

Association between breast cancer susceptibility and variation in the catalase gene in women from Babylon, Iraq

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Abstract: Catalase represents a critical enzyme in the organism's defense system against oxidative stress, a physiological state implicated in the pathogenesis of various cancers. As a primary antioxidant defense enzyme, catalase plays a vital role in mitigating oxidative damage. Substantial evidence from numerous studies indicates that genetic polymorphisms in the CAT gene are significant in cancer etiology. This investigation aimed to elucidate the influence of the CAT gene polymorphism (rs7943316) on breast cancer susceptibility. Genomic DNA was isolated from blood samples of breast cancer patients and a control cohort. The specified single-nucleotide polymorphism (SNP) was analyzed using polymerase chain reaction (PCR) coupled with restriction fragment length polymorphism (RFLP) analysis. Genotype distribution in the control group was as follows: the heterozygous A/T genotype was most prevalent (55 %), followed by the homozygous T/T genotype (36.7%), while the homozygous A/A genotype was not observed (8.3 %). In the breast cancer (BC) patient group, the A/T genotype was also the most frequent (51.7%), followed by T/T (31.7%) and A/A (16.6%). Analysis revealed that individuals carrying the A/T and T/T genotypes exhibited an elevated odds ratio for breast cancer development (OR = 2.129, P = 0.209 and OR = 2.315, P = 0.183, respectively). However, these associations were not statistically significant. Furthermore, comparative allele frequency analysis showed no significant difference in the distribution of the T allele of the CAT gene (rs7943316) between the breast cancer patients and the control group (P = 0.290). In conclusion, the findings of this study indicate that the development of breast cancer is not associated with polymorphisms of the CAT gene at the rs7943316 locus.

Keywords: rs7943316; SNP promoter; Breast Cancer Susceptibility; Oxidative Stress.

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Introduction

Breast cancer (BC) is the most common cancer in women worldwide, making up almost one-third of all malignant tumors in women. It is also the second most common cause of cancer-related death worldwide, behind lung cancer. The etiology of BC is complex, but accumulating evidence highlights the critical involvement of cellular homeostasis disruption (1-3).

A fundamental mechanism widely implicated in carcinogenesis is oxidative stress. This state, arising from an imbalance between pro-oxidant and antioxidant systems, promotes oncogenesis through the generation of DNA damage and the modulation of intracellular signaling pathways by Reactive Oxygen Species (ROS). Elevated levels of ROS are known to be instrumental in both the initiation and maintenance of oncogenic characteristics within the cellular environment (4-6).

To counteract the damaging consequences of elevated ROS levels, cells rely on a sophisticated antioxidant defense mechanism. This system is underpinned by essential enzymes, including myeloperoxidase (MPO), glutathione peroxidase (GPx), mitochondrial manganese superoxide dismutase (MnSOD), and the critical enzyme catalase. CAT functions as a principal antioxidant enzyme, efficiently catalyzing the dismutation of hydrogen peroxide (H₂O₂) into molecular oxygen (O₂) and water (H₂O), thereby neutralizing a primary source of oxidative damage (7,8). The gene encoding CAT is situated on chromosome 11p13, and its structure includes 13 exons and 12 introns. Given its role, CAT is a major enzyme activated in response to H₂O₂-specific oxidative challenge (9-11).

Genetic variability, particularly through Single Nucleotide Polymorphisms (SNPs), in antioxidant defense genes may significantly modulate cancer susceptibility. Previous research has identified several SNPs within the CAT gene that may be relevant to breast cancer pathogenesis. Of particular interest is the A-21T polymorphism (rs7943316) (Gene ID: 847) located in the promoter region of the CAT gene. This specific variant is functionally significant due to its proximity to the transcriptional promoter site and transcription factor binding elements. It is hypothesized that the A-21T polymorphism may impair the gene's regulatory capacity, potentially diminishing antioxidant stress activity and consequently increasing susceptibility to disease. While CAT is one of the most important enzymes in intracellular detoxification, its gene polymorphisms require further validation before routine clinical application (12-15).

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To elucidate population-specific genetic risk factors for breast carcinogenesis, this study was designed to examine the association between CAT gene polymorphisms (rs7943316) and disease susceptibility. Utilizing a case-control methodology and genotyping analysis in a cohort of Iraqi women, this research aims to provide insights into the genetic underpinnings of breast cancer development in this demographic.

Materials and Methods

Study Design and Population

This investigation employed a hospital-based case-control design to evaluate the potential association between the single nucleotide polymorphism (SNP) CAT (rs7943316) and susceptibility to breast cancer (BC) in the Iraqi female population. The study cohort was comprised of 60 female patients with newly diagnosed, histopathologically confirmed primary breast carcinoma (the Case Group), recruited from a major oncology center in Iraq (Hillah, Babylon). The Control Group consisted 60 apparently healthy, unrelated women matched for age and geographical residence, with no personal or family history of any malignant disease. The BC group's ages ranged from 20 to 80, whereas the control group consisted of healthy women between the ages of 20 and 75. January 2024 to the September 2024 was the research period. All participants provided confirmation of their Iraqi descent. Comprehensive demographic and clinical data were systematically collected from all enrolled subjects.

Ethical Compliance and Sample Collection

The University of Babylon's (Hillah, Babylon, Iraq) Ethics Committee approved the research protocol (Approval number: DSM-5324). All participants gave written informed consent prior to inclusion. A volume of approximately 5 mL of peripheral venous blood was drawn from each participant using aseptic techniques. The samples were collected into vacutainer tubes containing EDTA anticoagulant. These specimens were then immediately processed for molecular analysis.

Genomic DNA Extraction and Quality Control

Genomic DNA was isolated from the whole blood samples using a standard commercial purification kit (e.g., a silica-membrane-based kit), strictly following the manufacturers established protocol. The quantity and purity of the extracted DNA were subsequently assessed via spectrophotometry at the 260 and 280 nm wavelengths. Only DNA samples exhibiting an optimal purity ratio (A260/A280) between 1.7 and 1.9 were considered

acceptable for downstream polymerase chain reaction (PCR) and were stored at -20°C (16,17).

Genotyping of the CAT (rs7943316) Polymorphism

The CAT gene polymorphism (rs7943316), located within the promoter region, was genotyped using a PCR-RFLP assay. A specific genomic region encompassing the rs7943316 locus was amplified. The amplification was performed using the following primer pair, which was designed and synthesized by Macrogen Co. (Korea): forward primer, 5'-AAT CAG AAG GCA GTC CTC CC-3'; reverse primer, 5'-TCG GGG AGC ACA GAG TGT AC-3'. The PCR was conducted in a final reaction volume of 20 μL , containing 3 μL of template DNA, 1 μL of primers, 12.5 μL of master mix, and 2.5 μL of DNase-free water. The thermal cycling conditions comprised an initial denaturation step at 95°C for 3 minutes; followed by 30 cycles of denaturation at 94°C for 30 seconds, annealing at 60°C for 30 seconds, and extension at 72°C for 30 seconds; with a final extension at 72°C for 5 minutes. Subsequently, the amplified PCR products were digested with the restriction endonuclease *HinfI* (Promega, Madison, WI, USA). To establish the optimal digestion conditions, aliquots of the PCR product were incubated with 2 units of the enzyme at 37°C for durations ranging from one to four hours. The enzymatic reaction was terminated by heat inactivation at 65°C for 20 minutes (18). The resulting restriction fragments were resolved by agarose gel electrophoresis and visualized under ultraviolet light following staining.

Statistical Analysis

SPSS (Version 23) software was used for all statistical analyses. The chi-square (χ^2) test was used to assess the genotype distribution within the control group for divergence from Hardy-Weinberg Equilibrium (HWE). Via the χ^2 test, genotype and allele frequencies for the rs7943316 polymorphism were also compared between the groups of breast cancer patients and controls. By computing odds ratios (ORs) with matching 95% confidence intervals (CIs), the relationship between this genetic variation and breast cancer risk was measured. A priori, statistical significance was indicated by a threshold of $P < 0.05$ (19).

Results

Genotyping of the CAT (rs7943316) Gene Polymorphism

The PCR-RFLP analysis of the CAT (rs7943316) promoter polymorphism was successfully conducted. PCR amplification of the target region yielded a single band of 249 bp (Figure 1).

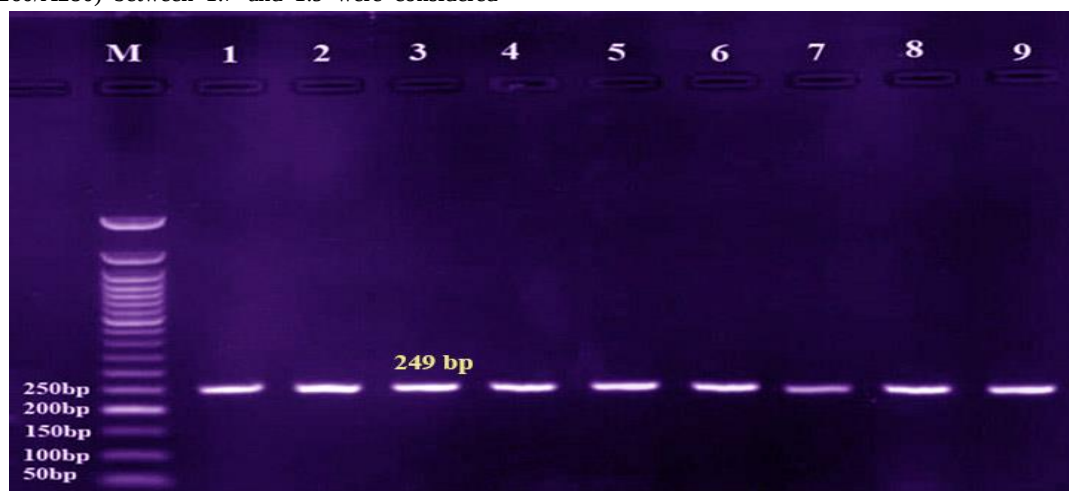


Figure 1. *Hinf*I-digested PCR product of the CAT gene (rs7943316); Lanes 1–7: patient samples; Lanes 8–9: control group samples. M: DNA marker.

Subsequent digestion with the *Hinf*I restriction enzyme (5'-G $\wedge$ ANTC-3' restriction site) revealed three distinct genotype

patterns: TT Homozygote (Mutant) that Characterized by a single undigested band of 249 bp, AT Heterozygote displayed three bands of 249 bp, 175 bp, and 74 bp, and AA Homozygote (Wild Type) showed two digested fragments of 175 bp and 74 bp (Figure 2).

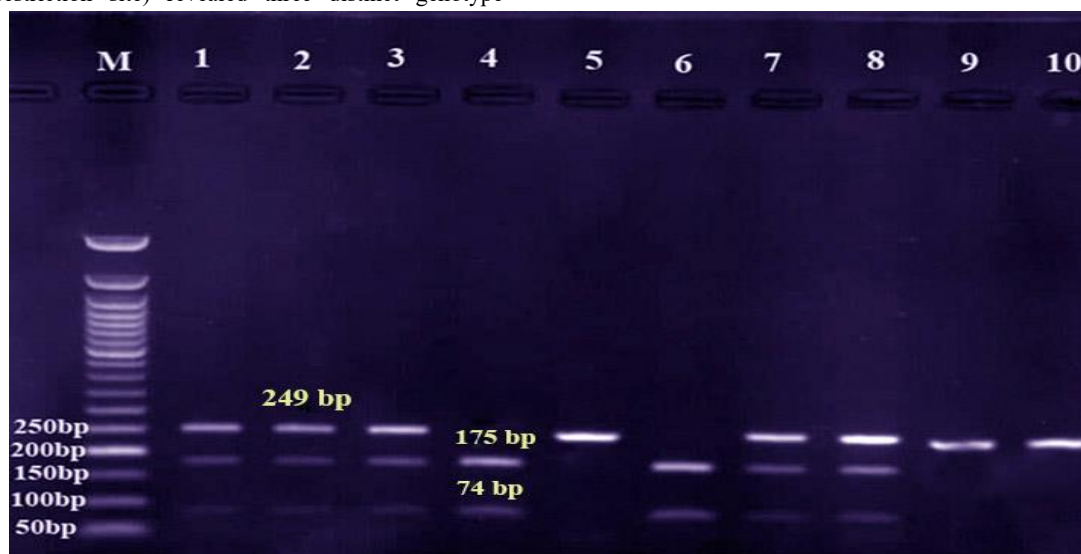


Figure 2. Allelotyping findings for the CAT gene (rs7943316) using RFLP. M: A marker for DNA. AT heterozygous allele (3 bands at 249, 175, 74 bp) is found in lanes 1-3 and 7-8. Lanes 5, 9, and 10: TT homozygous allele (single band at 249 bp). Lanes 4 and 6: AA homozygous allele (two bands at 175 and 74 bp).

Distribution and Association of CAT (rs7943316) Genotypes with Breast Cancer Risk

The investigation analyzed the distribution of the Catalase (CAT) gene promoter polymorphism A-21T (rs7943316) in a case-control cohort comprising 60 BC patients and 60 healthy control subjects (Table 1).

In the Control Group, the heterozygous AT genotype was the most prevalent, observed in 55.0% (n=33) of the subjects. This was followed by the homozygous mutant TT genotype (36.7%, n=22). The homozygous wild-type AA genotype was the least

frequent, detected in 8.3% (n=5) of controls. In the BC Patient Group, the AT genotype also showed the highest frequency (51.7%, n=31). The TT genotype followed with 31.7% (n=19), and the AA genotype was found in 16.6% (n=10) of patients. The analysis indicated that individual carriers of the A/T heterozygous genotype and the T/T homozygous mutant genotype were numerically more expected to develop BC, as evidenced by Odds Ratios greater than 1.0. Despite the OR values suggesting a potential increase in risk for the variant-carrying genotypes, the P-values (P=0.209 and P=0.183) are above the standard threshold of 0.05, indicating that the differences in genotype frequencies between the case and control groups were not statistically significant. Furthermore, the overall allelic association showed that the frequency of the T allele did not differ significantly between the BC patients and the healthy controls, yielding an OR of 0.755 (P=0.290).

Table .1: Frequency of CAT (rs7943316) Genotypes among BC Patients and Healthy Controls

Genotype allele	Patient group		Control group		P-value	OR	CI (95%)
	No.	%	No	%			
AA ^a	10	16.6	5	8.3			
AT	31	51.7	33	55	0.209	2.129	0.654 - 6.929
TT	19	31.7	22	36.7	0.183	2.315	0.672 - 7.975
Total	60		60				
A	51	42.5	43	35.8			
T	69	57.5	77	64.2	0.290	0.755	0.4493 - 1.2706

^a reference.

Discussion

The present study was undertaken to evaluate the role of a SNP in the promoter region of the CAT gene, rs7943316, in modifying breast cancer (BC) risk among Iraqi women. This research is predicated on the well-established role of oxidative

stress (OS) in carcinogenesis, where the accumulation of Reactive Oxygen Species (ROS), a byproduct of aerobic metabolism (21), drives cellular damage and transformation. CAT is a crucial antioxidant enzyme, primarily responsible for the efficient detoxification of hydrogen peroxide (H₂O₂) into water (H₂O) and oxygen (O₂) (22, 23). Therefore, genetic polymorphisms

that potentially impair CAT activity may elevate OS and consequently increase BC risk (24).

The rs7943316 polymorphism, one of the three commonly reported variants in the CAT promoter region (along with C-262T (rs1001179) and C-844T (rs769214)) (25–28), is hypothesized to influence CAT gene expression by altering the binding affinity of transcriptional factors (29,30).

The central finding of this investigation indicates the absence of a statistically significant association between the CAT rs7943316 genotypes and the risk of BC within this Iraqi population cohort ($P > 0.05$ for all comparisons). Specifically, the observed Odds Ratios (OR) for the variant-containing genotypes (A/T and T/T) were OR=2.129 and OR=2.315, respectively. These OR values, being greater than 1.0, suggest a potential, albeit non-significant, increased risk of BC associated with carrying the mutant T allele when compared to the wild-type AA genotype.

This study is explore the link between the CAT (rs7943316) polymorphism and BC susceptibility in the Iraqi population. The insignificant finding aligns with a limited number of prior studies that also reported a lack of association between rs7943316 and the risk of other malignancies, such as papillary thyroid carcinoma (31) or hepatocellular carcinoma (33). Conversely, many researchers have suggested that other CAT polymorphisms are associated with the risk of various cancers, including cervical, prostate, pancreatic, and colorectal cancers (32–35), highlighting the complex, site-specific, and population-dependent nature of these genetic associations.

The non-significant result may be attributed to the population-specific effect, where the polymorphism's impact is negligible in the Iraqi genetic background; or a limitation in statistical power to detect a subtle association, which may necessitate a larger sample size. In summary, while the OR results hint at a possible detrimental role for the T allele in increasing BC risk (due to OR>1 for the A/T and T/T genotypes compared to the AA reference), this association requires further validation in larger cohorts, as the current findings lack statistical power to establish a significant link.

Conclusion

The study found no statistically significant correlation between the CAT (rs7943316) polymorphism and the risk of Breast Cancer (BC) in the female cohort examined ($P > 0.05$). The variant genotypes (A/T and T/T) showed Odds Ratios (OR=2.129 and OR=2.315) greater than 1.0, suggesting a potential (but statistically insignificant) increased risk associated with the T allele. The rs7943316 polymorphism is an insignificant predictor of BC risk in this group. Larger sample sizes and functional studies are required to confirm the true role of this genetic variant in BC development.

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