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# Association Between BARD1 Polymorphisms, Immunological and Hormonal Markers, and Breast Cancer Risk in Iraqi Women

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ABSTRACT

**Background:** Breast cancer is a leading cause of cancer-related mortality worldwide. Genetic factors, including polymorphisms in DNA repair genes such as BARD1, may influence susceptibility. Inflammatory and tumor markers also play a role in cancer progression. This study aimed to investigate the association between BARD1 exon mutations, immunological and hormonal markers, and breast cancer risk in Iraqi women.

**Methods:** This case-control study comprised 100 patients with early-onset breast cancer and 100 healthy controls, frequency-matched for age and Body Mass Index (BMI). Serum levels of BARD1, MUC-1, CEA, CA15-3, estrogen, progesterone, prolactin, IL-1β, and TNF-α were measured using ELISA. Five BARD1 SNPs were genotyped using direct sequencing, and their association with breast cancer risk was assessed using logistic regression. The discriminative potential of the biomarkers was evaluated using Receiver Operating Characteristic (ROC) curve analysis.

**Results:** Significantly elevated levels of IL-1β, TNF-α, CEA, BARD1, and MUC-1 were observed in the patients ($p < 0.0001$). ROC analysis showed discriminative potential for IL-1β (AUC = 0.88, 95% CI: 0.83–0.94), CEA (AUC = 0.78, 95% CI: 0.70–0.86), BARD1 (AUC = 0.77, 95% CI: 0.69–0.85), and MUC-1 (AUC = 0.73, 95% CI: 0.65–0.81). Three SNPs (rs2106145710, rs1695783243, and rs1574847014) were associated with increased breast cancer risk (rs1574847014 OR = 11.67, 95% CI: 3.5–38.8), whereas rs10498023 showed a protective effect (OR = 0.33, 95% CI: 0.22–0.51).

**Conclusion:** Elevated levels of inflammatory and tumor markers, along with specific BARD1 polymorphisms, are associated with breast cancer risk in Iraqi women. These biomarkers may serve as noninvasive diagnostic tools, and genetic screening could aid in early risk stratification.



## INTRODUCTION

Cancer cells frequently invade adjacent tissues or metastasize to distant organs via the bloodstream or the lymphatic system. Cancer can arise in several tissues and organs. The initial phase of cancer development involves genetic mutation, referred to as the "Initiation" phase. “Initiators” that induce or facilitate genetic changes include hormones, chemicals, radiation, infections and hypoxia. [1] Breast cancer is the most prevalent type of cancer in Iraq, and is regarded as an exceedingly diverse disease. The incidence of this type of cancer has increased in recent years in Iraq.[2] Cancer predominantly affects the elderly, but in recent years, there has been an inexplicable increase in cancer diagnoses among younger individuals. Numerous theories, including exposure to escalating levels of environmental carcinogens, have been suggested; however, there is a lack of definitive data to substantiate these claims. [3] There is significant inter-individual variation in the age at diagnosis among BRCA1 and BRCA2

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mutation carriers, which continues to be evident, even among relatives sharing the same mutations. [4] The fundamental function of BRCA1 in breast cancer can be ascribed to its impact on chromatin modification, thereby linking BRCA1 dysregulation to both epigenetic and genetic instability. [5]

Genetic variations that interact with BRCA1 and BRCA2 in the detection and repair of DNA damage are prime candidates for investigation as genetic modifiers of cancer risk associated with BRCA1 and BRCA2. The BRCA1-BARD1 heterodimer is crucial for BRCA1 functionality, with contacts facilitated by the ring-finger domains of both proteins. [6] BARD1 is a crucial protein that associates with BRCA1 to create a functional complex integral to DNA repair, specifically in addressing double-strand breaks, thus significantly contributing to tumor suppression and the prevention of cancer progression. Mutations in BARD1 or BRCA1 can impair protective mechanisms and facilitate carcinogenesis, as observed by BARD1 mutations in breast and ovarian malignancies. [7] BARD1 and BRCA1 collaboratively preserve genomic integrity and their functional loss may result in cancer. Although BRCA1 has been recognized in gliomas, especially as a germline mutation, BARD1 has exclusively been documented in glioblastoma cell lines. [8]

A tumor biomarker is defined as a chemical produced by a tumor or in response to a tumor. Biomarkers, including those in breast tissue, can be identified in any tissue. Therefore, they may have prognostic, diagnostic, and/or predictive significance. Current serological breast cancer markers include cancer antigen 15-3 (CA 15-3) and carcinoembryonic antigen (CEA). Despite their discovery decades ago, CA15-3 and CEA are the most widely utilized tumor markers in breast cancer management.[9] Mucin-1 (MUC-1) protein provides similar clinical information. CA 15-3 is the most widely used test to assay MUC-1, and is considered the gold standard. [10] Breast cancer is the most prevalent malignancy affecting Iraqi women, and patients with elevated serum levels of CA 15-3 are more likely to have breast cancer metastases. [11]

Hormones significantly influence the regulation of breast epithelial proliferation; breast cancer is more prevalent in women due to the continuous growth and changes in mammary glands, influenced by hormones such as estrogen and progesterone than in men (1% of cases). [12] Thus, progesterone may affect the initial phases of breast cancer progression. Similarly, estrogen may play a direct role in carcinogenesis. Estrogen metabolites can cause mutations or generate DNA-damaging free radicals in cell and animal models. It has also been proposed that variants of genes involved in estrogen synthesis and metabolism could elevate the risk of breast cancer. Such variants are analogous to cytochrome (P-450) alleles that alter the metabolism of tamoxifen in some women. [13] Prolactin and ovarian hormones influence breast formation and lactation, resulting in nourishment and increased neonatal benefits. Its major actions in mammary epithelial proliferation and differentiation suggest its involvement in breast cancer. [14]

Cytokines serve as communicators of the immune system, enabling them to orchestrate robust responses to various stressors. Cytokines can either promote or suppress inflammation, and attract or deter immune cells. [15] Over the last 30 years, the significance of cytokines in cancer-related inflammation has been well-established. The interleukin-1 (IL-1) family is one of the most well-defined families of cytokines. [16] IL-1 promotes cancer cell proliferation and invasiveness in various malignancies such as breast and colorectal cancers. The functional roles of IL-1β (IL1B) and the inhibitory effect of celastrol on IL1B expression have been investigated in triple-negative breast cancer (TNBC) cells. [17] TNF-α is an essential pro-inflammatory cytokine found in the TME of breast cancer patients and is secreted by stromal cells, mainly tumor-associated macrophages, and by the cancer cells themselves. [18] This study aimed to evaluate the role of immunological biomarkers and *BARD1* in the early detection and prognosis of breast cancer among Iraqi female patients.

## METHODS

### *Study Subjects*

A case-control study involving 200 female Iraqi participants was conducted. The case group comprised 100 women with pathologically confirmed early-onset breast cancer (aged 20–60 years, mean age: 47.92 ± 9.95 years), recruited from the Clinic for Early Detection of Breast Cancer at AL-Amal National Hospital for Cancer Management in Baghdad, Iraq, between November 2024 and February 2025. The control group consisted of 100 healthy female volunteers with no personal history of cancer, recruited from the same hospital. These individuals attended the hospital for routine health check-ups or visited non-oncology departments and were frequency-matched to cases based on 5-year age groups and Body Mass Index (BMI) categories.

Written informed consent was obtained from all the participants prior to their inclusion in the study. The study protocol, including the consent procedure and forms, was approved by the Human Ethics Committee of the College of Science of Baghdad University (Ref.: CSEC/0225/0028). All the procedures were performed in accordance with the ethical standards of the Declaration of Helsinki.

The inclusion criteria for breast cancer patients were as follows: (a) patients with early-onset breast cancer confirmed by pathological diagnosis; (b) patients who did not undergo mastectomy; (c) patients who did not receive radiotherapy or chemotherapy before surgery; and (d) patients with complete basic clinical information. The exclusion criteria were as follows: uncertain pathological diagnosis, incomplete medical records, hematological diseases, immune system diseases, or those who received any antitumor treatment before surgery.

*Samples collection*

In this study, 200 blood samples were collected from patients with BC and healthy controls from November 2024 to February 2025. All samples were divided into two 2-mL EDTA tubes for genetic analysis and a 3-mL gel tubes for immunological analysis (ELISA Test).

*Serum Biomarker Quantification via ELISA*

Serum MUC-1, CEA, CA15-3, estradiol (E2), progesterone (PROG), prolactin (PRL), IL-1β, TNF-α, and BARD1 levels were quantified using commercial sandwich enzyme-linked immunosorbent assay (ELISA) kits. All assays were performed in accordance with the manufacturer's instructions. Kits for BARD1 (SL4487Hu) and MUC-1 (cat. no. SL2212Hu), CEA (SL2426Hu), CA15-3 (SL0383Hu), E2 (SL0686Hu), PROG (SL1452Hu), TNF-α (SL1761Hu), IL-1β (SL0984Hu) were obtained from SunLong Biotech (China). The prolactin (PRL assay was conducted using a kit from ELK Biotechnology Co. (China) (Cat. ELK1224). All kits were operated based on comparable methodological principles for sandwich ELISA. To ensure assay reproducibility, all samples and internal quality controls were run in duplicates. The intra-assay and inter-assay coefficients of variation (CV%) for all biomarkers were maintained below 10% in accordance with the manufacturers' specifications and standard quality control protocols.

*DNA Polymorphism*

*DNA extraction and BARD1 gene genotyping*

One hundred patients with early-onset breast cancer (aged 20–60 years) and 100 healthy women (aged 20–60 years) were included. Exon mutations in *BARD1* were identified and examined by direct sequencing.

*Genomic DNA isolation and quantification*

Genomic DNA was isolated from peripheral blood using the standard phenol/chloroform technique [19,20] and DNA extraction was performed according to the kit manual, Whole Blood DNA MiniPrep Kit (ELK China). A Quantus Fluorometer was used to detect the concentration of the extracted DNA to determine the quality of the samples for downstream applications. To 2 µL of DNA, 198µl of diluted Quantifluor Dye was added. After 5 min of incubation at room temperature in the dark, DNA concentration values were determined. Conventional PCR was used to amplify the (268 bp) region of *BARD1* SNPs. Primers were optimized using the identical primer pair (forward and reverse) at 55, 58, 60, 63, and 65°C to identify the optimal primer annealing temperature. The primer pair for amplifying a 268 bp region of exon 2 of the BARD1 gene was designed with the forward sequence GTTGGGCCTTGGATGAAATA and reverse sequence CAATAGGTTACTTTGCAGACTTTGA. The optimal annealing temperature (Tm) for this primer set was determined to be 58°C and the primer design was based on a previous study. [21]

PCR was performed in a 25 µl reaction volume containing 12.5 µl GoTaq Green Master Mix, 5 µl nuclease-free water, 1 µl each of forward and reverse primer (10 µM), and 5.5 µl DNA template (20-29 ng). After confirming successful DNA amplification via agarose gel electrophoresis, PCR products were purified and sent to Macrogen Corporation (Korea) for Sanger sequencing on an ABI3730XL analyzer. Reference sequences for *BARD1* were obtained from NCBI. Sequencing reads were aligned to the reference, and single nucleotide polymorphisms (SNPs) were identified using Geneious software.

The PCR cycling conditions were as follows: initial denaturation at 95 °C for 5 min; 30 cycles of denaturation at 95 °C for 30 s, annealing at 60 °C for 30 s, and extension at 72 °C for 30 s; followed by a final extension at 72 °C for 7 min.

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*Statistical Analysis*

Data were analyzed using SPSS (version 26) and WinPepi software. The normality of all continuous variables (e.g., age, BMI, and biomarker levels) was assessed using the Shapiro–Wilk test; thus, data are presented as mean ± standard deviation. Comparisons of these parameters between the patient and control groups were performed using the independent sample t-test. Categorical data are expressed as numbers (percentages) and compared using the chi-square testThe potential discriminative value of the serum biomarkers was evaluated using Receiver Operating Characteristic (ROC) curve analysis.

Genotype and allele frequencies for BARD1 polymorphisms were assessed for Hardy-Weinberg equilibrium, and their associations with breast cancer risk were calculated as odds ratios (ORs) with 95% confidence intervals (CIs). Pairwise linkage disequilibrium (LD) between the five SNPs was

analyzed using the SHeSIS online platform [http://analysis.bio-x.cn]. The algorithm automatically computes both the standardized disequilibrium coefficient (D′) and correlation coefficient ($r^2$) for each SNP pair. Pearson's correlation coefficients between biomarker levels were also analyzed. Statistical significance was set at $P \leq 0.05$. To account for multiple comparisons of the five SNPs and nine biomarkers tested, Bonferroni correction was applied. A post-hoc power analysis was conducted using the G*Power software. With a sample size of 100 cases and 100 controls, the study had over 80% power to detect an odds ratio of 2.0 for genetic variants with a minor allele frequency of ≥0.20 at a significance level (α) of 0.05.

## RESULTS

The study included 100 women diagnosed with breast cancer (mean age: 47.92 ± 6.95 years) and 100 healthy women (mean age: 41.27 ± 8.91 years) with no significant differences between the groups (*p = 0 .081*). The mean BMI of the patients was not significantly higher than that of the controls (*p = 0.371*), with the majority classified as overweight or obese, as shown in Table 1.

**Table 1.** Comparison of demographic parameters under study between patients and healthy control.

| Parameters | | patients No.= 100 | Controls No.=100 | *p*-value |
|---|---|---|---|---|
| Age (years) (mean±SD) | | 47.92 ± 6.95 | 41.27 ± 8.91 | 0.081 ns. |
| BMI (kg/m²) (mean±SD) | | 29.91 ± 4.66 | 29.26 ± 5.48 | 0.371 ns. |
| Normal (<25 kg/m²) | | 15 (15%) | 20 (20%) | 0.452 ns |
| Overweight (25–29.9 kg/m²) | | 48 (48%) | 45 (45%) | |
| Obese (≥30 kg/m²) | | 37 (37%) | 35 (35%) | |
| Disease onset (days) (mean±SD) | | 9.850 ±1.358 | - | - |
| Family history | Yes | 63% | 0% | |
| | No | 37% | 100% | <0.0001 **** |
| | Yes | 14% | 0% | |
| Smoking | No | 86% | 100% | <0.0001 **** |
| Tumor Grade | Tumor grade I | 9% | - | - |
| | Tumor grade II | 91% | | |
| Tumor size | < 3 cm | 71% | - | - |
| | > 3 cm | 29% | | |

Highly significant differences (**** $p$ <0.01) and nonsignificant differences ($p$>0.05): ns.

Patients exhibited significantly elevated levels of IL-1β, TNF-α, CEA, BARD1, and MUC-1 in their serum compared to controls (*p < 0.0001*). Non-significant alterations were observed in estrogen (*p = 0.613*), progesterone (*p* = 0.055), prolactin (*p* = 0.872), and CA15-3 (*p* = 0.709). The results are presented in Table 2.

**Table 2.** Comparison of biomarkers in patients with breast cancer and healthy control

| Parameters | Patients No.= 100 | Controls No.=100 | *p*-value |
|---|---|---|---|
| IL-1β ( pg/ml) | 21.66 ± 5.973 | 11.98 ± 4.044 | <0.0001 **** |
| TNF-α (pg/ml) | 32.49 ± 6.172 | 28.22 ± 3.697 | <0.0001 **** |
| CEA (pg/ml) | 29.31 ± 7.147 | 23.12 ± 4.664 | <0.0001 **** |
| BARD1 (pg/ml) | 15.49 ± 5.276 | 10.63 ± 3.794 | <0.0001 **** |
| MUC-1 (ng/ml) | 0.9856 ± 0.3202 | 0.7354 ± 0.1949 | <0.0001 **** |
| Progesterone (ng/ml) | 4.186 ± 0.6670 | 2.547 ± 0.5294 | 0.055 ns. |
| Estrogen (pg/ml) | 92.73 ± 10.18 | 86.88 ± 5.448 | 0.613 ns. |
| Prolactin (ng/ml) | 4.834 ± 1.931 | 4.879 ± 2.036 | 0.872 ns. |
| CA15-3 (U/ml) | 7.932 ± 3.299 | 8.108 ± 3.353 | 0.709 ns. |

An independent sample t-test was used to assess Significant differences (**** $p$ <0.01) and non-significant differences ($p$ > 0.05). ns.

ROC curve analysis was used to assess the diagnostic potential of biomarkers. IL-1β demonstrated outstanding performance with an AUC of 0.88, showing 85% sensitivity and specificity. A strong diagnostic utility was also observed for TNF-α (AUC=0.75), CEA (AUC=0.77), BARD1 (AUC=0.77), and MUC-1 (AUC=0.73). Progesterone showed statistical significance, but with modest accuracy (AUC=0.66). In contrast, estrogen, prolactin, and CA15-3 did not demonstrate a significant diagnostic value in distinguishing patients from controls (Table 3).

**Table 3.** Potential discriminative value of Serum Biomarkers for Breast Cancer Based on ROC Curve Analysis

| Biomarker | AUC (95% CI) | p-value | Cut-off Value | Sensitivity (%) | Specificity (%) |
|---|---|---|---|---|---|
| IL-1β | 0.88 (0.83 – 0.93) | < 0.0001 | 15.19 pg/ml | 85 | 85 |
| TNF-α | 0.75 (0.68 – 0.83) | < 0.0001 | 29.34 pg/ml | 74 | 74 |
| CEA | 0.77 (0.70 – 0.84) | < 0.0001 | 24.29 pg/ml | 74 | 85 |
| BARD1 | 0.77 (0.70 – 0.84) | < 0.0001 | 10.63 pg/ml | 81 | 81 |
| MUC-1 | 0.73 (0.6629 – 0.79) | < 0.0001 | 0.795 ng/ml | 65 | 65 |
| Progesterone | 0.66 (0.58 – 0.74) | < 0.0001 | 0.207 ng/ml | 64 | 64 |
| Estrogen | 0.56 (0.48 – 0.64) | 0.103 | - | - | - |
| Prolactin | 0.51 (0.42 – 0.59) | 0.739 | - | - | - |
| CA15-3 | 0.506 (0.42 – 0.58) | 0.883 | - | - | - |

AUC, Area Under the Curve; CI, Confidence Interval.

Pearson's correlation coefficient analysis revealed several significant relationships between the studied parameters in women with breast cancer (Figure 1). Positive significant correlations included: IL-1β to BARD1 ($r = 0.376$, $p < 0.001$) and MUC-1 ($r = 0.495$, $p < 0.001$); TNF-α to CEA ($r = 0.644$, $p < 0.001$), BARD1 ($r = 0.686$, $p < 0.001$), and MUC-1 ($r = 0.569$, $p < 0.001$); CEA to BARD1 ($r = 0.674$, $p < 0.001$) and MUC-1 ($r = 0.550$, $p < 0.001$); BARD1 to MUC-1 ($r = 0.698$, $p < 0.001$); progesterone to estrogen ($r = 0.623$, $p < 0.001$); and prolactin to CA15-3 ($r = 0.271$, $p = 0.006$). Significant negative correlations were observed between MUC-1 and prolactin ($r = -0.204$, $p = 0.042$) and between progesterone and prolactin ($r = -0.300$, $p = 0.002$).

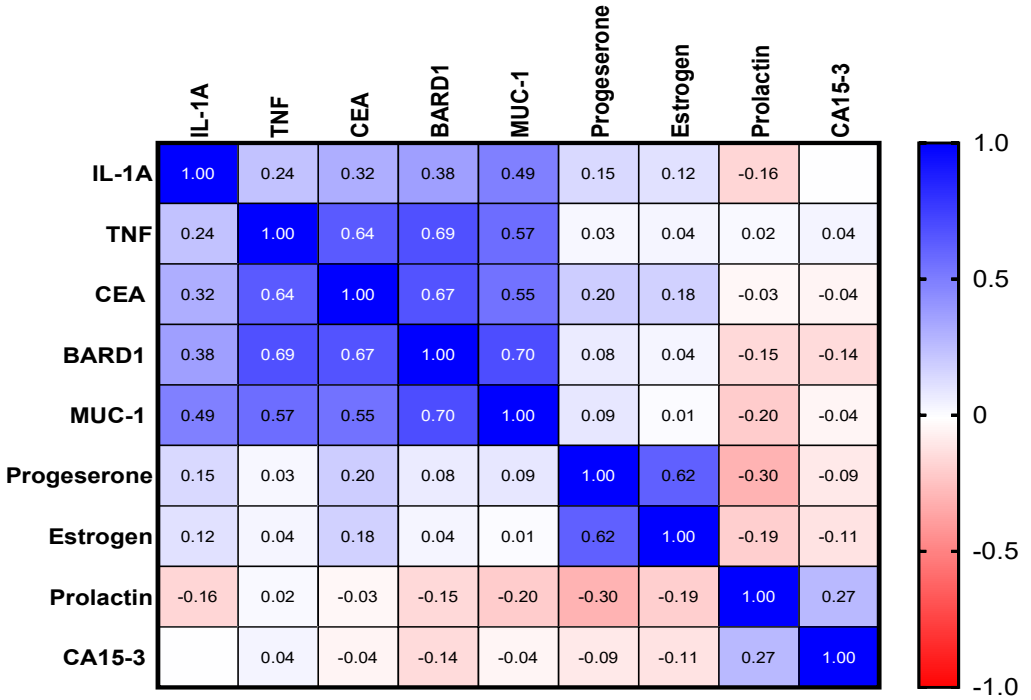


**Figure 1.** Heat–map matrix or correlation analysis between biomarkers data in patients with breast cancer. Values inside boxes indicate the correlation coefficient. Blue color indicates a positive correlation. Red color indicates a negative correlation.

Genetic analysis of the five BARD1 polymorphisms revealed significant associations with breast cancer risk in Iraqi (Table 4). Three SNPs were identified as risk factors: rs2106145710 (GG genotype OR = 3.33, p = 0.008), rs1695783243 (CC genotype OR = 6.60, $p = 1.6 \times 10^{-5}$), and rs1574847014 (CC genotype OR = 11.67, $p = 1.1 \times 10^{-5}$). Their minor alleles (G, C, and C, respectively) also conferred significantly increased risk. In contrast, the SNP rs10498023 demonstrated a substantial protective effect, where both the heterozygous TC (OR = 0.25, $p = 2.9 \times 10^{-5}$) and homozygous CC (OR = 0.17, $p = 2.3 \times 10^{-3}$) genotypes were associated with reduced disease risk; the C allele was likewise protective (OR = 0.33, $p = 3.7 \times 10^{-7}$). The remaining SNP, rs169578112, showed no significant association with the risk of breast cancer. These findings underscore the critical role of specific BARD1 genetic variants in modulating susceptibility to breast cancer in this population.

**Table 4**. Association of BARD1 Polymorphisms with Breast Cancer Risk: Consolidated Genotype and Allele Frequencies.

| SNP ID | Genotype/ Allele | Patients (n=100) n (%) | Controls (n=100) n (%) | OR (95% CI) | p-value |
|---|---|---|---|---|---|
| rs169578112 | TT | 30 | 28 | Reference | - |
| | TC | 43 | 48 | 0.84 (0.43-1.61) | 0.595 NS |
| | CC | 25 | 24 | 0.97(0.46-2.07) | 0.942 NS |
| HWE *p*-value | | 0.234 NS | 0.700 NS | - | - |
| | T Allele | 103(0.53) | 104(0.52) | Reference | - |
| | C Allele | 93(0.47) | 96(0.48) | 0.98(0.66-1.45) | 0.913 NS |
| *rs2106145710* | TT | 30 | 50 | Reference | - |
| | TG | 50 | 40 | 2.08 (1.13 - 3.84) | 0.019 * |
| | GG | 20 | 10 | 3.33 (1.39 - 8.00) | 0.008 ** |
| HWE *p*-value | | 0.919 NS | 0.633 NS | | |
| | T allele | 110 (0.55) | 140 (0.70) | Reference | - |
| | G allele | 90 (0.45) | 60 (0.30) | 1.91 (1.27 - 2.88) | 0.002 ** |
| *rs1695783243* | AA | 25 | 55 | Reference | - |
| | AC | 45 | 35 | 2.38 (1.49 - 5.38) | 0.002 ** |
| | CC | 30 | 10 | 6.60 (2.82 - 15.45) | $1.6 \times 10^{-5}$ **** |
| HWE *p*-value | | 0.328 NS | 0.221 NS | | |
| | A allele | 95 (0.48) | 145 (0.73) | Reference | - |
| | C allele | 105 (0.52) | 55 (0.27) | 2.91 (1.92 - 4.41) | $4.8 \times 10^{-7}$ **** |
| *rs1574847014* | AA | 15 | 50 | Reference | - |
| | AC | 50 | 40 | 4.17 (2.06 - 8.44) | $8.4 \times 10^{-5}$ **** |
| | CC | 35 | 10 | 11.67 (4.76 - 28.60) | $1.1 \times 10^{-7}$ **** |
| HWE *p*-value | | 0.676 NS | 0.633 NS | | |
| | A allele | 80 (0.40) | 140 (0.70) | Reference | - |
| | C allele | 120 (0.60) | 60 (0.30) | 3.50 (2.32 - 5.29) | $3.0 \times 10^{-9}$ **** |
| *rs10498023* | TT | 60 | 25 | Reference | - |
| | TC | 30 | 50 | 0.25 (0.13 - 0.48) | $2.9 \times 10^{-5}$ **** |
| | CC | 10 | 25 | 0.17 (0.07 - 0.39) | $2.3 \times 10^{-3}$ ** |
| HWE *p*-value | | 0.045* | 1.000 NS | | |
| | T allele | 150 (0.75) | 100 (0.50) | Reference | - |
| | C allele | 50 (0.25) | 100 (0.50) | 0.33 (0.22 - 0.51) | $3.7 \times 10^{-7}$ **** |

OR, Odds Ratio; CI, Confidence Interval; HWE, Hardy-Weinberg Equilibrium; NS, not significant. Significance codes: *p ≤ 0.05; **p ≤ 0.01; **p ≤ 0.0001

LD shows how closely linked these SNPs are: High D′ and r² values indicate strong LD between rs1574847014 and rs10498023 (D′ = 0.944, r² = 0.585), rs2106145710 and rs1695783243 (D′ = 1.000, r² = 0.815), rs1574847014 & rs1695783243 (D′ = 1.000, r² = 0.733). This implies that these SNPs may co-segregate, implying that they are inherited together more often than by chance, as shown in Table 5 and Figure 2.

**Table 5**. LD Analysis Between Five SNPs of the BARD1 Gene.

| LD | rs169578112 | rs2106145710 | rs1695783243 | rs1574847014 | rs10498023 |
|---|---|---|---|---|---|
| rs169578112 | | D′ 1.000 | D′ 0.896 | D′ 0.711 | D′ 0.944 |
| | | r² 0.657 | r² 0.551 | r² 0.452 | r² 0.585 |
| rs2106145710 | D′ 1.000 | | D′ 0.943 | D′ 1.000 | D′ 0.535 |
| | r² 0.657 | | r² 0.800 | r² 0.733 | r² 0.286 |
| rs1695783243 | D′ 0.896 | D′ 0.943 | | D′ 1.000 | D′ 0.457 |
| | r² 0.551 | r² 0.800 | | r² 0.815 | r² 0.188 |
| rs1574847014 | D′ 0.711 | D′ 1.000 | D′ 1.000 | | D′ 0.460 |
| | r² 0.452 | r² 0.733 | r² 0.815 | | r² 0.155 |
| rs10498023 | D′ 0.944 | D′ 0.535 | D′ 0.457 | D′ 0.460 | |
| | r² 0.585 | r² 0.286 | r² 0.188 | r² 0.155 | |

D′: Scaled D value (D value represents linkage disequilibrium for each pair of SNP) with an interval between (0-1). r²: Correlation coefficient between each pair of SNP (0-1).

## DISCUSSION

This study identified significant elevations in specific immunological markers (IL-1β, TNF-α, CEA, BARD1, and MUC-1) and associations between BARD1 polymorphisms and breast cancer risk in an Iraqi cohort. While demographic factors

such as age and BMI were not significantly different between our groups, a strong family history and smoking were prominent risk factors, aligning with the established epidemiological data.

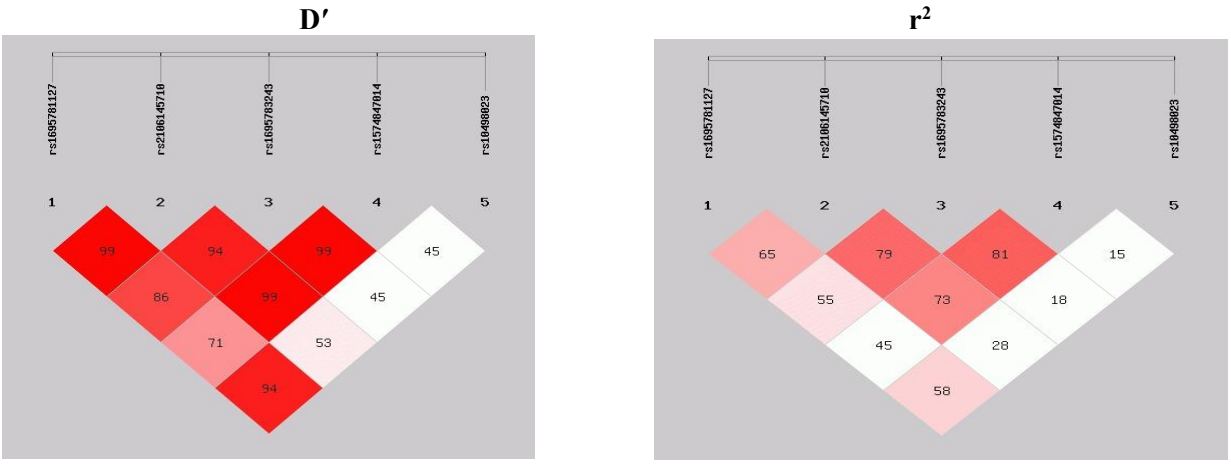


**Figure 2.** Pairwise LD coefficient (D') and correlation coefficient ($r^2$) between *BARCA* SNPs in women with breast cancer and controls.

The significantly elevated IL-1β levels observed in patients with breast cancer compared to controls align with extensive literature supporting IL-1β as a critical player in breast cancer pathogenesis. This finding demonstrates a potential discriminative value, with an AUC of 0.8849, a sensitivity of 85%, and a specificity of 85% at a cut-off value of 15.19 pg/ml. Recent studies have established IL-1β as a multifaceted oncogenic mediator of breast cancer progression. Cytokines promote primary tumor growth, regulate inflammation within the tumor microenvironment, and facilitate the epithelial-to-mesenchymal transition (EMT), which is crucial for metastasis. [22,23] The role of IL-1β in bone metastasis is particularly significant, where it creates a conducive niche for metastatic breast cancer cells and stimulates a vicious cycle of bone destruction. Studies have shown that patients with breast cancer with primary tumors expressing IL-1β are more likely to experience relapse in the bone or other organs. [23,24] The strong association observed between IL-1β and other inflammatory markers (BARD1: r=0.376, MUC-1: r=0.495) suggests a coordinated inflammatory response in breast cancer. This is consistent with recent findings that tumor-infiltrating B cells enhance IL-1β-driven invasiveness in triple-negative breast cancer through NF-κB activation. [25]

Similarly, in addition to IL-1β, the pro-inflammatory cytokine TNF-α, a recent systematic review comprising nine studies has consistently demonstrated that patients with breast cancer exhibit higher TNF-α levels than healthy controls. TNF-α significantly affects breast cancer progression through multiple mechanisms: it promotes tumorigenesis via the TNF-TNFR2 axis, upregulates TAZ expression through the non-canonical NF-κB pathway (increasing breast cancer stem-like cells), and induces metastasis-related gene expression changes. [26] Importantly, TNF-α levels are elevated in metastatic breast cancer and are associated with a poor chemotherapy response and reduced survival times. The strong positive correlations observed with CEA (r=0.644), BARD1 (r=0.686), and MUC-1 (r=0.569) suggest that TNF-α is involved in coordinated inflammatory and metastatic pathways. [18,27] While our results and those of several studies have shown elevated TNF-α levels, it is important to note that some reports, such as those by Krajcik *et al.* (2003) found no association.[28] This discrepancy may be attributable to the differences in the study population, sample size, or assay methodology. Our data, showing strong correlations with other markers, supports a role for TNF-α in the Iraqi cohort. [28]

CEA levels were significantly elevated and demonstrated strong diagnostic utility (AUC=0.78, 74% sensitivity, 85% specificity), supporting their role as valuable biomarkers, particularly for monitoring treatment response and detecting metastasis. CEA functions as a cell adhesion molecule, and elevated blood CEA levels are typically associated with subclinical breast cancer metastasis. Recent studies have emphasized that post-treatment CEA levels are more clinically significant than the pre-treatment levels. In responders to treatment, the median post-treatment CEA level have been observed to drop significantly to 2.07 ng/mL, while non-responders have been found to show elevated levels at 11 ng/mL. [29,30]

Elevated MUC-1 levels with a moderate discriminative value (AUC=0.7311, 65% sensitivity and specificity) are consistent with the well-established role of MUC-1 as a glycoprotein involved in tumor metastasis and invasion. MUC-1 is frequently overexpressed in multiple breast cancer

subtypes, including tubular, invasive lobular, invasive ductal, and mucinous breast carcinomas. [31] Overexpression is associated with poor prognosis, decreased overall, disease-specific, and relapse-free survival.[32] Interestingly, MUC-1 expression patterns vary by molecular subtype, and are significantly increased in ER+ and PR+ tumors, but downregulated in triple-negative breast cancer.[33] This selective expression pattern suggests that MUC-1 is involved in hormone-dependent breast cancer pathways, which is consistent with the observed correlations with other inflammatory markers. Amoako *et al.* reported MUC-1 expression in 59% of Ghanaian breast cancer cases, with significant associations with HER2 overexpression and triple-negative breast cancer, suggesting that MUC-1 may be particularly relevant in aggressive breast cancer subtypes across different populations. [34]

Serum CA15-3 levels did not differ significantly between the study groups ($p = 0.709$). This finding may be explained by the limited sensitivity of CA15-3 in early-stage or non-metastatic disease, as supported by local and international evidence. An Iraqi study using similar samples found no significant difference in CA15-3 levels between controls and early-stage patients, consistent with literature indicating its primary utility lies in monitoring advanced disease and recurrence. [35] Consistent with its known clinical role, CA15-3 demonstrates utility primarily in advanced breast cancer for monitoring therapeutic response and detecting recurrence, due to its limited sensitivity in early-stage disease. [36]

Elevated BARD1 protein levels demonstrated moderate discriminative value (AUC=0.7770, 81% sensitivity and specificity). BARD1's role extends beyond simple biomarkers to critical tumor suppressors involved in DNA repair and genomic stability. BARD1 functions in conjunction with BRCA1 to form heterodimers that are essential for DNA damage repair, replication fork protection, and tumor suppression. Overexpression of oncogenic isoforms BARD1β and BARD1δ permits cancer development, making BARD1 a potential target for both diagnostic screening and therapeutic intervention. [7]

Beyond the observed elevation in serum BARD1 protein levels, we investigated the potential genetic underpinnings of risk by analyzing polymorphisms within the BARD1 gene itself. Recent bioinformatics analyses corroborate our protein findings, linking BARD1 overexpression to poor prognosis, particularly in luminal A subtypes.[37,38] The divergent association of the BARD1 rs2070096 variant in our Iraqi cohort compared to other studies in a Chinese population underscores the critical influence of population-specific genetic architecture. [39,40] The associations between specific BARD1 SNPs and breast cancer risk are likely mediated by mechanisms that alter the structure, function, or expression of BARD1 protein. Non-synonymous SNPs in critical domains (RING, ANK, and BRCT) can impair BARD1-BRCA1 heterodimerization, disrupt its ubiquitin ligase activity, or hinder its recruitment to DNA damage sites, compromising genomic integrity. Furthermore, SNPs in regulatory regions can modulate splicing, potentially favoring the expression of oncogenic isoforms (e.g., BARD1β and BARD1δ) that act in a dominant-negative manner. The strong LD observed between several risk-associated SNPs (e.g., rs2106145710 and rs1695783243) suggests that they may tag haplotypes that harbor such functionally deleterious variants.

Interestingly, the present study identified rs10498023 as having a protective effect against breast cancer, with the C allele showing a reduced frequency in patients (OR = 0.33, 95% CI: 0.22-0.51, $p = 3.7 \times 10^{-7}$). This finding aligns with previous observations that certain BARD1 variants are protective. [7,39] The protective nature of this SNP may be related to a mechanism that enhances BARD1's tumor-suppressor function. For instance, the rs10498023 variant can stabilize the BARD1-BRCA1 complex, improve its efficiency in DNA damage repair, or protect against aberrant splicing that produces oncogenic isoforms. Alternatively, its strong LD with other SNPs ($D' = 0.944$ with rs1574847014) indicates that it may be a marker for a co-inherited yet unidentified protective haplotype that optimizes BARD1 activity.

LD analysis revealed strong correlations between several SNPs, particularly rs2106145710 and rs1695783243 ($D = 1.000$, $r = 0.815$). Similar LD patterns were observed in neuroblastoma studies, where BARD1 SNPs in introns 1, 3, and 4 showed strong LD ($r^2$=0.47–0.96). This suggests that these variants may co-segregate and represent common haplotype blocks that influence susceptibility to breast cancer.

Over one million women worldwide are afflicted with breast cancer each year, making it a prevalent illness. [41] Approximately 5% to 10% of breast cancer cases are hereditary; however, this figure varies according to the study population and the specific genes assessed.[42] If the analysis of mutations is confined to familial cases, the proportion will be elevated. [42] Risk models for various breast cancer subtypes indicate that susceptibility genes (*BRCA1, BRCA2, BARD1, RAD51D, and PALB2*) confer a lifetime breast cancer risk exceeding 20%. [43] The *BARD1* gene was discovered in 1996 to elucidate the biological role of the BRCA1 protein. Potentially pathogenic *BARD1* mutations have been documented.

[44] A recent study employed a panel of 34 candidate susceptibility genes to sequence samples from 60,466 women with breast cancer and 53,461 healthy controls. They demonstrated that protein-truncating variations in four genes (*BARD1, RAD51C, RAD51D, and TP53*) were linked to an increased risk of breast cancer. [45] Several studies have shown that BARD1 gene variation enhances the risk of breast cancer. The BARD1 Cys557Ser variant is associated with an increased risk of both single and multiple primary breast cancers. The Cys557Ser allele frequency was significantly higher in invasive breast cancer patients (0.028) than in controls (0.016) (OR = 1.82, 95% CI 1.11–3.01, p = 0.014). The frequency was further elevated to 0.037 in a high-risk subpopulation (familial history, early onset, or multiple cancers), corresponding to an increased odds ratio (OR = 2.41, 95% CI 1.22–4.75, p = 0.015).[7] In contrast, a separate meta-analysis found no significant association between the BARD1 Cys557Ser variant and breast cancer risk in single-SNP analyses. The pooled odds ratios were 0.90 (95% CI: 0.71–1.15) among BRCA1 carriers and 0.87 (95% CI: 0.59–1.29) among BRCA2 carriers. [46]

The present study showed that BARD1 polymorphisms are important in Iraqi breast cancer risk, whereas other studies have linked it to glioblastoma pathogenesis. BARD1-expressing glial cells are strongly associated with cancer-associated fibroblasts in glioblastoma and may increase the risk of progression. [47] In mesothelioma, a study found that approximately 1.8% of all mesothelioma patients and 4.9% of individuals under 55 years of age possess rare germline mutations of (*BARD1*) gene, which were expected to be deleterious by computational analysis [21], suggesting that *BARD1* may play a broader role in cancer biology beyond breast cancer.

We acknowledge that the diagnostic accuracy of the biomarkers, as assessed by ROC analysis in this case-control study, may be overestimated due to spectrum bias. The clear distinction between cases and healthy controls may not reflect the clinical reality of the use of these biomarkers. Therefore, the results should be interpreted as demonstrating the potential discriminative value of these markers, which requires validation in a prospective cohort or clinical diagnostic setting.

*Limitations*

Despite the significant associations observed, this study has certain limitations. As this was a case-control study, it was susceptible to confounding bias. While we accounted for age, smoking, and family history, other potential confounders, such as detailed dietary patterns, physical activity levels, reproductive history, and specific environmental exposures, were not collected. The influence of these factors on immunological markers and genetic risk cannot be ruled out and should be the focus of future studies.

## CONCLUSION

This study demonstrated a significant association between specific immunological biomarkers and BARD1 gene polymorphisms and early-onset breast cancer risk in Iraqi women. Elevated serum levels of IL-1β, TNF-α, CEA, BARD1, and MUC-1 were observed in the patients, and ROC analysis confirmed their diagnostic utility, particularly IL-1β, which showed high sensitivity and specificity. Genetic analysis revealed that three BARD1 SNPs (rs2106145710, rs1695783243, and rs1574847014) were associated with increased breast cancer risk, whereas rs10498023 appeared to be protective. Critically, our findings highlight the potential of a combined diagnostic strategy that integrates non-invasive biomarker profiling with genetic screening. Such a multimodal approach could significantly improve early detection and enable refined risk stratification. This paves the way for personalized medicine in the Iraqi population and similar cohorts, allowing tailored surveillance protocols for high-risk individuals and more informed clinical management decisions. Further validation in larger multi-ethnic cohorts and functional studies of BARD1 variants are warranted to elucidate their mechanistic roles in breast carcinogenesis.

## CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

## ETHICAL CONSIDERATIONS

All procedures performed in this retrospective data analysis involving human participants were in accordance with the ethical standards of the institutional review board, which did not require informed consent. This study was approved by the University of Miami Institutional Review Board (IRB #00000738).

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None.

## DATA AVAILABILITY

The datasets used and analyzed in the current study are available from the corresponding author upon reasonable request.

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## AI DISCLOSURE

AI tools were used only for language editing. The authors take full responsibility for the content.

## AUTHOR CONTRIBUTIONS

Z.A.A: Study design, experimental work, data acquisition, study conception, data analysis, proposal writing, drafting and revision of the manuscript, and submission of the final manuscript. M.F.A: Study design, planned methodology, and read, revised, and approved the manuscript.

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