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Association of catalase gene polymorphism (rs1001179) with the enzyme activities and breast Cancer Susceptibility in a sample of Iraqi women in Babylon City

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Abstract: Background: The development of breast cancer is influenced by variations in the antioxidant enzymes. An essential part of the body's fundamental antioxidant defense mechanism is catalase. Numerous studies indicate that the catalase gene polymorphism rs1001179 acting a critical role in the genesis of cancer.

Objective: In order to determine how the rs1001179 polymorphism affects catalase (CAT) activity and the progress of breast cancer (BC) in individuals with BC, this study was designed.

Methods: A spectrophotometric test was used to measure the amount of CAT enzymatic activity in serum samples. Following the extraction of genomic DNA from the blood samples, we used restriction fragment length polymorphism, polymerase chain reaction, and electrophoresis to evaluate the presence of single nucleotide polymorphism (SNP) rs1001179.

Results: According to the findings, the BC group had lower CAT activity (10.605±8.490 U/ml) than the control group (16.895±8.100 U/ml). The homozygous mutants (TT) and heterozygous carriers (CT) were fewer likely to acquire BC, with odd ratios of 1.48 and 22.2, with P value 0.64 and 0.032 for the two genotypes, respectively. Between the study groups, there was a significant difference in the incidence of the T allele (P value 0.025 and OR 3.5). The wild-type CC genotype and C allele exhibited higher CAT activity than the mutant (TT and CT) and the T allele within the patient group. The results of this study propose that the CAT (rs1001179) polymorphism may contribute to the hereditary risk of BC and might be utilized as a possible tumor susceptibility marker.

Conclusion: The rs1001179 SNP increased reactive oxygen species, particularly hydrogen peroxide, via decreasing CAT enzyme activity. The key factor that contributes to the development and spread of cancer is DNA damage, which can result from this buildup.

Keywords: *Breast Cancer; Catalase; Catalase activity; ROS; Oxidative Stress.*

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Introduction

Malignant neoplasms currently rank as the leading global cause of mortality, presenting a major public health crisis with life-threatening implications. These aggressive growths contribute substantially to worldwide death rates, underscoring their status as a critical medical and societal burden.[1] According to biology and epidemiological research, carcinogenesis is a multifaceted, intricate process that arises from the interplay of hereditary and environmental variables.[2] Among female malignancies, breast carcinoma represents the predominant oncological diagnosis, comprising roughly one-third of all cancer cases affecting women globally. Epidemiological data indicate that this illness is the primary cause of cancer incidence in women. It is the second leading cause of mortality in the medical sector, behind lung cancer.[3] An imbalance between antioxidant defense systems and reactive oxygen species can lead to oxidative stress, which has been related to the development of a range of chronic and metabolic illnesses, including cancer .[4]

ROS can activate proto-oncogenes, block tumor suppressor genes, induce chromosomal aberrations, and cause substantial DNA damage .[5,6] Nevertheless, an antioxidant defense-related enzyme, such as catalase (CAT), myeloperoxidase, glutathione peroxidase, or superoxide dismutase, may lessen the negative effects of ROS .[7, 8]

Catalase (CAT) serves as a crucial enzymatic antioxidant responsible for the dismutation of hydrogen peroxide (H2O2) into molecular oxygen and water, effectively neutralizing reactive oxygen species (ROS)-mediated oxidative damage .[9] The CAT gene, spanning approximately 34 kb on chromosomal region 11p13, exhibits a genomic architecture of 13 protein-coding exons

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interspersed with 12 non-coding intronic sequences .[10] Extensive genetic variation studies have revealed multiple single nucleotide polymorphisms (SNPs) distributed throughout this locus, with potential functional implications for enzyme activity .[11, 12] The CAT gene's C262T polymorphism (rs1001179) is particularly noteworthy since it is situated close to the transcription promoter region. Furthermore, studies indicate that it is relevant to the transcription factor binding site .[13] The CAT SNP's mutant allele (T) (rs1001179) has been linked to reduced enzyme activity, which raises ROS levels and may be a factor in the progress or spread of cancer, in contrast to the variant C allele .[14] The CAT rs1001179 polymorphism has been associated in recent studies with a higher risk of BC and other malignancies.[15,16] The purpose of the current study was to assess how the CAT SNP (rs1001179) genotype affected the CAT activity and susceptibility to BC in Iraqi patients.

Materials and Methods

Study Design and Ethical Approval

The present study involved a case-control study of 100 women. The ages of BC patients are distributed between 26 and 80 years, from Merjan University Hospital in Iraq. Additionally, the ages of the control group ranged from 20 and 71 years. Between September 2021 and January 2022, the sample was collected, and each participant gave their written consent.

Participants

- **Patient Population**: This study comprised 70 breast cancer patients.
- **Control Group**: This study included 30 women who appeared to be healthy and free of chronic conditions, chosen from a list of known volunteers. **Inclusion Criteria:** This study covered cases that had usual symptoms or underwent early detection screening with ultrasound.
- **Exclusion criteria:** Women who had previously had chemotherapy or had benign tumors were excluded.

Blood Samples

Every individual donated 4 milliliters of venous blood. For molecular analysis, the samples were separated into two parts. The first part, which included 2 milliliters, was meticulously collected in tubes containing EDTA. However, the serum was separated using the second part (2 ml) by centrifuging it for 15 minutes at 3,000 rpm. Until it was required, the serum was then kept in Eppendorf tubes at -20 ℃ .[17, 18]

CAT assay

Catalase enzymatic activity in serum samples was quantified through a spectrophotometric assay employing hydrogen peroxide as the primary substrate, where the reaction product forms a stable molybdate complex. Concurrently, glutathione concentration was determined via a colorimetric method based on the formation of a chromogenic yellow product, achieved through the interaction of acid-soluble thiol groups with 5,5' -dithiobis-(2-nitrobenzoic acid) (DTNB), following established protocols. [19]

DNA Extraction and Genotyping

Whole blood specimens were collected in EDTA-coated vacutainers to prevent coagulation, with subsequent genomic DNA isolation performed using the Genaid Genomic DNA Mini Kit (Blood/Cultured Cell; Taiwan) according to the supplier's standardized protocol. DNA purity and concentration were determined spectrophotometrically through dual-wavelength optical density measurements at 260 nm and 280 nm using an Implen NP80 NanoPhotometer (Germany). The biotechnology Laboratory at the University of Babylon's College of Science (Iraq) served as the site for all molecular biology procedures. Isolated DNA aliquots were preserved at -20°C in temperature-controlled environments to ensure nucleic acid stability for downstream molecular applications [20]. The CAT gene-specific oligonucleotide primers were computationally designed using reference sequences obtained from the NCBI genomic database. Commercial synthesis of each primer pair was carried out by Macrogen, Inc. (Seoul, South Korea). The forward and reverse primer sequences are 5'-AGAGCCTCGCCCCGCCGGCCCG-3' and 5'-TAAGAGCTGAGAAAGCATAGCT-3', respectively. Using a Biometra heat cycler (Germany), PCR amplification was carried out in 20 µL reaction mixtures with 12.5 µL of PCR master mix, 3 µL of genomic DNA template, 1 µL of forward and reverse primers, and 2.5 µL of nuclease-free water. The conditions were as follows: initial denaturation at 94°C for 2 min; 30 cycles of denaturation (94°C, 30 sec), annealing (52°C, 1 min), and extension (72°C, 1 min); then final elongation at 72°C for 5 min. In accordance with manufacturer instructions, amplification products were next digested using 2 U of SmaI restriction enzyme (Promega, USA) at 37°C for 1-4 hours. Enzyme inactivation was then accomplished by incubating at 65°C for 20 minutes.

Gel Electrophoresis Techniques

In order to prepare a 1% agarose gel, 1 g of agarose powder was suspended in 100 mL of 1× TAE buffer in a microwave-safe flask, and it was then heated for one to three minutes until it completely dissolved. Ethidium bromide was added at a final concentration of 0.2–0.5 µg/mL after chilling for 5 minutes in order to aid in the visualization of nucleic acids by intercalation.

The homogeneous solution was cast into a gel tray and allowed to polymerize at 4° C for 10-15 minutes. Following solidification, the gel was moved to an electrophoresis chamber containing 1× TAE/TBE running buffer. Electrophoresis was conducted at 75-80 V after loading the DNA molecular weight marker in the first well and experimental samples in adjacent lanes. DNA band separation patterns were subsequently documented using a Cleaver Scientific (UK) UV transillumination system .[21]

Ethical approval

The University of Babylon's local ethics committee accepted the experiment and allowed sample collection (Approval ID: 1657-SM963).

Statistical Analysis

IBM SPSS (version 23) was used to analyze the data of the current study. Sample means from the patient and healthy groups were used and compared using the dependent samples t-test. We used one-way analysis of variance (ANOVA) to compare several groups. The Hardy-Weinberg equilibrium assumption and chi-square test were used to determine the genotype distribution, allele frequency, odds ratio (OR), 95% confidence interval (CI), and p-value. One definition of statistical significance is the probability threshold ($P \leq 0.05$) .[22]

Results

In the present study, the BC group's CAT activity significantly dropped to 10.605 U/ml from 16.895 U/ml in the control group. Figure 1.

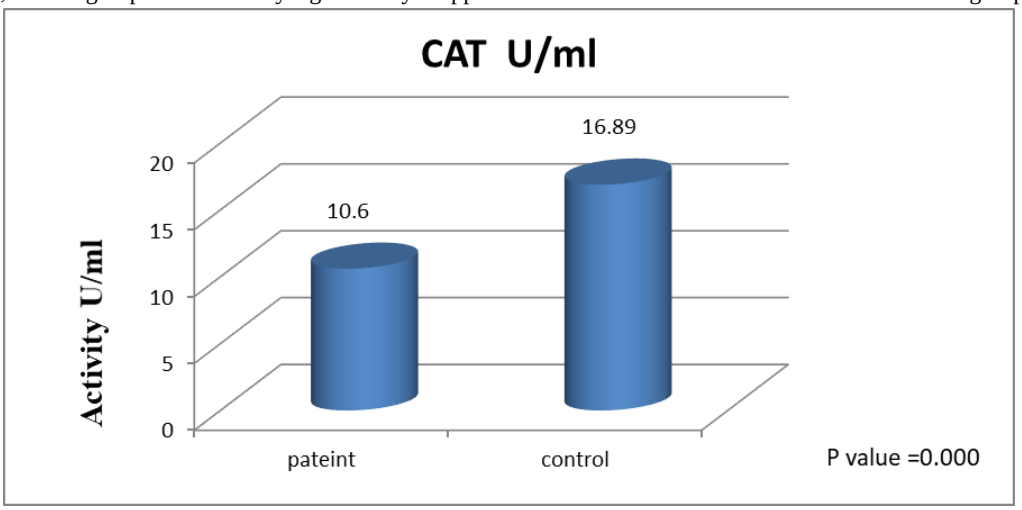

Figure 1: shows the levels of catalase (CAT) in the patient and control groups (U/ml).

As a preliminary step in CAT gene amplification, genomic DNA was isolated from whole blood specimens using standardized extraction protocols. An agarose gel polymerase chain reaction revealed the existence of a single band that matched the target sequence (185 bp). As seen in Fig. 2, three genotypes were found by restriction fragment length polymorphism (RFLP) genotyping: CT with 185, 155, and 30 bps (mutant heterozygote), TT with 185 bp (mutant homozygous), and CC with 155 and 30 bps.

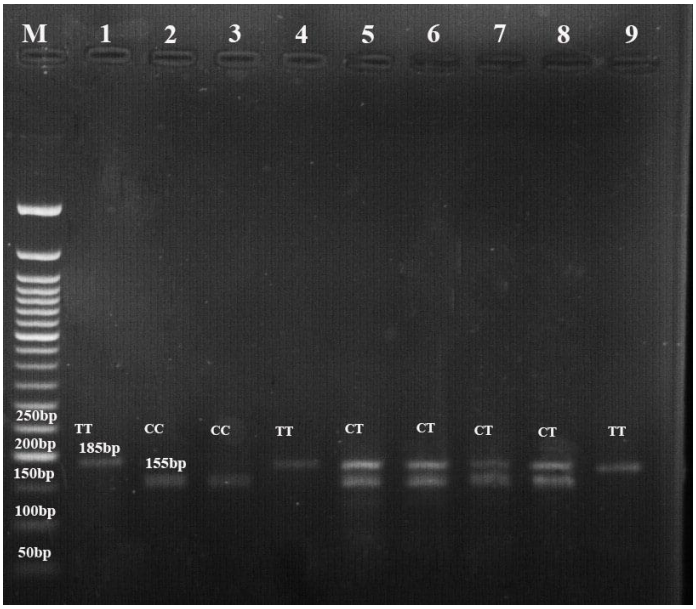

Figure 2: Shows the rs1001179 allelotyping in study groups. The lanes 2 and 3 are each CC (155 and 30 bp) ; lanes 5, 6, 7, and 8 are each CT (185, 155, and 30 bp) ;Lanes 1, 4, and 9 are each TT (185 bp). M: DNA marker (50 bp). The figure doesn't demonstrate the little band (30 bp).

With 28 participants (93%), The most prevalent genotype among healthy individuals was CC homozygote. two subjects (7%), the homozygote mutant genotype (TT), and no cases of the heterozygote genotype (CT) were found. With 47 (66%) patients, the CC homozygote was likewise the most prevalent genotype in the BC group. Next in line were TT (the mutant homozygote genotype) in 5 (7%), and the CT (mutant heterozygote genotype) in 18 (27%).

Significant relationships were found between susceptibility to BC, the T allele, and the CT genotype. According to Table 1, the T allele of rs1001179 and the CT and TT genotypes were substantially related with a higher danger of having breast cancer (odd ratio 22.2; 95% CI 1.287- 381.71; P value 0.032, 1.48, 95% CI=0.270-8.19, P value 0.64 and odd ratio 3.5, P value 0.025, 95% CI 1.17-10.46, respectively).

Table 1: shows the allele frequency and rs1001179 genotyping for the research groups.

Alleles	Patients No:70)		Controls (No: 30)		P Value	OR	95% CI
	No.	%	No	%			
Co-dominant							
CC_a	47	66	28	93			
CT	18	27	0	0	0.032	22.20*	1.287 to 381.71
TT	5	7	2	7	0.640	1.480*	0.270 to 8.190
Recessive							
CC+CT	65	93	28	93			
TT	5	7	2	7	0.931	1.070*	0.1970 to 5.8870
Dominant							
CC	47	66	28	93			
CT+TT	23	34	2	7	0.013	6.850*	1.500 to 31.287
Over-dominant							
CC+TT	52	73	30	100			
CT	18	27	0	0	0.034	21.490 *	1.250 to 369.488
Alleles							
C	112	80	56	93			
T	28	20	4	7	0.025	3.5	1.170 to 10.469

OR: Odd ratio; P≤0.05; (95% CI); $_a$: reference

In the group of patients with breast cancer, the CAT activity study showed that the CAT activity of the persons with the CT and TT (mutant genotypes) was considerably lower than that of the individuals with the CC genotype. The CAT activity of the T allele was also significantly lower than that of the C allele as shown in Table 2.

Table 2: BC patients' catalase activity according on genotypes for the CAT gene (rs1001179)

Pattern of genotypes	The activity of Catalase (u/ml) Mean ± SD	P value
CT Vs. CC	7.45±2.18 Vs10.84±6.20	0.002*
TT Vs. CC	7.97±2.13 Vs 10.84±6.20	0.048*
TT Vs. CT	7.97±2.13 Vs 7.45±2.180	0.644
T allele Vs. C allele	7.75±2.15 Vs 9.90±5.590	0.011*

Discussion

It is the first study to show that the rs1001179 CAT gene variants are associated with BC in a sample of the Iraqi population. The rs1001179 SNP genotypes CT and TT were shown to have 1.48 and 22.20 higher odds of developing BC , respectively, compared to CC (wild-type). Additionally, a 3.5 fold higher danger of BC was associated with the T allele than with the C allele.

The generation of ROS constitutes an essential outcome of anaerobic metabolic activities .[23] To counteract the deleterious effects of ROS accumulation, biological systems have evolved sophisticated antioxidant defense mechanisms .[24] Catalase (CAT), a ubiquitous heme-containing antioxidant enzyme, demonstrates particularly high expression levels in renal, hepatic, and erythrocytic tissues .[25] This critical enzyme mediates the detoxification of hydrogen peroxide (H2O2) through its dismutation into molecular oxygen and water, thereby preventing oxidative cellular damage .[26] Genetic polymorphisms in the CAT gene may compromise enzymatic activity, potentially leading to impaired ROS neutralization, increased oxidative DNA damage, and heightened disease susceptibility .[27] Current genomic research has catalogued approximately 245 SNPs in the CAT gene, with significant scientific attention directed toward the rs1001179 variant in the transcriptional regulatory region due to its potential disease associations .[25]

Recent studies' findings demonstrated that CAT enzyme gene polymorphisms affect transcription and, in turn, gene expression. The oxidative status of the cells and their environment are subsequently impacted by this change. As a consequence, this variation plays a key role in cancer development. [28, 29] The current investigation found a substantial drop in CAT activity among BC patients compared to the healthy group and a decrease in CAT activity in BC patients with CT and TT (mutant genotypes) . Numerous organs, including the breast, lung, liver, and colon regions, have been the subject of research on the link between the polymorphism of *CAT* rs1001179 gene and cancer .[24] 35 case-control studies were meta-analyzed, and the results showed a correlation between CAT polymorphisms and cancer risk. [30] In particular, the C262T polymorphism in the CAT gene may be a risk factor for cancer. According to reports, those with TT

homozygosity had a 17% higher chance of getting cancer than people with the C allele .[31]

Although surveys have indicated that the *CAT* gene polymorphism increases the risk of prostate and cervical cancers [10,35], some investigations have indicated that it may serve as a protective factor in lowering the risk of hepatocellular carcinoma .[33,34] Other investigations have found no association between the polymorphism and the risk of BC [16] or hepatocellular carcinoma .[32] According to the scientific underpinnings of epidemiological research, the CT and TT genotypes are connected to lower CAT activity, whereas the CAT (rs1001179) genotype is linked to the risk of disorders related to oxidative stress .[36]

Reduced CAT activity and increased generation of oxygen-free radicals support the oxidative stress theory in breast cancer .[37] Oral squamous cell carcinoma , prostate cancer, adenocarcinoma, and colorectal cancer are among the malignancies for which several studies have shown a considerable decrease in CAT activity .[38, 39]

"The relatively small sample size is an important limitation of the current study. Although our findings revealed statistically significant relationships among the CAT rs1001179 polymorphism and both enzyme activity and breast cancer susceptibility, a bigger cohort might improve the results' statistical power and adaptability to the greater Iraqi population. To validate these relationships, determine their exact effect sizes, and offer a more thorough knowledge of the CAT rs1001179 polymorphism's function in breast cancer etiology within this area, future research with larger participant numbers—particularly in the control group—is essential.

Conclusion

Because the CAT (rs1001179) polymorphism is found in promoter region and affects both DNA transcription and mRNA levels, the results of this study provide evidence that it lowers the activity of the CAT enzyme. One of the mechanisms influencing the beginning and progression of cancer is DNA damage, which may be caused by ROS, particularly H2O2, which rises as a result of this decrease in CAT activity.

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