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Original Research Article

Association of CYP3A4 and CYP3A5 Polymorphisms with Indian Breast Cancer Patients& Its Clinical Implications

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ABSTRACT

Introduction: Polymorphisms of the different genes have been reported to be associated with a variety of cancers including breast cancer. CYP genes belongings to Cytochrome P450 (CYP450), have been implicated in various cancer formation and development due to their roles such as oxidative stress, activating procarcinogens, and inactivating anticancer drugs.

Objective: This study aimed to examine whether polymorphisms in the CYP3A4 and CYP3A5 genes affect the risk of developing breast cancer.

Results: CYP3A4*1B gene polymorphism revealed that in breast cancer patient group 96 had the *1A/*1A genotype (64%), 39 (26%) had the *1A/*B genotype and 15 (10%) had the *1B/*B genotype. In the control group 131 (87%) had the *1A/*1A genotype, 16 (10.66%) had the *1A/*B genotype and 3 (2%) had the *1B/*B genotype. Concerning genotype distribution, a significant difference between the breast cancer patients and the controls was observed where $p<0.05$) The genotype frequency of the CYP3A5*3 *1/*1, *1/*3, and *3/*3 polymorphisms in breast cancer and healthy control group were analysed. Frequencies of CYP3A4*3 *1/*1, *1/*3 and *3/*3 genotypes were 67.3%, 24% and 8.7 % in breast cancer patients and 79.3%, 14% and 6.7% in the controls, respectively. The distribution of CYP3A5*3 *1/*3 genotypes was significantly associated with breast cancer patients when compared with controls.

Conclusion: In conclusion, the results of our study found a positive association between CYP 3A4*1B and CYP3A5*3 polymorphisms and predispositions to breast cancer risk.

Keywords: Breast cancer; CYP3A4; CYP3A5; Polymorphism

INTRODUCTION

Breast cancer is the most common malignancy among women worldwide[1]. Cytochrome P450 (CYP450), belonging to ω-hydroxylase, participates in the metabolism of a wide variety of carcinogens and anticancer drugs. Certain CYP genes have been implicated in cancer formation and development due to their roles in promoting oxidative stress, activating procarcinogens, and inactivating anticancer drugs

The CYP3A isoenzymes represent the predominant cytochrome P450 enzymes in the human liver and gastrointestinal tract. CYP3A subfamily has four members (CYP3A43, CYP3A7, CYP3A5, and CYP3A4).CYP3A4 and CYP3A5 enzymes are the major enzymes for drug metabolism, both enzymes making up nearly 30% of the total CYP enzymes expressed in the human liver. CYP3A4 has been identified and one of the most common variants is in the 5′promoter region (−290), marked as CYP3A4*1B (rs2740574) [2]. Badavi et al., 2015, in his study showed that compared with the wild-type CYP3A4*1A, CYP3A4*1B shows about a 2-fold increase in enzyme activity [3]. A decrease in the catalytic activity of the enzyme was also determined and some even linked this SNP with increased cancer risk[4]. Frequent single nucleotide polymorphism (SNP) of CYP3A5 gene is a substitution of adenine with guanine in intron 3: 6896A>G (rs776746). The mutated allele CYP3A5*3 results in a splicing defect of the mRNA and produces an unstable and non-functional protein with decreased activity, or loss of activity of the encoding enzyme, which according to some studies could play a role not only in interindividual variations[5]. CYP3A4 and CYP3A5 polymorphism frequencies also differ remarkably among different human populations [6]. SNP in the genes encoding drug-metabolizing enzymes (DME) could have a critical role in individual responses to therapy.

CYP3A enzyme activity shows interindividual variability due to the combined effect of genetics and interaction with drugs. Both CYP3A4 and CYP3A5 genes are polymorphic and several variant alleles have been described for either the CYP3A4 or the CYP3A5 gene Only two variant alleles are at linkage disequilibrium, namely CYP3A4*1B and CYP3A5*3 are common across diverse ethnic populations and have functional relevance. The present study aimed to determine whether any association existed between certain single nucleotide polymorphisms (SNPs) of the CYP3A4, and CYP3A5 genes and Breast cancer & therapy in the south Indian population.

MATERIAL & METHODS

Study Population& Sample Collection

A total of 150 female subjects who have confirmed diagnosis of breast cancer included in this study aged between 22 to 69 years and 150 unrelated subjects aged between 21to 66 years were also enrolled as the control group. We collected the tissue samples (n=150) to investigate the association between CYP3A4*1B (rs 2740574) and CYP3A5*3 (rs 776746) and with different combinations of chemotherapy. This study was approved by the Institutional Ethics Committee, between the period June 2017 to August 2021 Hyderabad.

DNA Extraction

Genomic DNA was extracted from tumor tissues and blood samples of the cases and controls by a rapid non-enzymatic method by salting out cellular proteins with saturated solution and precipitation by dehydration. Gel electrophoresis was performed using 2% agarose to ensure the best quality and yield of the DNA was obtained. These DNA samples were used further for PCR amplification.

Genotyping of the CYP3A4*1B (rs 2740574) Polymorphism

CYP3A4*1B (--392G>A) Polymorphism was analyzed using a set of gene-specific primers forward: 5'-GGAATGAGGACAGCCATAGAGACAAGGGGA-3' and Reverse: 5'-CCTTTCAGCTCTGTG TTGCTCTTTGCTG-3' synthesized at Bioserve Biotechnologies (Hyderabad, India). A three-step PCR amplification was performed. Briefly, a 25 µl reaction was set up containing 0.2 mM of each dNTP, 1X buffer, 1.5 mM MgCl2, and 2U of Taq DNA polymerase (Bioserve, India). 30 cycles of PCR procedure were performed with denaturation at 95°C for 35 seconds, annealing at 55°C for 30 seconds, and elongation at 72°C for 30 seconds. Final elongation was performed at 72°C for 5min After amplification, 5µl of PCR product was subjected to restriction digestion with one unit of Hhf1 (from Fermentas, USA)restriction enzyme. The restriction fragments were then visualized by 2%

agarose gel electrophoresis, with ethidium bromide staining. Three types of band patterns were obtained: Wild type homozygote (A/A), one band corresponding to 207bp; Polymorphic homozygote (G/G), two bands corresponding to 18 and 189 bp and Heterozygote (A/G), three bands corresponding to 18, 189, and 207 bp.

Genotyping of the CYP3A5*3 (rs 776746) Polymorphism

CYP3A5*3 (6986A>G) polymorphism was analyzed using the following gene-specific primers Forward: 5' - CATGACTTAGTAGACAGATGAC-3' and Reverse:5'- GGTCCAAACAGGG AAGAAATA-3' synthesized at Bioserve Biotechnologies (Hyderabad, India). 35 cycles of PCR procedure were performed with denaturation at 94°C for 35 seconds, annealing at 55°C for 30 seconds, and elongation at 72°C for 30 seconds. Final elongation was performed at 72°C for 5 minutes. After amplification, 5µl of PCR product was subjected to restriction digestion with one unit of Ssp1 restriction enzyme. The restriction fragments were then visualized by 2% agarose gel electrophoresis, with ethidium bromide staining. The 293 bp amplicon was digested with a restriction enzyme and cut the wild genotype into three fragments of 148 bp, 125 bp, and 20 bp. However, A to G transition at position 6986 results in loss of restriction site and result in two fragments of 168 bp and 125 bp in the case of mutant genotype.

Statistical Analysis

Clinico-demographic data was collected from the patients' records and data was summarised by using descriptive statistics.

The clinical parameters were calculated and were expressed as a percentage, mean, and standard deviation, and the allele frequencies were expressed in frequencies and percentages with 95% confidence intervals (CI). Using the chi-squared test, the distribution of the genotype frequencies for patients and control subjects was compared; and the Odds ratio (OR) with corresponding confidence interval (95% CI) for disease susceptibility was also calculated. The observed frequency of the allele frequencies was also analyzed for the Hardy– Weinberg equilibrium using the v2 method. All the statistical analyses were done at a 5% significance level, and the p-value of <0.05 was considered significant.

RESULTS

Clinic pathological characteristics in Breast cancer patients.

General characteristics of the breast cancer patients and controls are given in table 1. Breast cancer patients (n=150) were histopathologically confirmed and healthy controls (n=150) who were age and sex-matched only enrolled in this study. In breast cancer cases age ranged from 20-70 years and the mean age was found to be 44.89 ± 14.30, whereas in control groups age was 22-70 years and the mean age was found to be 41.98 ± 13.91 years. Breast cancer patients were divided into 5 groups according to age at diagnosis; these were 21-30, 31-40, 41-50, 51-60, and 61-70 years. A higher percentage of cases and subjects were found in the age groups 41-50 (38%) years when compared to other age groups. It was observed the majority of sporadic breast cancer cases were higher in the postmenopausal (62%) group than compared in the premenopausal group (38%). The frequency of overweight and obese patients were found to be 28% and 9.33% in this study. The frequency of ER+/PR+ and Her2 positive tumor types were found to be 60% and 78%. According to histological differentiation of tumor grades, I to III were found to be 34.66%, 48.66%, and 16.66%, patients were classified into three grades, poor, moderate, or well-differentiated grade, respectively. In our study majority of patients (57%) received both chemo and hormonal therapy (FAC/Tamoxifen) (Table 1).

Table 1 Breast Cancer Patients Characteristics

Patients Characteristics	Cases N=150(%)
Age of Diagnosis	
21-30	27 (18%)
31-40	33 (22%)
41-50	57 (38%)
51-60	23 (15.33%)
61-70	10 (6.66%)
Menopausal Status	
Pre Menopausal	57 (38%)
Post Menopausal	93 (62%)
Age at Menarche	
11-12	47(31.33%)
13-14	80(53.33%)
15-16	23(15.33%)
Body mass index(BMI) status	
Normal weight >18.50 to <24.99	94 (62.66%)
Overweight>25 to <29.99	42 (28%)
Obese >30.00	14 (9.33%)
Hormone Receptor Status	
ER+ /PR+	90 (60%)
ER+/PR-	15 (10%)
ER-/PR+	9 (6%)
ER-/PR-	36 (24%)
Her-2 Status	
Positive	117(78%)
Negative	33(22%)
Histological Grade	
Grade-1	52 (34.66%)
Grade II	73 (48.66%)
Grade III	25 (16.66%)
Chemotherapy & Hormonal Therapy	
FAC/Tamoxifen	85 (56.66%)
FAC/Non Tamoxifen	50 (33.33%)
Unknown	15 (10%)

CYP3A4*1B (rs 2740574) Genotype Distribution in Breast cancer patients and control subjects

The investigation of the CYP3A4*1B gene polymorphism revealed that in the breast cancer patient group 96 (64%) cases had the *1A/*1A genotype, and 39 (26%) had the *1A/*B genotype and 15 (10%) had the *1B/*B genotype. In the control group 131 (87.33%) had the *1A/*1A genotype, 16 (10.66%) had the *1A/*B genotype and 3 (2%) had the *1B/*B genotype. Concerning genotype distribution, we found a significant difference between the breast cancer patients and the controls observed for all genotypes where ($p<0.05$) [Table 2].

Table -2 Genotyping of the CYP3A4*1B in breast cancer patients and controls

Genotypes	Cases n=150	Controls n=150	Odds Ratio	95% CI	P value
*1A/*1A	96 (64%)	131(87.33%)	0.25	0.143-0.463	0.0001
*1A/*1B	39(26%)	16(10.66%)	2.64	1.395- 5.014	0.0029
*1B/*1B	15(10%)	3(2%)	5.444	1.542 to 19.221	0.0085

CYP3A5*3 (rs 776746) Genotype Distribution in Breast cancer patients and control subjects

The genotype frequency of the CYP3A5*3 *1/*1, *1/*3, and *3/*3 polymorphisms in breast cancer and healthy control groups. Frequencies of CYP3A5*3*1/*1, *1/*3 and *3/*3 genotypes in cases were 67.3%, 24% and 8.7 % whereas in control group 79.3%, 14% and 6.7% respectively. The

distribution of CYP3A5*3 *1/*1, *1/*3 genotypes in cases and controls showed statistical significance in this study (Table 3).

Table -3 Genotyping of the CYP3A5*3 in breast cancer patients and controls

Genotypes	Cases n=150	Controls n=150	Odds Ratio	95% CI	P value
*1/*1	101(67.3%)	119(79.3%)	0.537	0.318 to 0.905	0.0196
*1/*3	36(24%)	21(14%)	1.939	1.070 to 3.514	0.0289
*3/*3	13(8.7%)	10(6.7%)	1.328	0.563 to 3.131	0.5162

DISCUSSION

The CYP3A4 and CYP3A5 genes are known to perform a mono-oxygenase reaction, which is involved in several drug-related reactions such as bio-activation of medicines, excretion of drug compounds, and deactivation of drug compounds[7]. Approximately 30% of CYP enzymes showed a high expression level in the liver and intestine, and activities of CYP3A4 and CYP3A5 constituted approximately 36% of all CYP3A enzyme activities. CYP enzymes showed genetic variation across individuals, with deficiencies that differ from each other and are occurring in 1% to 30% of populations, depending on ethnicity [8]. Therefore, a large number of studies were conducted to validate the effect of single-nucleotide polymorphisms (SNPs) of CYP3A4 and CYP3A5 on these polymorphic expressions and the risk of various diseases[9] including cancers**.** In the present study, we found that CYP3A4*1B (rs 2740574) Genotype showed significance when compared between cases controls, where *1A/*1A genotype proved to be the protective role in the control group where p showed a significance. Higher frequencies of *1A/*1B and *1B/*1B were also found in the patients' group when compared with the control group and indicating that the risk of eighter one genotype variant may increase the incidence of breast cancer risk.

When we compared the frequencies of the CYP3A4*1B variant in the present study with previous studies like African Americans [10], and Caucasian [11] differed from each other results, the p-value was significant but no studies are done in breast cancer patients. In a study from the Iran population, the genotype frequency was found to be in controls 99% (n= 198), whereas in cases 98% (n= 49) groups respectively [12].

To date there is no such study supports that the frequencies of CYP3A4*1B are associated with breast cancer incidence (P-value> 0.1). These results show no association between breast cancer and CYP3A4*1B polymorphism and comparison between Incidence rates of breast cancer[13].

In our study, we also found that CYP3A5*3 (rs 776746) *1/*1 genotype plays a protective role in breast cancer development where the *1/*1 genotype frequency was higher than cases in controls and it is statistically significant. Other CYP3A5*3 (rs 776746) *1/*3 genotype frequency showed a significant in breast cancer development where p=0.028 and no association between breast cancer risk was found with *3/*3 genotype in this study when compared between cases and controls.

Our results have differed from previous studies, where Jin et al 2005[14] and Tucker et al, 2005 [15] and Surekha D 2009 did not find any significant association between CYP3A5*3 (rs776746) but Surekha D et al., 2009 [16] studies suggest that CYP3A5*3 polymorphism might influence the breast cancer risk which mainly influence by the type of exposure.

CYP3A5 is an important member of the CYP3A family. It participates in the catalytic oxidation of many exogenous substances, including toxins, carcinogens, the metabolism, and clearance of some drugs. Studies have shown that CYP3A5 plays an important role in the development of different cancers [17,18]. The allele frequency of CYP3A5*3 reported between East Asian populations such as Korea, China, and Japan is 71- 75%, South Asian countries like India 65%, whereas in Malay

(55%), in African American population it was reported between 29-35%, Caucasians (91-94%) and Hispanic population 60-66% [19,20]. In this allele, it reported that cryptic splicing site in intron 3 created by guanine at the position of 6986, which leads to aberrant splicing of the transcript to cause truncation of the CYP3A5 protein, which resulted no enzyme activity [21]. This polymorphic allele is strongly associated with the reduced drug clearance of CYP3A substrates, assuming higher drug levels of the CYP3A substrates in the subjects possessing this allele. Because it has a relatively high frequency and occurs in diverse populations, its roles have been extensively investigated. Our results revealed that there is an association between risk of the breast cancer and CYP3A4*1B CYP3A5 polymorphism in south Indian women. The presence of CYP3A4*1B and CYP3A5*3 polymorphisms in some patients might also influence the metabolism of drugs used in chemotherapy and thus exacerbate the prognosis Limitations of our study, we did not consider the associations of CYP3A5 and CYP3A4 genetic polymorphisms with the response to treatment and chemotherapeutic drug toxicity which most of the studies done so far. Thus, the participants received combination chemotherapy with excessive potential confounding factors limited further analysis. The size of the current study was only a relatively small number in the specific population.

CONCLUSION:

Our study suggests that the CYP3A5 gene may be related not only to the risk and prognosis of breast cancer but also to the treatment and drug selection of breast cancer. It may be a predictor of the occurrence, development, and prognosis of breast cancer, but it needs a larger sample of research to further confirm the findings.

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