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Research Article

Sulfotransferase 1A1 (*SULT1A1*) Polymorphism and Breast Cancer Risk: A Case-control Study in South India

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Abstract

Introduction: Breast cancer is the most common cancer among women worldwide. Sulfotransferase plays an important role in the formation of estrogen which is usually conferred as a risk factor for breast cancer. The SULT1A1*2 polymorphism is likely to play an important role in the susceptibility to breast cancer. This polymorphism (G638A) in the sulfotransferase 1A1 (SULT1A1) gene may causes Arg213His amino acid change and consequently results in significantly reduced enzyme activity and thermostability.

Materials and Methods: In this case-control study, we investigated the role of SULT1A1 G638A polymorphism in breast cancer patients. We genotyped 144 patients with breast cancer and 116 healthy control subjects, using a Polymerase chain reaction –Restriction Fragment length polymorphism (PCR-RFLP) method.

Results: The frequencies of SULT1A1 G638G, G638A and A638A were 83.5%, 17.8%, and 1.4% in the breast cancer group and 89.5%, 4.0% and 0.0% in the control group. The results of our study indicate that the SULT1A1 G638A genotype showed 4.6 folds increased risk of breast cancer ($p = 0.002$).

Conclusion: In conclusion, our results suggest that, SULT1A1 G638A variant is significantly associated with risk of breast cancer in south Indian women.

Keywords: Breast Cancer; Sulfotransferase; Polymorphism
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Introduction

The risk of hormone dependent cancer is directly or indirectly related to reproductive hormones in particular estrogen. Estrogen stimulation plays an important role in human breast cancer cell growth and development. It was reported that estrogen could affect breast cancer risk through stimulating cellular proliferation and promoting tumor progression [1]. To date, 13 human cytosolic SULT isoforms have been identified and grouped as four major families: *SULT1, SULT2, SULT4* and *SULT6* [2] Sulphotransferase (*SULT1A1*) gene locates in chromosome 16p11.2 - p12.1. *SULT1A1* gene belongs to the superfamily and catalyzes sulfate conjugation and plays a significant role in the phase II metabolism of a large number of endogenous and exogenous compounds [3]. It is evident from previous studies that *SULT1A1* gene also acts as an isoform for the sulfation of estrogen and catecholestrogen in breast cancer forming hydrophilic sulphates of estrogen which are excreted in urine [4].

Cancer susceptibility has been proposed to be influenced by functional polymorphism in enzyme metabolizing carcinogenic substrates. A common polymorphism in exon 7 of the *SULT1A1* gene results in the substitution of a histidine for an arginine at position 213 of the translated protein, which in turn produces an enzyme with much lower catalytic activity and decreased thermal stability [5]. The variant *allele SULT1A1*2* with reduced sulfotransferase activity might enhance the risk of bone marrow cancer [6]. Two previous studies [7, 8] reported the *SULT1A*1*2 polymorphism associated with breast cancer. Recently Wang et al. meta-analyzed the relationships between *SULT1A1* and breast cancer risk [9] and concluded that there was no significant relationship between *SULT1A1* R213 H polymorphism and the risk of breast cancer. Previous studies have shown inconsistent results for the association between the *SULT1A1* G638A polymorphism and several malignancies including bladder cancer, ovarian cancer and prostate cancer [10]. Therefore, we conducted case-control study to explore the role of *SULT1A1* G638A polymorphism in the development of breast cancer in south Indian population.

Materials & 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 Mahavir hospital 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. We obtained informed consent from the study participants.

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.

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 refused 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 [11]. 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 was 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 *SULT1A1**2 Polymorphisms

Polymerase chain reaction (PCR) was performed, with specific primers synthesized from bioserve Biotechnologies Ltd. (Hyderabad, India): 5'-TCCAGAATCTGTTCCAGAGCGTGC-3'(forward) and 5'-CTTGGGGAGAACCATCCTCA -3' (reverse). A three-step PCR was standardized using an takara thermocycler 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 were visualized after electrophoresis in an ethidium-bromide-stained 2% agarose gel. RFLP was conducted with PCR amplified product using *Hha1* (***Fermentas***)restriction enzyme for 37^0C overnight PCR products subjected to enzyme digestion were visualized on 3% agarose gel stained with ethidium bromide (Fig-3).

Statistical Analysis

All statistical analysis odds ratio(OR), 95% confidential intervals (CI) and Probability(P) values were done using the MedCalc for Windows, version 7.4.1.0 (MedCalc Software, Mariakerke, Belgium). Values less than $P<0.05$ were regarded as statistically significant.

Results

The present study was carried out in 144 sporadic Breast Cancer patients and 116 healthy age matched women volunteers. The study was approved by ethical committee and informed consent was taken. Age range for BC patients was 28 – 78 years. The mean age at which Breast Cancer was identified 49.87 years. 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. It was observed that the majority of sporadic that breast cancer cases were high in postmenopausal group when compared to premenopausal group. In the present study all cases are invasive ductal carcinomas 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%) and ER-PR- (49.53%) breast tumors were high when compared to other tumor subtypes like ER+/PR- (9.34%) , ER-/PR+ (9.34%), ER+/PR-, ER-/PR+ tumor showed equal distribution. In the present study Grade II showed the highest frequency followed by (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 (Table 1).

Table 1 Clinical and pathological features

Total cases	144(100%)
Age Range	
20-39	46(32%)
40-59	92(64%)
Menopausal	
Status	91(63%)
Postmenopausal	53(37%)
Hormonal Status	
ER+/PR+	51(36%)
ER-/PR-	69(48%)
Nuclear Grade	
Grade-I	25(17%)
Grade-II	88(62%)

Sulfotransferase 1A1 (*SULT1A1*) G638A Genotyping Analysis

SULT1A1 G638A Gene polymorphisms were analyzed in DNA obtained from Tissue samples of 144 Breast Cancer patients and compared to 116 healthy age matched women volunteers blood samples. *SULT1A1* G638A Polymorphism was analyzed by PCR –RFLP and the PCR product (200bp) was digested with *Hha1* 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 200bp fragment, indicates A allele, heterozygous GA genotype shows 200, 160 and 40bp (Figure 1 & 2) bands and wild type GG genotype shows 160 and 40 bp bands.

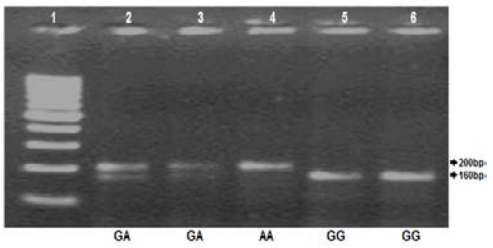

Figure 1 *SULT1A1* PCR products after restriction digestion with HhaI on 2% agarose gel.
Lane 1 = 100bp DNA Ladder, **Lane 2&3** = GA genotype, **Lane 4** = AA genotype and **Lane 5** =GG genotype

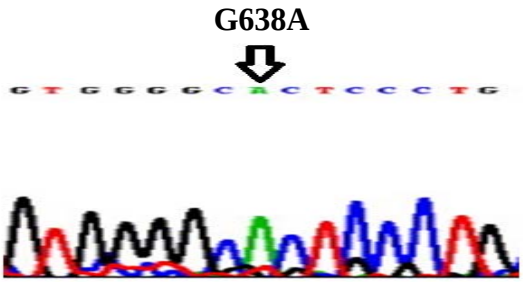

Figure 2 G-A alteration at nucleotide position 638 in Exon 4 Junction

Frequencies of GG, GC, and CC genotypes were 83.5%(n=117), 17.8%(n=25), 1.4%(n=2) in the breast cancer cases and 89.5%(n-111), 4%(n=5), 0.00%(n=0) in the controls respectively. Table 2 shows results for the *SULT1A1* G638A Polymorphism, GA, and AA genotypes showed higher frequency in breast cancer patients than controls. From *SULT1A1* Genotyping Analysis, it was observed that there was no significant difference in the distribution of GG genotype between cases (83.5%) and controls (89.5%). AA genotype was observed only in 1.4% of breast cancer cases and it was absent in the control group, While the distribution of GA genotype was higher in breast cancer cases (17.8%) when compared to controls (4.0%). Odds ratios (OR: 4.66, 95% CI: 1.72-12.60) and p values (0.002) showed an association of IM genotype with Breast Cancer. In this study *SULT1A1* G allele frequency was 0.899%, A allele frequency was 0.11%in the breast cancer patients and G allele frequency was 0.978%, A allele frequency was 0.021% in the controls suggesting that individual alleles were associated (p-value 0.001) with breast cancer (Table 12). In *SULT1A1* (G638A) genotype, a total of 288 alleles were reported of which, 29 were mutant and 259, wild type (Table 2)

Table 2 Distribution of *SULT1A1* gene G638A polymorphism in Breast Cancer patients and controls

Genotype	Cases (n=144)	Controls (n=116)	OR	95%CI	X2	P-value
G638G	117(83.5%)	111(89.5%)	0.19	0.07 - 0.52	11.11	0.001
G638A	25(17.8%)	5(4.0%)	4.66	1.72 -12.60	9.48	0.002
A638A	2(1.4%)	0(0.00%)	4.08	0.19 – 85.9	0.31	0.365

Correlation analysis of *SULT1A1* G638A polymorphism and breast cancer clinical characteristics

From this analysis it was observed that, GG genotype distribution was lower in both pre and post menopausal breast cancer patients when compared to controls. The difference in the distribution of GA genotype between breast patients and controls was significant; these were elevated in premenopausal breast cancer patients. Whereas only 2% of the post menopausal breast cancer patients showed PM (Poor Metabolizer) genotype, none of the pre menopausal breast cancer patients and controls showed this genotype. Genotyping analysis of *SULT1A1* with respect to ER/PR status showed that distribution of GA and AA genotypes of *SULT1A1* between ER/PR positive and ER/PR negative breast cancer patients. High frequency of GA genotype was found to be ER-ve patients. From the correlation analysis of *SULT1A1* genotyping, it was observed that, the AA genotype was not seen in both Grade 1 and Grade II. Slight increase of GA genotype from Grade I to Grade III was observed, however the difference was not significant in all the 3 grades of GA & GG genotypes (Table 3).

Table 3 Correlation analyses of *SULT1A1* G638A polymorphism and breast cancer clinical characteristics

Clinical Data	GG(n=117)	GA(n=25)	AA(n=2)
Menopausal Status			
Post Menopausal	85% (99)	12% (14)	3% (4)
Pre Menopausal	70%(82)	30%(35)	0%(0)
Hormonal Status			
ER+/PR+	76%(89)	19%(22)	5%(6)
ER-/PR-	60%(70)	38%(45)	2%(2)
Nuclear Grade			
Grade-I	75%(87)	25%(30)	0%(0)
Grade-II	70%(82)	26%(32)	4%(3)
Grade-III	69%(81)	31%(36)	0%(0)

Discussion

SULT1A1 is the primary sulfotransferase isoform responsible for the sulfation of 4-OH TAM [12](Falany CN et al., 1997). Sulfation of compounds such as estradiol is generally considered to result in their inactivation, in that sulfated estrogens are poor ligands for the estrogen receptor. The most common polymorphism in the *SULT1A1* gene being a G • A transition at nucleotide 638, which results in an arginine to histidine substitution at the conserved amino acid 213, and has been associated with a decreased sulfotransferase activity. From the analysis of *SULT1A1* genotypes among breast cancer patients, we found that the distribution of heterozygous mutant genotype between breast cancer patients and controls was significant; this appears to be associated with breast cancer. While the distribution of homozygous mutant genotype was more in breast cancer patients than in controls, however the result was not statistically significant. An A allele showed significant association with breast cancer. Correlation analysis of *SULT1A1* genotypes with menopausal status, observed a significant association of GA genotypes with premenopausal women breast cancer patients, suggesting that this may be associated with early onset of the disease. This finding is consistent with earlier report which shows that His *SULT1A1* allele increased the risk in premenopausal patients. Breast cancer patients with ER/PR negative tumors showed an association with heterozygous *SULT1A1* genotype. Significant association of this polymorphism with disease progression was observed hence there was difference in the distribution of *SULT1A1* genotypes between breast cancer patients with different grades. Breast cancer patients with *SULT1A1* genotype showed a significant association with response. Moreover there was a positive association Arg/His genotypes with premenopausal women and with ER/PR negative cases which puts the cases at increased risk.

According to previous studies, his allele of *SULT1A1* was found to involve in the inactivation of estrogen, the hormone is believed to play important role in etiology of breast cancer. Wei Zheng in a case – control study reported that postmenopausal women who were with His/His allele at codon 213 of *SULT1A1* gene may be at an increased risk of breast cancer, and patients with Arg/His were also found to be at higher risk with breast cancer [7]. Tang D et al. found no risk in Arg/His breast cancer patients [8]. Ding-Fen Han et al. suggest that the *SULT1A1* His allele was positively associated with the risk of breast cancer in Chinese women [13]. It appears that there is an interaction between *SULT1A1* polymorphism and related exposure factors. Monique Saintot et al. reported that His *SULT1A1* allele increased the risk only in premenopausal patients [14]. One in Indian study reported that the genetic polymorphisms of the *SULT1A1* gene is associated with an increased risk of breast cancer among postmenopausal Kerala women [15]. It was found that this allele lowers sulfation of the tobacco carcinogens among women with His *SULT1A1* could increase exposure to genotoxic compounds; *SULT1A1* induces the individual susceptibility to breast cancer among current smokers. Nowell and colleagues showed that the high-activity allele *SULT1A1*1* contributed significantly to tamoxifen response [16]. They suggested that sulfation may affect bioavailability of 4-OH-tamoxifen by reduced clearance of the sulfated metabolite. This may provide a genotype-dependent reservoir of inactivated metabolite, which can be desulfated by steroid sulfatase expressed in breast tumours and can be recovered to the active 4-OH-tamoxifen, leading to a prolonged anti-oestrogen effect [17]. The majority of studies suggest that genetic polymorphisms in xenobiotic metabolizing enzymes may play an important role in the susceptibility of individuals to cancer [18]. In our previous study, we found that the *CYP2D6* genotype may play an important role as a risk factor for breast cancer development in the south Indian population [11]. In conclusion the *SULT1A1* G638A was significantly associated with an increased risk for early onset of the breast cancer. However the detection of *SULT1A1* polymorphisms has important clinical implications for diagnosis, and therapy.

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