to the activity of the enzyme, patients can be classified into three different phenotypes: poor metabolizes (PMs), extensive metabolizers (EMs) and ultra-rapid metabolizers (UMs). The different phenotypes have profound effects on the efficacy of a drug and on its adverse reactions. Inter- individual differences in CYP2D6 can produce adverse effects or lack of therapeutic effect with an altered risk for some cancers (Coezy et al., 1982; Dehal et al., 1997). In view of this background, the aim of our present study was to investigate the genotypes of *CYP2D6* in breast cancer patients in a two-arm study where we wish to compare tamoxifen treated cases

against non tamoxifen treated cases, in arm-1 the 2 types of cases were investigated and in arm 2 cases against controls were studied.

MATERIALS AND METHODS

Study population

Breast cancer patients were diagnosed based on clinical examinations as well as mammography and pathological examinations. This study is a Hospital-based case-control study conducted in South Indian population. All incident 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, to participate in a face-to-face interview using a structured questionnaire.

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, menopausal status, smoking, 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, Grade, Axillary nodes and whether they had exposed to chemotherapy, Hormonal therapy and radiotherapy. Patients were recruited following certain inclusion and exclusion criteria, which were determined before the beginning of the study.

Inclusion and exclusion criteria

Patients must be women with breast cancer having positive (tumor-involved) axillary lymph nodes at the time of surgery. Patients must have adequate blood counts and adequate kidney and liver function. Patients cannot be pregnant. Patients cannot have significant pre-existing medical or psychiatric conditions, including history of heart disease.

Protocol of the study

The study group was segregated into 3 sub-groups of breast cancer cases:

1. Group-1 consisted of 140 Tamoxifen receiving breast cancer patients.
2. Group-2 consisted of 140 breast cancer patients receiving other drugs (non-tamoxifen).
3. Group-3 consisted of 124 healthy Controls.

These groups could be further classified according to the extent of metabolism of the drugs used such as extensive metabolizers (EM), Intermediate metabolizers (IM) and poor metabolizers (PM).

Sample collection

Based on the above criteria, a total of 282 breast cancer patients formed our study population; out of 282 cases 140 cases were tamoxifen treated postmenopausal women, 142 cases were non tamoxifen treated and 124 age-matched controls. The selection of the study group was from three major Hospitals in Hyderabad, Andhra Pradesh between the periods March 2005 to Jan 2008, that is, Indo American Cancer Institute and Research Center, Mahavir Hospital and Research Centre and MNJ Cancer Hospital, Red Hills, Hyderabad.

Collection of biopsy and blood samples

Tumor breast tissues were collected in normal saline at the time of surgery. The tissues were, immediately transferred and stored at -80°C till further processing was done.

The tumor samples obtained were of various tumor sizes and diagnosed mainly as invasive ductal Carcinoma by the Pathologist. About 3 ml Blood samples were collected from healthy women (Voluntarily) by venipuncture. These samples were used as controls, in various experiments.

Patients treatment modality/ clinical evaluation methods

140 breast cancer cases that received tamoxifen therapy were categorized as group-1, and another 140 cases that received various combinations of chemotherapy like FAC (5-Fluorouracil, Adriamycin and Cyclophosphamide) 51.74% and FEC (5-Fluorouracil, Epirubicin and Cyclophosphamide) 28.82%, FED (5-Fluorouracil, Epirubicin and Doxorubicin,) and CMF (Cyclophosphamide, Methotrexate and 5-Fluorouracil) 7.2 and 3.6% respectively were categorized as group-2. It was observed that in the present study CAF combination therapy showed high frequency than the other combinations. In this study 5FU was common in all the combination chemotherapy agents. This study was conducted in two medical centers (Mahavir Hospital and Indo American cancer Hospital). Assessment of response was made according to the WHO criteria. Responders to 5FU were classified as those patients whose tumor burden decreased by 50% or more (partial response) or completely disappeared (complete response). Non-responders (Poor response) included those with stable disease or cancer progression.

Genotype analysis

DNA was isolated from the cancer biopsy samples and blood samples from healthy volunteers by a rapid non-enzymatic method by salting out cellular proteins with saturated solution and precipitation by dehydration (Alluri et al., 2005). PCR of CYP2D6 G1934A was performed using the following primers:

Forwad 5'ATGAGAGCTGCCAACCTT3

Reverse 5'ATGTGAACCAGCTCCCTGTC3'.

Standard PCR method was performed, in thermal cycler. A 3 step PCR with an initial denaturation at 94^0C for 5 min followed by cycling at 94^0C for 30 s, annealing for 30 s at 60^0C temperature, 72^0C for 45 s and a final extension at 72^0C for 5 min was carried out for about 35 cycles. This was followed by RFLP using BstN1 restriction enzyme (Fermentas).

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 0.05 were considered statistically significant. Odds ratio, were calculated using MedCalc for Windows, version 7.4.1.0 (MedCalc Software, Mariakerke, Belgium).

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

CYP2D6 Genotyping	Tamoxifen treated cases (n = 140)	Tamoxifen –Non treated cases (n = 142)	Odds ratio	95% CI	Chi square	P - value
EM	98(70%)	113(79.57%)	0.23	0.11 – 0.47	7.31	0.0001
IM	42(30%)	20(14.08%)	2.29	1.28 – 4.11	7.31	0.005
PM	NIL	9(6.33%	-	-	-	-

EM = GG, IM = GA, PM = AA genotypes. * p = < 0.05 (Significant).

RESULTS

The Characteristics of the Breast Cancer (BC) patients is given below

Age range for BC patients was 28 – 78 years. The mean age at which Breast Cancer identified was 49.87 years. As mentioned above breast cancer patients were divided into 3 groups according to age at diagnosis, these are 20 - 39, 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 and the incidence was very low in the age group 21 - 30 followed by 71 - 80 years. Depending on the menopausal status, breast cancer patients were categorized into premenopausal (37%) and postmenopausal groups (63%). We found that the majority of cases were sporadic breast cancer cases and were high in postmenopausal group when compared to premenopausal group. Estrogen and progesterone receptor status in breast cancer patients served as a good prognostic and predictive marker for response to therapy. ER, PR statuses were done by immunohistochemistry (IHC) in some centers from where samples were collected. Hence the categorization according to hormone receptor status which was determined by IHC was as follows ER-/PR-, ER+/ PR+, ER+/PR- and ER-/PR+. The percentage of ER+PR+ (36.36%) ER-PR- (49.53%) and breast tumors were high when compared to other tumor subtypes like ER+/PR- (9.34%) and ER-/PR+ (9.34%) and ER+/PR-, ER-/PR+ tumor showed equal distribution. Tumor grade is a system used to classify cancer cells in terms of how abnormal they look. 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.

Genetic polymorphisms analysis

Arm – 1 Study: CYP2D6, G1934A Genotyping Analysis in Tamoxifen treated and non tamoxifen treated Breast Cancer Patients In the arm-1 study we compared the tamoxifen treated patients with patients receiving other treatment but not tamoxifen. The liver enzyme CYP2D6 catalyses transformation of tamoxifen into 4-OH-tamoxifen. CYP2D6 gene polymorphism may be an important pharmacogenetic determinant of predicting response of tamoxifen therapy. The most frequent inactivating polymorphism in *CYP2D6* is the *CYP2D6*4* allele, which generates a G → A transition at nucleotide 1934, we analyzed this SNP in the present study. DNA was obtained from tissue biopsy samples of 140 BC patients all were postmenopausal and were treated with tamoxifen and compared to 142 cases of the other treatment (but not tamoxifen) cases consisting of pre and postmenopausal women.

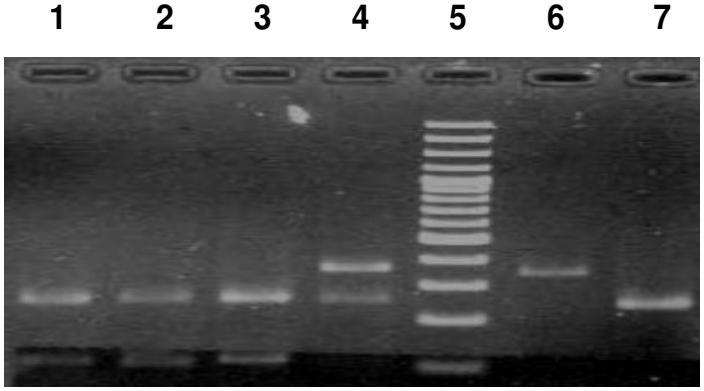

Figure 2. CYP2D6 PCR products after restriction digestion with BstN1 on 2% agarose gel. Lane 1, 2 3 and 7 = EM Genotype, Lane 4 = IM Genotype, Lane 5 = 100 bp DNA Ladder and Lane 6 = PM genotype.

The CYP2D6 G1934A polymorphism as determined by PCR – RFLP and the PCR product (334 bp) was digested with *BstNI* restriction enzyme. The DNA fragments were then separated using 2% agarose gel and detected by ethidium bromide staining. The absence of restriction site, gave a 334 bp fragment, indicating A allele, in which heterozygotes showed IM genotypes with 334, 200 and 134 bp bands and wild type EM genotypes showed 200 and 134 bp fragments (Figure 2). Frequencies of *CYP2D6* EM, IM, and PM genotypes were 70% (n = 98), 30% (n = 42), 0.00% (n = 0) in the breast cancer tamoxifen treated cases and 85% (n - 118), 15% (n = 22), 0.00% (n = 0) in the non tamoxifen treated cases respectively (Table 1). Yearly Interval follow up in tamoxifen treated Postmenopausal breast cancer women with CYP2D6*4 polymorphisms. Out of total 140 tamoxifen treated cases 42 cases showed CYP2D6*4 polymorphism. In this group we found 21.62% (n = 8) cases who

Table 2. Distribution of CYP2D6 gene G1934A polymorphism in Breast Cancer patients and controls.

Genotype	Cases (n = 142)	Controls (n = 124)	OR	95%CI	X^2	P-value
EM	113 (79.57%)	116 (93.54%)	0.26	0.11 – 0.61	9.65	0.001
IM	20 (14.08%)	7 (5.64%)	2.74	1.11 - 6.72	4.28	0.03
PM	9 (6.33%)	1 (0.80%)	8.32	1.03 - 66.6	4.17	0.04

(EM- Extensive metabolizer, IM- Intermediate Extensive metabolizer, PM- Poor metabolizer)* p = < 0.05 (Significant).

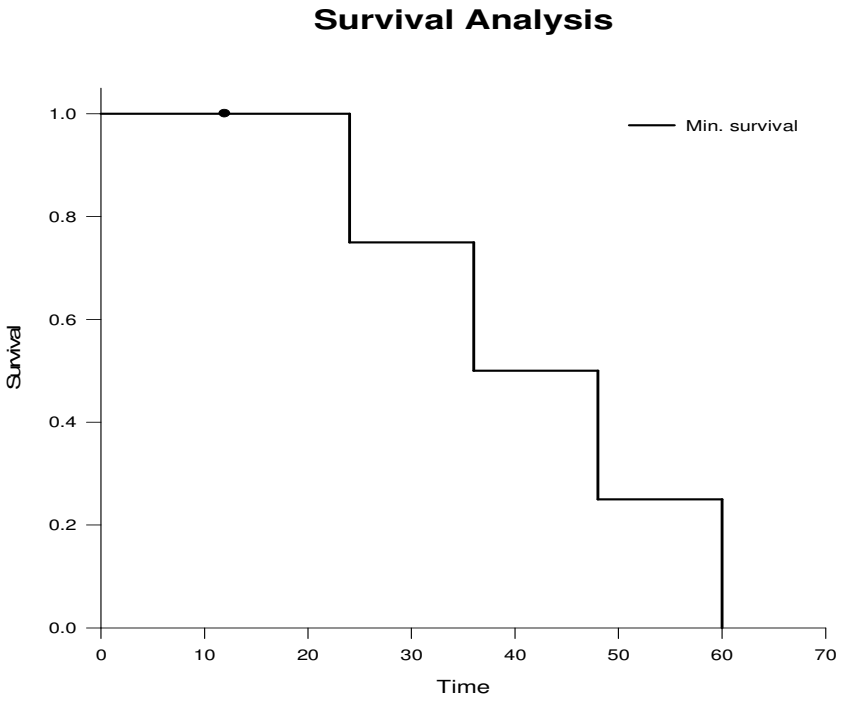

Figure 3. Survival graph in tamoxifen treated patients with CYP2D6 Genotype (Mean survival time 42 months).

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 for 2 years and 16.21% (n = 6) cases were receiving for less than 1 year. In our study we found 9 cases with recurrence having CYP2D6*4 polymorphism, we also found 2 cases were no more after 5 years of treatment and in non tamoxifen cases we found 4 recurrent cases which were showing CYP2D6*4 polymorphisms.

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. This prognostic study indicated that patients with CYP2D6 GA polymorphisms had shown shorter survival periods than others. Survival details are presented in Figure 3. Arm-2 Study: CYP2D6 G 1934 A Genotyping Analysis in non tamoxifen treated Breast Cancer Patients and controls samples Polymorphisms in CYP2D6 G 1934 A Gene were analyzed in DNA obtained from tumor tissue samples of 142 BC patients and compared to 124 healthy age matched women volunteers' blood samples. CYP2D6 G 1934A Polymorphism was analyzed by PCR –RFLP and the PCR product (334 bp) was digested with *BstNI* restriction enzyme. The DNA fragments were then separated using 2% agarose gel and detected by ethidium bromide staining. The absence of restriction site, which gave a 334 bp fragment, indicates A allele, heterozygous IM genotype shows 334, 200 and 134 bp bands and wild type EM genotype shows 200 and 134 bp (Figure 2) bands. Frequencies of *CYP2D6* EM, IM and PM genotypes were 79.57% (n = 113), 14.08% (n = 20), 6.33% (n = 9) in the breast cancer cases and 93.54% (n = 116), 5.64% (n = 7), 0.80% (n = 1) in the controls respectively.

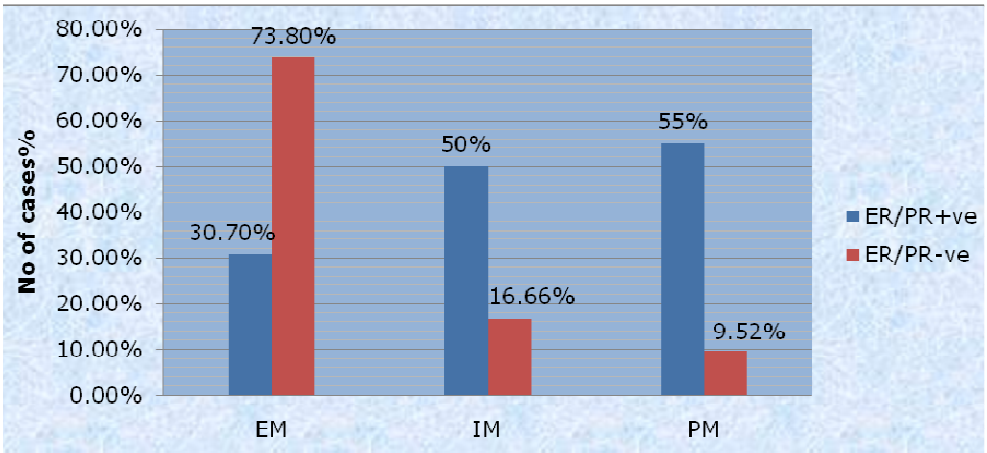

Figure 4. Depicting the Correlation Analysis of CYP2D6 genotypes vs. ER/PR status of Breast Cancer Cases.

Table 2 shows results for the CYP2D6 G1934A Polymor-phism, Extensive metabolizers (EM), Interme-diate meta-bolizers (IM) and poor metabolizers (PM) showed high frequency in breast cancer patients than controls. In the present study both Intermediate metabo-lizers (IM) and poor metabolizers (PM) were found to be statistically significant [OR 2.74, 95% CI 1.11 - 6.72 x 2 4.28, p-value 0.03] and [OR 8.32, 95% CI 1.03 - 66.6, x2 4.17, p-value 0.04]. The homozygous and heterozygous genotypes were found to be associated with breast cancer. *CYP2D6* G allele frequency was 0.866%, A allele frequency was 0.133% in the breast cancer patients and G allele frequency was 0.96%, A allele frequency was 0.036% in the controls.

Correlation of tamoxifen metabolizing genes polymorphisms with demographic factors

In pre and postmenopausal breast cancer patients, EM genotype frequency was lower when compared to controls. Whereas IM genotypes were elevated in BC patients than in controls, however the difference was significant for premenopausal BC patients. Similar to IM genotypes, PM genotype distribution was high in BC patients than in controls. However, the results were not statistically significant. Estrogen and progesterone receptors serve as prognostic markers and predict response to endocrine therapy.

In the present study genotypes of CYP2D6 showed an association with ER//PR status. It was observed that IM and PM genotypes of CYP2D6 were high in ER/PR positive BC when compared to ER/PR negative Breast Cancer (Figure 4). Genotypic frequencies correlated with the grade of the disease where they showed an effect on the disease progression.

Data from the correlation analysis of CYP2D6 vs. Grades of the disease showed that, PM were completely absent from the patients of Grade I status but were found in patients with Grade II and III. The percentage of IM were increased from Grade I to Grade III, suggesting that PM and IM genotypes were associated with advanced stages of the disease (Figure 5).

DISCUSSION

Tamoxifen metabolizing enzyme like CYP2D6 is part of the P450 enzyme system and is responsible for meta-bolizing tamoxifen to its most active form, endoxifen. CYP2D6 gene is the most important polymorphic genes and its genotype frequency, allelic frequency and clinical relevance have been extensively investigated in different ethnic groups.

To date, no data on breast cancer patients in the south Indian population is available. Hence this is the first presentation of this two arm study where tamoxifen treated cases were compared with non tamoxifen treated cases and also with controls.

In arm-1 study we found most of the tamoxifen treated (30%) were intermediate metabolizers, tamoxifen treated IMs carriers showed 24.32% (n = 9) recurrence. We found that carriers of the CYP2D6*4 variant allele were at decreased risk of recurrences in tamoxifen treated patients. Among IMs 30% (n = 42) tamoxifen patients the average survival period observed was 42 months. The prognostic study indicates that patients with CYP2D6 GA polymorphisms had shown shorter survival periods. But the significance of these findings has to be assessed in a larger number of patients. However reports are available which are in line with our observations; In breast cancer, patients treated with adjuvant tamoxifen, non-functional and severely impaired CYP2D6 variants are associated with a worse DFS and with a higher frequency of severe and mild toxicities (Cajal, 2009). Wegman et al. (2005) observed a significantly improved benefit from tamoxifen

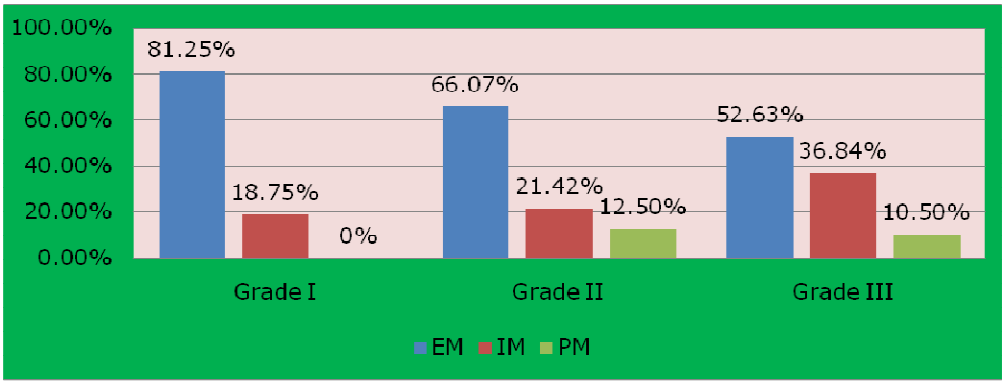

Figure 5. Graph depicting the percentage of CYP2D6 genotypes vs. Grades of breast cancer cases.

in patients carrying the *CYP2D6*4* allele. Breast cancer patients with the CYP2D6 *4/*4 or wt/*4 genotypes could have lower benefit of TAM treatment and tend to have a higher risk of disease relapse. Homozygotes for Cyp2D6 *4 are genetically responsible for about 75% PMs. Goetz et al. (2006) reported that, in tamoxifen-treated breast cancer patients, women with the cytochrome P450 (CYP) *2D6 *4/*4* genotype tend to have a higher risk of disease relapse indicating a possible role of CYP2D6 in the metabolic activation of tamoxifen to endoxifen. Coller et al. (2002), have demonstrated in experimental studies that the *CYP2D6* genotype is a determinant of the ability to form 4-OH-tamoxifen. Stearns et al. (2003), reported that inhibition of CYP2D6 had no significant effect on 4-OH-tamoxifen concentration.

PMs were not found in tamoxifen treated cases but PMs were found in nine non tamoxifen treated cases. PMs Lack CYP2D6 protein in their liver and hence, insufficient to activate carcinogens to electrophilic DNA-reactive moieties. Therefore, it is evident from this study that metabolites rather than the parent compounds could be the cause of tumurogenesis. PM may show poor therapeutic outcome, which may be due to low level of the metabolite-endoxifen. The risk of breast cancer mortality is also increased in tamoxifen users with decreased CYP2D6 activity, consistent with the model in which endoxifen formation is dependent on CYP2D6 activity (Bijl, 2009).

In the present study we found significant association in both heterozygous (Intermediate metabolizes, the p value was 0.03) and homozygous mutant (poor metabolizers the p value was 0.04) are significantly associated with the risk of breast cancer. The CYP2D6 polymorphism G1934A leads to a disruption of the reading frame and a truncated non functional protein Therefore, individuals with heterozygous or mutant homozygous CYP2D6 genotypes have poor or no enzyme activity respectively. De Jong et al. (2002) also found that homozygous mutant CYP2D6 genotype increased the risk of breast.

A study from south India suggests that, CYP2D6 enzyme activity was found to decreased in individuals carrying *4 alleles (Naveen et al., 2006). CYP2D6*4 genotype polymorphism interacting with plasma malon-dialdehyde (MDA) oxidative stress and plasma ferretin level may have a role in the pathogenesis of breast cancer (Mansoura University Information 2007).

When CYP2D6*4 polymorphism was analyzed for influence of menopausal status of breast cancer patients IM (Heterozygous) genotypes were higher in BC patients than in controls, however the difference was significant for premenopausal BC patients and this suggests that this genotype may be associated with early age at onset of BC. But in earlier studies Ladona et al. (1996) reported a significant association between the heterozygous CYP2D6 genotype and breast carcinoma risk among postmenopausal patients. It was observed that IM and PM genotypes of CYP2D6 were high in ER/PR positive BC patients when compared to ER/PR negative cases, suggesting an association of these genotypes with ER/PR + tumors. However the functional relevance is not known. The correlation data analysis of CYP2D6 with grades of the disease showed that, PM were completely absent from the patients of Grade I tumors but it was found in patients with Grade II and III tumors. The percentage of IM increased from Grade I to Grade III, suggesting that PM and IM genotypes may be associated with tumor aggressiveness for breast cancer. BC patients with CYP2D6 homozygous genotypes ($P < 0.03$,) showed a significant association with response

In conclusion this study suggests that gene polymorphisms may be susceptibility biomarker for breast carcinoma. Assessment of CYP2D6 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. CYP2D6 might be useful as a guide for better diagnosis of breast cancer cases. But to the best of our knowledge, this is the first study reporting genotype-phenotype relationship in breast cancer patients in Indian population. The frequency of IM are much higher therefore, tamoxifen pharmacogenomics for breast cancer patients of Indian requires a more focus on IM. Larger studies of the

CYP2D6 genotype-clinical outcomes associations are needed. Since about 25% of commonly prescribed drugs are metabolized by *CYP2D6*, the findings of this study may be clinically important.

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REFERENCES

Ali S, Coombes RC (2002). Endocrine-responsive breast cancer and strategies for combating resistance. Nat Rev. 2: 101-112.

Bijl JM (2009). The CYP2D6*4 polymorphism affects breast cancer survival in tamoxifen users. Breast cancer research and treatment 118(1): 125-30.

Boocock DJ, Brown K, Gibbs AH, Sanchez E, Turteltaub KW (2002). Identification of CYP forms involved in the activation of tamoxifen and irreversible binding to DNA. Carcinogenesis 23: 1897-1901.

Cajal R (2009). Impact of CYP2D6 polymorphisms in tamoxifen adjuvant breast cancer treatment. Breast cancer research and treatment 0167-6806 1573-7217.

Cholerton S, Daly AK, Idle JR (1992). The role of individual human cytochromes P450 in drug metabolism and clinical response. Trends Pharmacol. Sci. 13: 434-439.

Coezy E, Borgna JL, Rochefort, H. (1982). Tamoxifen and metabolites in MCF7 cells: correlation between binding to estrogen receptor and inhibition of cell growth. Cancer Res. 42: 317-323.

Coller JK, Krebsfanger N, Klein K, Endrizzi K, Wolbold R, Lang T, Nüssler A, Neuhaus P, Zange UM, Eichelbaum M, Mürdter TE (2002). The influence of CYP2B6, CYP2C9, and CYP2D6 genotypes on the formation of the potent antioestrogen Z-4-hydroxytamoxifen in human liver. Br. J. Clin. Pharmacol. 54: 157-167.

Danielson PB (2002). The cytochrome P450 superfamily: biochemistry, evolution and drug metabolism in humans. Curr. Drug Metab. 3: 561–597.

Dehal SS, Kupfer D (1997). CYP2D6 catalyzes tamoxifen 4-hydroxylation in human liver. Cancer Res. 57: 3402-3406.

Desta Z, Ward BA, Soukhova, NV, Flockhart DA. (2004). Comprehensive evaluation of tamoxifen sequential biotransformation by the human cytochrome P450 system in vitro: prominent roles for CYP3A and CYP2D6. J. Pharmacol. Exp. Ther. 310: 1062–75.

Fabian C, Tilzer L, Sternson L (1981). Comparative binding affinities of tamoxifen, 4-hydroxytamoxifen, and desmethyltamoxifen for estrogen receptors isolated from human breast carcinoma: correlation with blood levels in patients with metastatic breast cancer. Biopharma Drug Dispos. 2: 381-390.

Gough AC, Smith, CAD, Howell, SM, Wolf CR, Bryant SP, Spurr NK (1993). Localization of the CYP2D gene locus to human chromosome 22q13. 1 by polymerase chain reaction, in situ hybridization, and linkage analysis. Genomics. 15: 430-432.

Hanioka N, Kimura S, Meyer UA, Gonzalez FJ (1990). The human CYP2D locus associated with a common genetic defect in drug oxidation: a G1934 → A base change in intron 3 of a mutant CYP2D6 allele results in an aberrant 3' splice recognition site. Am. J. Hum. Genet. 47: 994-1001.

Jin Y, Desta Z, Stearns V, Ward B, Skaar T, Storniolo AM (2005). Association between CYP2D6 genotype, antidepressants, and tamoxifen metabolism during adjuvant breast cancer treatment. J. Natl. Cancer I. 97: 30-39.

Kroemer HK, Eichelbaum M (1995). Molecular basis and clinical consequences of genetic cytochrome P450 2D6 polymorphism. Life Sci. 56: 2285-2298.

Lien EA, Solheim E, Lea OA (1989). Distribution of 4-hydroxy-N-desmethyltamoxifen and other tamoxifen metabolites in human biological fluids during tamoxifen treatment. Cancer Res. 49: 2175-2183.

Lien EA, Solheim E, Uela PM (1991). Distribution of tamoxifen and its metabolites in rat and human tissues during steady-state treatment. Cancer Res. 51: 4837-4844.

Lien EA, Anker G, Lonning PE (1990). Decreased serum concentrations of tamoxifen and its metabolites induced by aminoglutethimide. Cancer Res. 50: 5851-5857.

Matthew P, Goetz JM, RaeVera J, Suman SL, Safgren MM (2005). A. Pharmacogenetics of Tamoxifen Biotransformation Is Associated With Clinical Outcomes of Efficacy and Hot Flashes J. Clin. Oncol. 23: 9312-9318.

Naveen AT, Prasanna T, Farzana BL, Rajan S, Adithan. (2006). CYP2D6 genotype and phenotype relationship in South Indians. JPGM. 52: 253 – 256.

Nebert DW (1997). Polymorphisms in drug-metabolizing enzymes: what is their clinical relevance and why do they exist? Am. J. Hum. Genet. 60: 265-271.

Rendic S (2002). Summary of information on human CYP enzymes: human R450 metabolism data. Drug Metab. Rev. 34: 83–448.

Sachse C, Brockmöller J, Bauer S, Roots I (1997). Cytochrome P450 2D6 variants in a caucasian population: allele frequencies and phenotypic consequences. Am. J. Hum. Genet. 60: 284-295.

Shaswati K, Kaiser J, Prabhavathy D, Mohana VCH, Sudha M (2007). Polymorphic sites (1236 and 3435) in mdr1 gene influencing drug response in breast cancer patients. Int. J. Pharmacol. 3(6): 453-460.

Stearns V, Johnson MD, Rae JM (2003). Active tamoxifen metabolite plasma concentrations after coadministration of tamoxifen and the selective serotonin reuptake inhibitor paroxetine. J. Natl. Cancer Inst. 95: 1758–1764.

Suman G, Kaiser J (2006). Novel CYP3A4 gene polymorphisms in post chemo breast cancer patients, Int. J. Cancer Res. 2006 2(4): 358-366.

Wegman P, Vainikka, L, Stal O, Nordenskjold B, Skoog L, Rutqvist LE, Wingren S (2005). Genotype of metabolic enzymes and the benefit of tamoxifen in postmenopausal breast cancer patients. Breast Cancer Res. 7: 284–290.

West WL, Knight EM, Pradhan S, Hinds TS. (1997). Interpatient variability: genetic predisposition and other genetic factors. J. Clin. Pharmacol. 37: 635-648.

Zanger UM, Raimundo S, Eichelbaum M (2004). Cytochrome P450 2D6 Overview and update on pharmacology, genetics, biochemistry. Naunyn Schmiedebergs Arch. Pharmacol. 369: 23–37.