

The Correlation between Catalase C262T Gene Polymorphism and Catalase Enzyme Activity in Breast Cancer Patients

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ABSTRACT

Oxidative stress is known to be one of the major factors involved in the development and progression of cancer. Oxidative stress is significant in numerous types of cancer. Catalase plays important roles in the primary defense against oxidative stress. The purpose of this study was to investigate the effects of the CAT C-262T (rs1001179) gene polymorphisms and catalase activity on the susceptibility to breast cancer. The present results revealed that significant difference was observed in the rs1001179 CAT C-262T SNP between the patients and controls. We found that the genotypic frequency of heterozygous C/T with homozygous C/C (OR=22.20, 95% CI=1.287 to 382.71, P= 0.0328). and the genotypic frequency of homozygous variant T/T with the homozygous C/C (OR=1.48, 95% CI= 0.270 - 8.19, P= 0.64). There were significant differences in T allele frequencies in patients as compared to the controls in the CAT C-262T polymorphism (OR= 3.50, 95% CI: 1.1701 - 10.4, P=0.0250). There were no significant differences between catalase genotypes (CC, CT and TT) and its activity in serum of breast patients and control. Our findings showed that there was no significant association between CAT C-262T (rs1001179) polymorphism and Breast cancer susceptibility.

Keywords:

Breast cancer; Antioxidant polymorphism ; rs1001179; CAT C-262T; CAT activity; PCR-RFLP.

Introduction

Breast cancer is the most commonly occurring cancer in women, comprising almost one third of all malignancies in females. It is second only to lung cancer as a cause of cancer mortality, and it is the leading cause of death for American women between the ages of 40 and 55 [1]. Oxidative stress plays an important role in carcinogenesis because of induction of DNA damage and its effects on intracellular signal transduction pathways [2]. ROS may also play a key role in cancer development by inducing and maintaining the oncogenic phenotypes of cancers [3]. CAT C-262T polymorphism is located 262 bp upstream of the CAT transcription site and the association between this polymorphism with the gene expression level shows that the T allele is associated with a higher catalase expression level [4].

Catalase (CAT; OMIM: 115500), is the most potent enzyme [4] that is inducible by exposure to reactive species, particularly H₂O₂ [5]. Catalase is present in all aerobic cells. Catalase activity is likely to be affected by functional polymorphism of C-262 T (rs1001179) in the CAT gene. The C-262 T polymorphism in the promoter region of the CAT gene was found to be associated with altered catalase activity [4, 6]. Considering that CAT is one of the major enzymes involved in cellular detoxification [4, 5], several studies on the CAT C-262 T polymorphism associated with several types of multifactorial traits, including some types of cancers have been published [7,8].

Recent studies on antioxidant enzymes state that diseases like diabetes [9], age related diseases and cancers [10] are connected with defects in antioxidant pathways.

Material and Methods

Ethical statement

Every volunteer has informed written consent. The ethics committee of the MOH and MOHSER in Iraq's ethical approval for scientific research has accepted this research.

Study Population

The study subjects comprised from 70 patients selected from Merjan Teaching Hospital all were female as patients' group with age range (26-80 years). The control group study included 30 people apparently healthy that also were females with age range (20–71) years. All subjects in this study were taken written consent before participation in this study.

Analysis of catalase activity

Catalase activity was measured by the method of Aebi [11]. This method is based on the principle that spectrophotometric recording of the initial rate of hydrogen peroxide disappearance (0–60 s) is at a wavelength of 240 nm. Catalase enzyme activity is expressed as U/ml of plasma.

DNA extraction and genotyping

DNA of blood was extracted and purified using extraction and purification kit from Geneaid company (UK). The genotyping of the study groups was performed using the PCR-RFLP technique after DNA extraction from blood samples.

The targeted sites of DNA were amplified using design specific primers which used to identify CAT C-262T (rs1001179) , obtained from Macrogen company/south Korea . Forward primer: 5'-AGAGCCTCGCCCCGCCGGCCCG -3', and reverse primer: 5'-TAAGAGCTGAGAAAGCATAGCT -3'. PCR was carried out in 20 µl reaction volumes containing 1 µl of each forward and reverse primer, 12.5 µl of Green Master Mix, 3 µl of Genomic DNA, and 2.5 µl of nuclease-free water to bring the reaction volume up to 20 µl . Amplification was conducted in a thermocycler (Biometra, Germany) with the following settings: 2 min pre-denaturation at 94°C; 30 cycles with denaturation for 30 seconds at 94°C, annealing for 60 seconds at 52°C, extending for 60nseconds at 72°C; and a final extension of 5 min [12].

PCR products were electrophoresed in 1 percent agarose at 75 V using gel electrophoresis (cleaver science – UK) and visualized with ethidium bromide. A gel documentation system (Cleaver Scientific –UK) was used to take photos.

polymerase chain reaction-restriction fragment length polymorphism method (PCR-RFLP).

The PCR product was digested with 2 units of one of the specific endonucleases (SmaI) for 1-4 h at 37°C , according to manufacturer's instructions (Promega company) . PCR and digestion products

were analyzed by electrophoresis in 1 percent agarose at 75 V using gel electrophoresis (cleaver science – UK) and visualized with ethidium bromide.

Statistical analysis

All the statistical analyses were done with the SPSS statistical software (version 23; SPSS Inc., Chicago, IL), $p < 0.05$ was considered statistically significant

Results and Discussion

The genomic DNA (Fig.1) was extracted from the blood samples as a first step to amplify the target region of CAT gene.

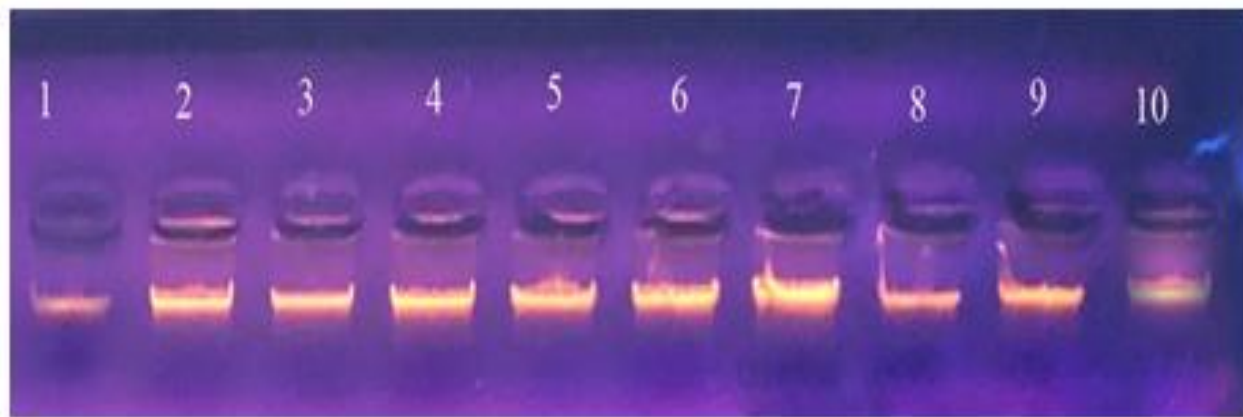


Figure 1. The electrophoresis pattern of genomic DNA extracted from blood samples of breast cancer patients and healthy control groups.

(Lane 1 -lane 10 refers to genomic DNA from blood samples; Electrophoresis conditions, 1% agarose, 75 V, 20 mA for 1h, stained with ethidium bromide).

Genotyping of CAT (rs1001179) Gene Polymorphisms

For CAT (rs1001179) genotyping, the genomic DNA was amplified using specific primers and accomplished by the Thermo-cycler apparatus under the optimal conditions. The results revealed that the presence of a single band (185bp) of the target sequence of CAT (rs1001179) gene in agarose gel (Fig. 1).

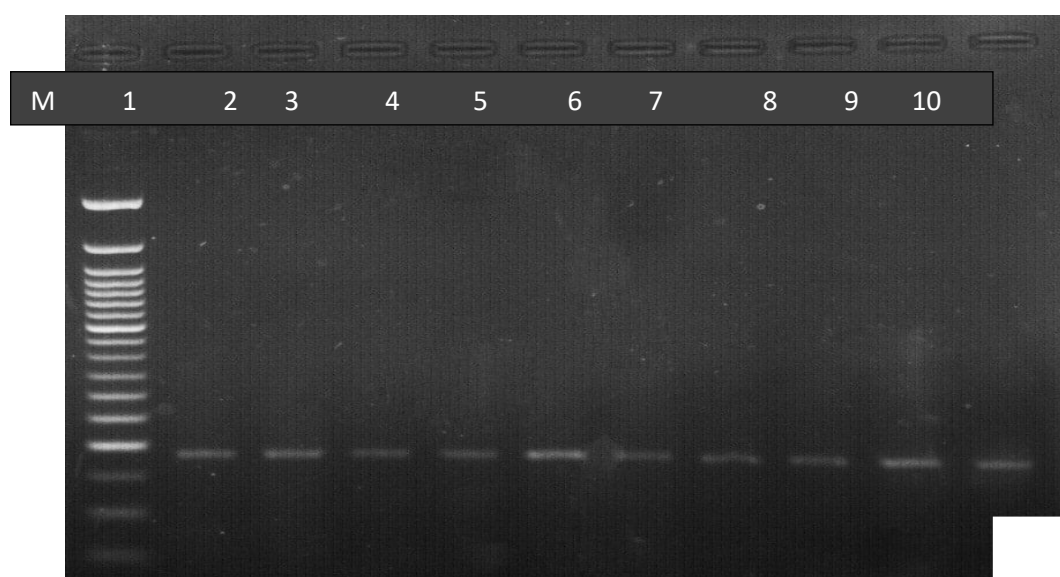


Figure 2. Agarose gel electrophoresis of an amplified product patterns of CAT (rs1001179) with specific primer.

(M: refers to DNA size marker; lanes 1 - 10 refer to PCR products of CAT (rs1001179) (185 bp) of breast cancer patients and healthy control groups. Electrophoresis conditions: 1% agarose concentration 1%; 75 V, 20 mA for 120 min. Staining method; precast ethidium bromide).

After that, the PCR products of the CAT (rs1001179) target sequences were digested with SmaI (5' CCC⁺GGG 3') restriction enzyme to detect the rs1001179 SNP in CAT gene. The genotypes of the studied subjected has been distributed into three groups based on the presence or absence of the Polymorphisms as in figure (4-19): T/T homozygous 185 bp, C/T heterozygote demonstrated 185, 155& 30 bp and C/C homozygous 155& 30 bp (Fig. 2).

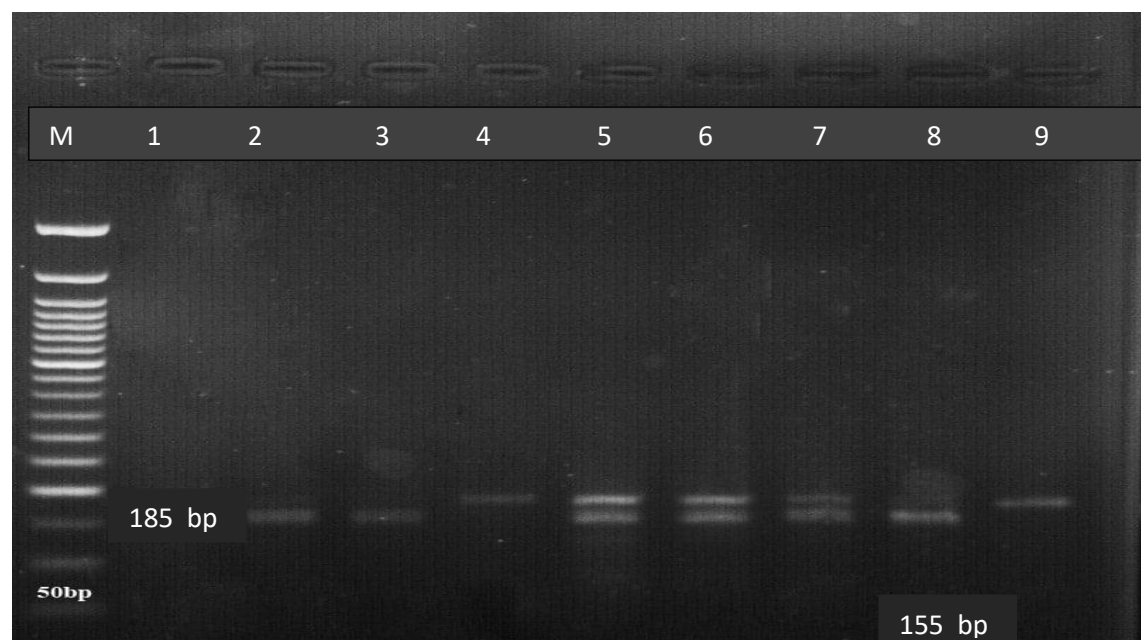


Figure 3. Electrophoresis patterns of allelotyping of CAT (rs1001179) gene of breast cancer patients and healthy control groups using MspI enzyme by PCR-RFLP method

(M: DNA ladder (50 bp); Lanes 1,3 ,4& 9 refer to a homozygous allele (TT) had a single band with 185 bp molecular size; Lanes 5,6,7&8 refer to a heterozygous allele (CT) had 3 bands with 185, 155 & 30 bp molecular size. Lanes 2&3 refer to a homozygous allele (CC) had a two band with 155 & 30 bp molecular size .Note: the band 30 bp was not visible).

The Genotypes Distribution of Rs rs1001179 Polymorphisms with Allele Frequency in Control and Case Groups.

The CAT rs1001179 gene polymorphism was studied in breast cancer cases and control. The distribution observed in CAT rs1001179 gene polymorphism in cases group and control group are showed in Table (1). The highest genotype in control group was CC homozygote genotype (93.3%) followed by homozygote genotype TT (6.7%), heterozygote genotype CT (0%). In breast cancer disease, the highest genotype was CC homozygote genotype (67%) followed by heterozygote genotype CT (26%), TT homozygote genotype (7%). analyses showed significant differences between cases and controls in the C-262 T polymorphism (CT vs CC: OR=22.20, 95% CI =1.287- 382.71, P=0.03). analyses showed no significant differences between cases and controls in the C-262 T polymorphism (TT vs CC: OR=1.48, 95% CI =0.270 to 8.19, P= 0.64).

Table 1. Genotype distribution and odd ratio of rs1001179 polymorphisms between the patients vs healthy control

Genotype rs1001179	Patients No. (%)	Control No. (%)	Significance level	O.R	CI (95%)
CC ^a	47 (67%)	28 (93.3%)			
CT	18(26%)	0 (0%)	0.0328	22.20	1.287to 382.71
TT	5 (7%)	2 (6.7%)	0.64	1.48	0.270 to 8.19
Total No.	70	30			
Allele	Frequency	Frequency			
C	0.8	0.93			
T	0.2	0.07	0.0250	3.50	1.1701 to 10.4

P ≤ 0.05 ; OR=(95%CI); ^a reference

There are several single nucleotide polymorphisms (SNPs) identified in the CAT gene, of which the rs1001179 polymorphism (C262T) was the most extensively studied [12,13]. Recently, a series of studies has demonstrated the associations between the CAT C262T polymorphism and risk for multiple cancers, such as breast cancer [14].

245 CAT SNPs have been identified, with most studies investigating the relationships between multiple diseases and rs1001179, a C > T substitution at position -262 from the transcription start site [15]. Previous studies indicated that CAT C262T gene polymorphism had an influence on transcription factors binding thus altering the basal transcription and consequent expression of this enzyme and hence influenced the oxidative status of cells and its microenvironment. Consequently, this polymorphism was believed to play a key role in the pathogenesis of cancer [16,17]. The growing studies investigated the relation of CAT C262T gene polymorphism to breast cancer, lung cancer, diabetic neuropathy, non-Hodgkin lymphoma, liver cancer and colorectal cancer [18]. Based on current accessible evidences, the individuals who carry the TT homozygote have 17% increased risk of cancer compared with the C allele carriers, revealing that the CAT C262T gene polymorphism may be a risk factor for cancer [19].

For tumor origin could influence the results from meta-analysis, we performed subgroup analyses by cancer type. However, we did not find any positive relationship in the studies of breast cancer, head and neck cancer, hematological malignancies, digestive system cancer or brain cancer. Interestingly, the significant association between the CAT C262T gene and prostate cancer [20,21], was the opposite in most genetic models.

There was association of CAT2 with gastric cancer and colorectal cancer [22]. 'T' allele of CAT C262T showed a protective effect against the risk of ovarian cancer [OR = 0.4 (95% CI 0.25–0.6), p value <.001], The present study reported a significant association between the distribution of CAT C262T gene polymorphism and ovarian cancer for the first time in a sample of the Iranian population [23]. The effect of catalase C262T gene polymorphism in susceptibility to ovarian cancer in Kermanshah province, Western Iran [23].

The correlation between CAT genotypes and activity in Control and Case Groups.

Analysis of allelic variants of the CAT gene and measurements of CAT activities can give a more complete view of CAT status, because they reflect the underlying genetic backgrounds and environmental effects of CAT. Our data showed that CAT activity in control was higher than its activity in case with breast cancer, there were no significantly differences in CAT activity between all allelic variants of the CAT gene in the Control and Case Groups (table.2).

Table 2. CAT genotypes and activity in Control and Case Groups.

Group	Genotype of rs1001179	CAT activity U\ml (Mean ± SD)
Patients	TT	10.9752±3.61 a
	CT	9.0734±4.105 a
	CC	9.8813±6.572 a
Control	TT	11.1265±3.98 a
	CC	17.1963±8.50 a

Other study showed that the TT variant had significantly lower CAT activity than CC and CT variants in our study group [24]. One study reported that CAT levels were significantly higher in donors carrying the T allele in comparison to donors homozygous for the C allele ($p < 0.03$) [25]. In contrast, another study showed that CAT TT homozygotes had significantly lower CAT activity than did CT and CC genotypes in Caucasians and African Americans [26].

Allelic variants of CAT gene may contribute to lower CAT enzymatic activity and higher sensitivity to ROS, and alter ROS detoxification and increase oxidative stress, thereby implicating oxidative DNA damage and modulating disease risk [27].

However, Meta-analysis by Wang et al., [28], reported no positive relationship between CAT2 polymorphism risk of breast cancer, head and neck cancer, haematological malignancies, digestive system cancer and brain cancer, however there was a significant association between the CAT C262T gene and prostate cancer.

The first study implying the possible association of CAT-262C/T polymorphism with juvenile idiopathic arthritis, suggested that the potential protective effect of the CC genotype, with regard to CAT activity and treatment outcome, the TT genotype (homozygous) was associated with a 4.36-fold higher likelihood of having juvenile idiopathic arthritis as compared to the CC genotype (wild-type) [29].

Conclusion

Based on the present statistical analysis, our study shows significant association of catalase C262T gene with the development of breast cancer, and no association between catalase C262T genotypes and catalase activity.

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