

RESEARCH ARTICLE

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Interleukin-1 β rs16944 Polymorphism and Human Cytomegalovirus Seropositivity as Genetic and Viral Risk Determinants of Female Breast Cancer

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Abstract

Background: Breast cancer is the most frequently diagnosed malignancy in women worldwide. Human cytomegalovirus (HCMV) has been implicated as an oncomodulatory agent in mammary carcinogenesis, and the Interleukin1 β -511C>T polymorphism (rs16944) is associated with altered inflammatory signaling and cancer susceptibility. **Objective:** To investigate the association between IL1 β rs16944 polymorphism and HCMV and breast cancer risk in patients with fibroadenoma compared to healthy controls. **Materials and Methods:** We conducted a hospital-based case-control study that enrolled 120 Iraqi women: 60 with breast cancer, 30 with fibroadenoma, and 30 healthy controls. HCMV IgG levels were quantified using the VIDAS® immunoassay. Serum IL-1 β and IL-6 levels were measured using sandwich ELISA. The rs16944 polymorphism was genotyped using Tetra-ARMS PCR, and HCMV DNA was confirmed using UL55 conventional PCR. Haematological parameters were assessed using automated analysis. **Results:** HCMV IgG seropositivity was significantly higher in breast cancer patients (81.7%) than in those with fibroadenoma (63.3%) and controls (60.0%) ($p = 0.04$), with HCMV DNA confirmed by UL55 PCR. Serum IL-6 levels were elevated in breast cancer (486.6 ± 228 pg/mL) and those with fibroadenoma (467 ± 225 pg/mL) compared to controls (184.5 ± 58.3 pg/mL; $p < 0.0001$). IL-1 β levels were paradoxically lower in breast cancer patients (891 ± 178.4 pg/mL; $p < 0.0001$). The rs16944 TT genotype was more common in breast cancer (35.0%) than in controls (20.0%) ($\chi^2 = 10.20$, $p = 0.0377$). **Conclusion:** HCMV seropositivity and the IL1 β rs16944 TT genotype are independently associated with breast cancer in an Iraqi female cohort, indicating that the convergence of viral infection and pro-inflammatory genetic predisposition may potentiate carcinogenic risk. The paradoxical suppression of systemic IL-1 β reflects cytokine compartmentalization in malignancy.

Keywords: Cytomegalovirus Infections; Interleukin-1beta; breast cancer; Interleukin-6

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Introduction

Breast cancer (BC) remains the most frequently diagnosed malignancy among women worldwide. According to the World Health Organization (WHO), an estimated 2.3 million new cases were diagnosed globally in 2022, accounting for approximately 670,000 deaths [1]. Human cytomegalovirus (HCMV) is a double-stranded DNA virus belonging to the family Herpesviridae, subfamily Betaherpesvirinae (HHV-5). With a genome of approximately 230 kilobase pairs encoding more than 200 proteins, HCMV represents one of the largest known human pathogenic viruses [2, 3]. The virus is ubiquitous, with seroprevalence estimates ranging from 50% to 90% of the global adult population [4]. Primary infection in immunocompetent individuals is typically asymptomatic

or manifests as a mild, self-limiting illness; however, following primary infection, HCMV establishes lifelong latency predominantly within CD34⁺ hematopoietic progenitor cells residing in the bone marrow [5]. Transmission occurs through direct contact with infected bodily secretions, including saliva and urine and through breastfeeding, sexual contact, blood transfusion, and organ transplantation [6]. Specific high-risk HCMV strains, most notably the HCMV-DB strain, have been demonstrated to induce a pro-oncogenic cellular environment in human mammary epithelial cells (HMECs), characterized by upregulated expression of oncogenes, genes involved in cell proliferation, DNA repair, cell survival, and epithelial-to-mesenchymal transition (EMT) [7, 8]. HCMV exerts its oncogenic influence through interference with multiple critical cellular pathways, including cell cycle

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regulation, apoptosis, angiogenesis, cellular invasion, and immune evasion [9]. Current evidence indicates a higher prevalence of HCMV proteins and nucleic acids in breast carcinoma tissues than in adjacent non-tumor tissues, further supporting a potential etiological role in breast carcinogenesis [10].

Viral infection, including HCMV, triggers inflammatory response and release of pro-inflammatory cytokines, most notably interleukin -1 and *IL-6*, which in turn modulate tumor biology and immune surveillance [11]. The *IL-1* family of cytokines is a principal mediator of innate immunity and pro-inflammatory signaling [12]. *IL-1 β* , encoded by the *IL1B* gene, is synthesized as an inactive precursor that requires cellular priming, followed by a secondary activating signal, to enable inflammasome-mediated caspase-1 cleavage and subsequent secretion of the mature cytokine [13]. Upon release, *IL-1 β* binds to its cognate receptor, IL-1R1, on target cells, initiating downstream transcriptional programs. In the context of malignancy, *IL-1 β* exerts pleiotropic effects, encompassing modulation of immune cell activity, promotion of angiogenesis, enhancement of cancer cell proliferation, migration, and metastatic dissemination [14].

Single-nucleotide polymorphisms (SNPs) represent the most prevalent form of genetic variation in the human genome, occurring at a frequency of approximately one per 800 base pairs [15]. Three functionally significant SNPs have been identified within the *IL1B* gene: rs1143627 (-31C>T) and rs16944 (-511C>T) in the promoter region and rs1143634 (+3954C>T) in exon 5. These polymorphisms have been extensively investigated with respect to their influence on *IL-1 β* transcriptional activity and their association with susceptibility to inflammatory diseases and various cancers [16].

Given the interplay between HCMV-induced inflammation, cytokine dysregulation, and genetic predisposition, investigating the combined role of HCMV infection and *IL1B* gene polymorphisms in BC represents a scientifically compelling and clinically relevant avenue of inquiry. This study aimed to investigate the association between the *IL1B* -511C>T (rs16944) single-nucleotide polymorphism and HCMV infection and to evaluate their potential roles as biomarkers in the risk and pathogenesis of BC in comparison with fibroadenoma patients and healthy controls.

Materials and Methods

Study Design and Participants

A hospital-based case-control study was conducted between September 1, 2025, and November 10, 2025, in the Babylon Governorate, Iraq. A total of 120 female participants were enrolled and stratified into three groups: (i) 60 histologically confirmed BC patients recruited from the Babylon Cancer Treatment Center; (ii) 30 patients with fibroadenoma recruited from the Breast Disease Screening Consultant Clinic at Imam Al-Sadiq Teaching Hospital, serving as a disease-comparison group; and (iii) 30 healthy females recruited from the same institution, serving as the control group. The participants were aged 32–76 years.

Inclusion Criteria

Participants were eligible for inclusion in the BC group if they met all of the following criteria: (i) a confirmed histopathologically diagnosed BC; (ii) any stage of BC; (iii) currently receiving or scheduled to receive chemotherapy; and (iv) aged ≥ 30 years.

Exclusion Criteria

Participants were excluded if they met any of the following criteria: (i) age < 30 years; (ii) pregnancy; (iii) presence of benign or malignant conditions other than BC (for the cancer group); or (iv) a history of genetic disorders.

All participants provided written informed consent prior to enrolment. This study was conducted in accordance with the ethical principles of the Declaration of Helsinki.

Blood Sample Collection and Processing

A venous blood sample (5 mL) was collected from each participant under aseptic conditions via standard venipuncture. The collected blood was divided as follows: 2 mL was transferred into an ethylenediaminetetraacetic acid (EDTA)-containing tube. Three milliliters were transferred into a gel-and-clot activator (serum separator) tube, allowed to coagulate at room temperature for 20 min, and subsequently centrifuged at 3,000 rpm for 4–5 min to obtain the serum. The samples were stored at -20°C until used for genomic DNA extraction and immunological biomarkers.

Immunological Assays

Measurement of *IL-1 β* and *IL-6* Serum Levels

Serum concentrations of interleukin-1 beta (*IL-1 β*) (BT Lab, Shanghai, China, E0143Hu) and interleukin-6 (*IL-6*) (BT Lab, Shanghai, China, E0090Hu) were quantified using commercially available sandwich enzyme-linked immunosorbent assay (ELISA) kits, following the manufacturer's instructions.

Detection of HCMV IgG Antibodies

Serum levels of HCMV-specific immunoglobulin G (IgG) antibodies were determined using the bioMérieux VIDAS® automated immunoanalysis system (bioMérieux, Marcy-l'Étoile, France) in accordance with the manufacturer's protocol. Seropositivity was defined according to the manufacturer's established cutoff values.

Molecular Analysis

Genomic DNA Extraction

Genomic DNA was extracted from EDTA-anticoagulated whole blood using the Geneaid Genomic DNA Mini Kit (Geneaid Biotech Ltd., New Taipei City, Taiwan), following the manufacturer's instructions. DNA concentration and purity were assessed spectrophotometrically, and the extracted DNA was stored at -20°C until further use.

Genotyping of *IL-1 β* rs16944 (-511C>T) Polymorphism by Tetra-ARMS PCR

The *IL1 β* rs16944 (-511C>T) polymorphism was genotyped using the tetra-amplification refractory

mutation system polymerase chain reaction (Tetra-ARMS PCR) technique [17]. This method employs four primers: two inner allele-specific primers and two outer primers, to simultaneously amplify allele-specific products in a single reaction, thereby enabling the discrimination of the CC, CT, and TT genotypes. The primer sequences used are presented in Table 1.

The outer primer pair (Fo/Ro) generates a control amplicon of 358 bp, confirming successful amplification. The inner C-allele-specific forward primer, combined with the reverse outer primer, produces a 141 bp product, whereas the inner T-allele-specific reverse primer, combined with the forward outer primer, produces a 217 bp product.

Each PCR reaction was performed in a total volume of 25 μ L comprising 12.5 μ L of 2 $\times$ GoTaq $^{\text{®}}$ Green Master Mix (Promega, Madison, WI, USA) to yield a final concentration of 1 $\times$; 2 μ L (10 pmol) of the forward inner primer; 2 μ L (10 pmol) of the reverse inner primer; 2 μ L (10 pmol) of the forward outer primer; 2 μ L (10 pmol) of the reverse outer primer; and 4.5 μ L of genomic DNA template.

Thermal cycling was performed under the following conditions: initial denaturation at 95 $^{\circ}$ C for 2 min; 35 cycles of denaturation at 95 $^{\circ}$ C for 30 s, annealing at 56 $^{\circ}$ C for 20 s, and extension at 72 $^{\circ}$ C for 30 s; followed by a final extension at 72 $^{\circ}$ C for 5 min.

Detection of HCMV UL55 Gene by Conventional PCR

HCMV DNA was detected in seropositive samples by conventional PCR targeting the UL55 gene, which encodes the highly conserved glycoprotein B (gB) transmembrane protein[18]. The primer sequences used are presented in Table 2.

Each PCR reaction was performed in a total volume of 25 μ L comprising 12.5 μ L of 2 $\times$ GoTaq $^{\text{®}}$ Green Master Mix (Promega, Madison, WI, USA); 2 μ L (10 pmol) of the forward primer; 2 μ L (10 pmol) of the reverse primer; and 8.5 μ L of genomic DNA template. Thermal cycling conditions were as follows: initial activation at 95 $^{\circ}$ C for 3 min; 40 cycles of denaturation at 95 $^{\circ}$ C for 30 s, annealing at 57 $^{\circ}$ C for 30 s, and extension at 72 $^{\circ}$ C for 60 s; followed by a final extension at 72 $^{\circ}$ C for 10 min.

Agarose Gel Electrophoresis

PCR-amplified products were resolved by agarose gel electrophoresis. Gels were prepared by dissolving 1.5 g of agarose powder in 100 mL of 1 $\times$ Tris-Borate-EDTA (TBE) buffer to yield a 1.5% (w/v) agarose solution. The mixture was heated in a microwave until the agarose was

completely dissolved and the solution became transparent. After cooling to approximately 50–60 $^{\circ}$ C, 5 μ L of ethidium bromide (10 mg/mL) was added and gently mixed. The gel was poured into a casting tray, combs were inserted, and the gel was allowed to solidify at room temperature. Electrophoresis was conducted at 80 V for 60 min in 1 $\times$ TBE running buffer. A DNA ladder of the appropriate size range was included in each gel run to facilitate accurate band size estimation.

Visualisation and Documentation

After electrophoresis, the gels were visualized under ultraviolet (UV) illumination using a gel documentation system. Images were captured using a gel documentation camera and analyzed for the presence and size of the amplification products.

Haematological Analysis

Complete blood count (CBC) parameters, including packed cell volume (PCV) and total white blood cell (WBC) count, were assessed for all participants using standard automated haematological analysis.

Statistical Analysis

Data were analyzed using GraphPad Prism 9. Continuous variables are expressed as mean $\pm$ standard deviation (SD). Differences between groups were assessed using one-way analysis of variance (ANOVA). Categorical variables were compared using the chi-squared (χ^2) test or Fisher's exact test. Genotype and allele frequencies were determined by direct counting, and Hardy–Weinberg equilibrium was assessed using the chi-squared test. A p-value of less than 0.05 was considered statistically significant, and the data have a normal distribution.

Results

Table 3 presents a comprehensive overview of the baseline characteristics of the 120 female participants enrolled across three study groups: 60 women with histologically confirmed BC, 30 women with fibroadenoma (a benign breast condition used as a disease comparison group), and 30 healthy women serving as controls. The table is organized into four sections. Section A presents demographic data; the three groups were broadly comparable in age (mean ages of 51.1, 47.7, and 50.6 years, respectively; $p = 0.085$), indicating no statistically significant age difference. Section B describes clinical features specific to the BC group, including tumor laterality (the left breast was affected in 50% of cases) and

Table 1. Primer Sequences and Expected Product Sizes for *IL-1 β* rs16944 (–511C>T) Genotyping by Tetra-ARMS PCR.

Primer	Sequence (5'→3')	Product Size
Forward Inner Primer (C-allele specific)	5'-CCT GCA ATT GAC AGA GAG CTA CC-3'	141 bp
Reverse Outer Primer (Ro)	5'-CTT AAC TTT AGG AAT CTT CCC ACT T-3'	—
Reverse Inner Primer (T-allele specific)	5'-CTT GGG TGC TGT TCT CTG CCG CA-3'	217 bp
Forward Outer Primer (Fo)	5'-ATC TGG CAT TGA TCT GGT TCA TCC-3'	—
Outer Control Amplicon (Fo/Ro)	—	358 bp

Table 2. Primer Sequences and Expected Product size for HCMV UL55 Gene Detection by Conventional PCR.

Primer	Sequence (5'→3')	Product Size
Forward Primer	5'-GCG GTG GTT GCC CAA CAG GA-3'	94 bp
Reverse Primer	5'-TTA AAG ATG ACC ACG GGT CGT-3'	

histological subtype (IDC was the most common [70%]). A family history of cancer was reported by 35% of BC patients. Section C reports haematological parameters: BC patients had significantly lower packed cell volume (PCV; mean 33.5% vs. 36.5% and 38.6% in the fibroadenoma and control groups, respectively; $p < 0.0001$), indicating anemia, and significantly higher WBC counts (mean $9.19 \times 10^9/L$ vs. 6.66 and $7.63 \times 10^9/L$; $p = 0.0016$). Section D summarizes HCMV serological status: BC patients had significantly higher mean CMV IgG antibody levels (77.15 UA/mL) and a higher seropositivity rate (81.7%) than the fibroadenoma (63.3%) and control (60.0%) groups ($p = 0.04$).

IL-1 β levels, concentrations were highest in healthy controls (1407 ± 580.6 pg/ml), intermediate in the fibroadenoma group (1102 ± 387 pg/ml), and lowest in patients with BC (891 ± 178.4 pg/ml), with a highly significant difference across groups ($p < 0.0001$). In contrast, *IL-6* levels were markedly elevated in both the BC group (486.6 ± 228 pg/ml) and fibroadenoma group (467 ± 225 pg/ml) groups relative to healthy controls (184.5 ± 58.3 pg/ml; $p < 0.0001$) Table 4.

Conventional PCR was used to confirm 48 IgG-positive samples (20 BC, 18 Fibroadenoma, 10 Control). For the subset of patients tested with PCR, all were positive, indicating active infection or latent virus (Figure 1).

At the genotype level, a statistically significant difference was observed across the groups ($\chi^2 = 10.20$, $p = 0.0377$). In the BC group, the heterozygous CT genotype was most prevalent (40.0%), followed by the homozygous variant TT genotype (35.0%) and the wild-type CC genotype (25.0%). In contrast, the control group showed a predominance of the CT genotype (53.3%), with lower frequencies of CC (26.7%) and TT (20.0%). Notably, the fibroadenoma group exhibited the highest frequency of the wild-type CC genotype (50.0%), followed by TT (30.0%) and CT (20.0%). At the allele level, the T allele was predominant in the BC group (55%), and the C allele was predominant across fibroadenoma. Control groups (45%, 60%, and 53.3% in the BC, fibroadenoma, and control groups, respectively) and the difference in allele frequencies did not reach statistical significance ($\chi^2 = 3.801$, $p = 0.091$), Table 5.

Table 3. Demographic, Clinical, Haematological, and HCMV Characteristics of Study Participants

Section	Parameter	Sub-parameter	Breast Cancer (n=60)	Fibroadenoma (n=30)	Control (n=30)	p-value
A. Demographic Characteristics	Age (years)	Mean ± SD	51.05 ± 10.19	47.67 ± 10.12	50.60 ± 14.53	0.085
		Range	32–76	29–66	33–75	
	Marital Status	Married	54 (90.0%)	26 (86.7%)	30 (100%)	—
		Single	3 (5.0%)	4 (13.3%)	0 (0.0%)	
		Widowed	3 (5.0%)	0 (0.0%)	0 (0.0%)	
B. Clinical Characteristics (Breast Cancer Group)	Tumour Site	Left	30 (50.0%)	—	—	—
		Right	25 (41.7%)	—	—	
		Both	5 (8.3%)	—	—	
	Histological Type	IDC	42 (70.0%)	—	—	—
		ILC	3 (5.0%)	—	—	
		Mixed	2 (3.3%)	—	—	
		Invasive solid papillary ca.	1 (1.7%)	—	—	
	Family History of Cancer	Yes	21 (35.0%)	—	—	—
		No	39 (65.0%)	—	—	
C. Haematological Parameters	PCV (%)	Mean ± SD	33.47 ± 4.14 ^a	36.53 ± 3.66 ^b	38.61 ± 4.18 ^b	<0.0001
		Range	20–42.9	28.5–42	29.9–47	
	WBC (×10 ⁹ /L)	Mean ± SD	9.19 ± 3.29 ^a	6.66 ± 1.59 ^b	7.63 ± 2.40 ^b	0.0016
		Range	2.91–16.4	3.9–11.2	4.8–12.1	
D. HCMV Status	CMV IgG (UA/mL)	Mean ± SD	77.15 ± 30.7	36.4 ± 14.2	32.47 ± 11.65	<0.0001
	CMV IgG Seropositivity	Positive n (%)	49 (81.7%)	19 (63.3%)	18 (60.0%)	0.04
		Negative n (%)	11 (18.3%)	11 (36.7%)	12 (40.0%)	

IDC, Invasive Ductal Carcinoma; ILC, Invasive Lobular Carcinoma; PCV, Packed Cell Volume; WBC, White Blood Cell count; HCMV, Human Cytomegalovirus; CMV IgG, Cytomegalovirus Immunoglobulin G antibodies. Superscripts (a, b) denote statistically distinct groups by One-way ANOVA post-hoc analysis. (—) indicates data not applicable to the respective group. * $p < 0.05$ is considered statistically significant.

Table 4. Cytokine Levels Concentrations Across Study Groups

Parameter	Breast Cancer (n=60)	Fibroadenoma (n=30)	Control (n=30)	p-value
<i>IL-1β</i> (pg/ml)	891 $\pm$ 178.4 ^a	1102 $\pm$ 387 ^b	1407 $\pm$ 580.6 ^c	<0.0001
Range	(585-1345)	(644 -1803)	(778.6-2802)	
<i>IL-6</i> (pg/ml)	486.6 $\pm$ 228 ^a	467 $\pm$ 225 ^a	184.5 $\pm$ 58.3 ^b	<0.0001
Range	(109-957)	(190.5-858)	(100 - 641)	

Superscripts (a, b ,c) denote statistically distinct groups by One-way ANOVA post-hoc analysis. * p < 0.05 is considered statistically significant.

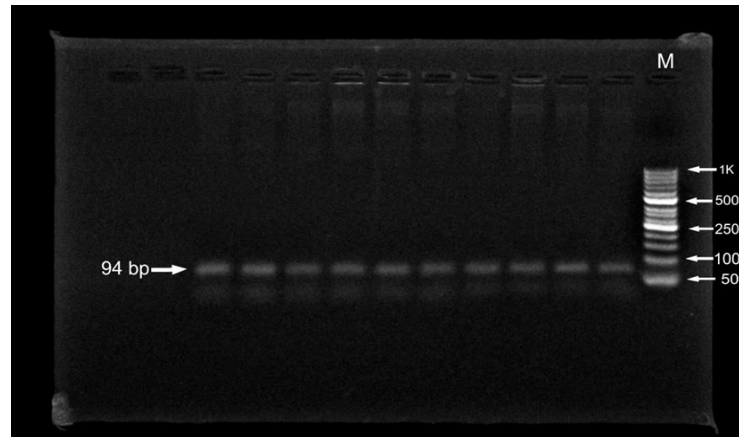


Figure 1. Gel Electrophoresis of HCMV UL55 Gene. Lane M contains a 50 bp DNA ladder as a molecular size standard. The observed bands correspond to the expected product sizes of 94 bp.

A representative electrophoretogram showing analyzed samples (lanes 1–22) and a 100 bp DNA ladder

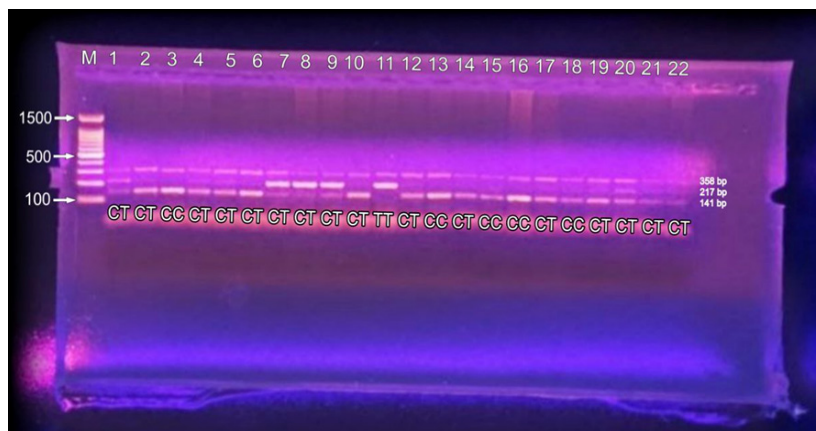


Figure 2: Gel electrophoresis of IL-1 β rs16944 genotyping. Amplified PCR products were separated on a 1.5% agarose gel at 80 V for 60 minutes. lane M contains a 100 bp DNA ladder as a molecular size standard. The observed bands correspond to the expected product sizes of 358 bp, 217 bp for the T allele, and 141 bp for C allele.

Table 5. Distribution of *IL-1 β* Gene Polymorphism (rs16944) in Study Groups

Genotype		Breast Cancer (n=60) n. (%)	Fibroadenoma (n=30) n. (%)	Control (n=30) n. (%)	X ²	P-Value
<i>IL-1β</i> rs16944	CC	15 (25 %)	15 (50.0 %)	8 (26.7 %)	10.2	0.0377*
	CT	24 (40.0 %)	6 (20 %)	16 (53.3 %)		
	TT	21 (35.0 %)	9 (30.0 %)	6 (20.0 %)		
Allele		Breast Cancer (n=60) n. (%)	Fibroadenoma (n=30) n. (%)	Control (n=30) n. (%)	X ²	P-Value
<i>IL-1β</i> rs16944	C	54 (45.0%)	36 (60.0 %)	32 (53.3 %)	3.801	0.091
	T	66(55.0%)	24 (40.0 %)	28 (46.7 %)		
	Total	120	120			

Discussion

This study investigated the association between the *IL-1β*-1 β-511C>T (rs16944) single-nucleotide polymorphism and CMV infection in the context of breast cancer risk, employing a hospital-based case-control design. The findings collectively support the hypothesis that both HCMV seropositivity and specific *IL-1β* genotypic variants contribute to the immunological milieu that characterizes breast carcinogenesis, while simultaneously revealing important paradoxes in cytokine biology that merit careful interpretation.

The significantly elevated HCMV IgG seropositivity rate observed in patients with BC (81.7%; $p = 0.04$) in the present study is consistent with a growing body of evidence implicating HCMV as a potential oncomodulatory agent in breast carcinogenesis. A comprehensive literature review by Blanco and Muñoz confirmed that HCMV prevalence is consistently higher in breast carcinoma tissues than in adjacent non-tumor or benign breast tissues, and that specific oncogenic HCMV strains, most notably the HCMV-DB strain, possess the capacity to transform human mammary epithelial cells (HMECs) through upregulation of oncogenes and genes involved in cell proliferation, DNA repair, and epithelial-to-mesenchymal transition (EMT) [9]. The detection of HCMV DNA in IgG-positive samples through conventional PCR targeting the UL55 gene encoding glycoprotein B (gB) in the present study further corroborates these observations, as the UL55/gB gene has been widely utilized as a highly conserved molecular target for HCMV detection in breast tumor tissues [19]. The confirmation of HCMV DNA in seropositive BC cases reinforces the notion that serological evidence of HCMV infection reflects genuine viral presence within the tumor microenvironment rather than incidental systemic exposure.

However, the oncogenic role of HCMV in BC remains a subject of active scientific debate. An ecological study by Jankovic et al. reported a strong and statistically significant inverse correlation between CMV seroprevalence and BC incidence at the global level, suggesting a potential oncoprotective role for CMV [8]. This paradoxical finding may reflect the complexity of HCMV–host interactions, in which the immunological consequences of chronic CMV infection, including the expansion of CMV-specific cytotoxic T lymphocytes and natural killer cells, may confer broader antitumor surveillance in populations with high seroprevalence. The present study's finding of elevated HCMV seropositivity in BC patients, rather than contradicting this global ecological pattern, may reflect a distinct pathophysiological context in which active or reactivated HCMV infection in an already immunocompromised or genetically predisposed host contributes to tumor promotion rather than protection. This interpretation is consistent with the dual oncomodulatory model proposed by Blanco and Muñoz, in which the net effect of HCMV on carcinogenesis critically depends on host immune status, viral strain, and tissue-specific factors [9]. We hypothesize that the Iraqi cohort may represent a distinct pathophysiological context where active HCMV reactivation in genetically

predisposed, immunocompromised individuals contributes to tumor promotion. Future studies, including viral load quantification, immune cell phenotyping, and longitudinal follow-up, are essential to distinguish between these mechanisms.

One of the most intriguing findings of the present study is the observation that serum *IL-1β* concentrations were significantly lower in the BC group than in both the fibroadenoma and control groups. This finding appears, at first glance, to contradict the established pro-tumorigenic role of *IL-1β* in cancer biology, wherein the cytokine is generally understood to promote angiogenesis, tumor cell proliferation, migration, and metastatic dissemination. However, this paradox is not without precedent in the literature. Kaplanov et al. demonstrated in a murine BC model that blocking *IL-1β* reversed tumor-associated immunosuppression, suggesting that in established tumors, *IL-1β* may paradoxically contribute to an immunosuppressive microenvironment that, once disrupted, restores antitumor immunity [20]. Furthermore, the reduced systemic *IL-1β* levels observed in the present study may reflect the well-documented phenomenon of cytokine compartmentalization in cancer, wherein proinflammatory cytokines are sequestered within the tumor microenvironment and are thus not reliably reflected in peripheral serum measurements. A study by Toney et al. [21] demonstrated that tumor-infiltrating B cells enhance *IL-1β*-driven invasiveness specifically in triple-negative BC, highlighting the importance of local rather than systemic cytokine dynamics. Additionally, the immunosuppressive effects of chemotherapy, to which all BC patients in the present study were exposed, may have contributed to the suppression of systemic *IL-1β* production, further confounding direct comparisons with the fibroadenoma and control groups.

In contrast to the *IL-1β* findings, serum *IL-6* levels were significantly elevated in both the BC and fibroadenoma groups relative to healthy controls ($p = 0.0001$), without a statistically significant difference between the two disease groups. This pattern suggests that *IL-6* elevation may represent a broader inflammatory response associated with breast pathology rather than a specific biomarker for malignancy. These findings are consistent with a 2025 systematic review and meta-analysis by De La Cruz-Vargas et al., which established that elevated serum *IL-6* is significantly associated with poor overall survival in patients with BC (hazard ratio 3.74; 95% CI: 1.84–7.6), positioning *IL-6* as a robust prognostic biomarker [22]. The parallel elevation of *IL-6* in patients with fibroadenoma observed in the present study may reflect the chronic low-grade inflammatory state associated with benign breast disease, which shares certain immunological features with malignant breast pathology. The role of HCMV in driving *IL-6* production within the tumor microenvironment is well-established; Mohamed et al. demonstrated that HCMV-infected tumor-associated macrophages (TAMs) express significantly elevated levels of *IL-6*, *IL-8*, and MCP-1 compared to their HCMV-negative counterparts, providing a mechanistic link between HCMV infection and the *IL-6* elevation observed in the present cohort [23].

Genotypic analysis of the *IL-1β* rs16944 (–511C>T)

polymorphism revealed a significantly higher frequency of the homozygous TT genotype in patients with BC (35%) than in controls (20%) ($p = 0.0377$), with the heterozygous CT genotype being the most prevalent in the BC group (40.0%). These findings align with and extend the existing literature on the association between rs16944 and cancer susceptibility. Pratap et al. reported in a 2024 case-control study that the CT genotype conferred a threefold increased risk of cervical cancer (OR, 3.60; 95% CI, 2.132–6.063), while the TT genotype was associated with a 3.34-fold increased risk (OR, 3.34; 95% CI, 1.952–5.713), with the T allele frequency significantly higher in cancer cases [24]. The mechanistic basis for this association lies in the capacity of the T allele at the –511 position to enhance *IL-1 β* promoter transcriptional activity, thereby potentially altering the inflammatory milieu in susceptible tissues. The paradox of elevated TT genotype frequency in patients with BC alongside reduced serum *IL-1 β* levels in the present study may be partially explained by the complex post-transcriptional regulation of *IL-1 β* , which requires inflammasome-mediated caspase-1 cleavage for the secretion of the mature cytokine, a process that may be dysregulated in the context of herpes simplex virus (HSV) infection or chemotherapy-induced immunosuppression. Furthermore, the manuscript explicitly acknowledges the ethnic-specific nature of the rs16944 association, positioning the findings within the context of Asian and Middle Eastern populations rather than presenting them as universal, as meta-analytic evidence demonstrates substantial ethnic differences in the association between rs16944 and cancer susceptibility.

The association between the *IL-1 β* –511C>T variant and BC predisposition has been substantiated by multiple meta-analytic investigations. A comprehensive meta-analysis by Harati-Sadeh et al., synthesized data from 3,331 BC cases and 3,609 controls, providing robust evidence of a significant association between the *IL-1 β* –511C>T polymorphism and BC susceptibility, with particularly pronounced effects observed in Caucasian and Asian patient subgroups [25]. This global pattern of association across diverse ethnic populations underscores the biological relevance of this polymorphism as a genetic risk factor for breast carcinogenesis. Furthermore, a case-control study by Eras et al., examining the *IL-1 β* –511C>T polymorphism in BC patients reported genotype frequencies of 22.1% for CC, 43.6% for CT, and 34.3% for TT among cases, compared to 24.3%, 55.7%, and 20.0% among controls, respectively [26]. Notably, this study observed a stronger and more pronounced relationship between the *IL-1 β* TT genotype and BC risk, a finding that is consistent with the elevated TT frequency observed in the present Iraqi cohort (35% in cases versus 20% in controls). The concordance between the genotypic distribution reported by Eras et al. and the present study's findings suggests that the rs16944 polymorphism may exert a relatively consistent effect on BC susceptibility across geographically and ethnically diverse populations, although the precise magnitude of this effect may be modulated by population-specific factors, environmental exposures, and interactions with other genetic or viral risk factors, such as HCMV infection.

The fibroadenoma group in the present study exhibited a markedly different genotypic profile, with the wild-type CC genotype being most prevalent (50%), followed by TT (30%) and CT (20%). This distribution contrasts with that of both the BC and control groups, suggesting that the fibroadenoma group may represent a genetically distinct subpopulation with respect to *IL-1 β* regulation. The elevated TT frequency in patients with fibroadenoma relative to controls, although not statistically significant in the present study, warrants further investigation in larger cohorts to determine whether the rs16944 polymorphism confers differential risk across the spectrum of breast pathology.

The haematological findings of the present study, specifically the significantly reduced PCV and elevated WBC counts in BC patients, are consistent with the well-documented haematological consequences of malignancy and its treatment. A case-control study by Abbas et al. confirmed that BC patients exhibit significantly reduced hemoglobin, PCV, and red blood cell counts, along with elevated WBC counts, compared to healthy controls, attributing these changes to tumor-induced hematopoietic suppression, chronic inflammation, and the myelosuppressive effects of cytotoxic chemotherapy [27]. However, this interpretation must not conflate disease effects with treatment effects, as chemotherapy status directly impacts hematological profiles. The Abbas et al. study may not have fully controlled for chemotherapy status. The elevated WBC count observed in the present study may additionally reflect HCMV-driven immune activation, as HCMV infection is known to induce characteristic lymphocytosis and monocytosis as part of the host immune response to viral replication.

Limitations and Future Directions

The present study has several limitations that should be acknowledged. The relatively small sample size, particularly in the fibroadenoma and control groups, is a limitation. The cross-sectional nature of the serological and cytokine measurements precludes causal inferences regarding the temporal relationship between HCMV infection, *IL-1 β* dysregulation, and BC development. Furthermore, the confounding effect of chemotherapy on cytokine levels and haematological parameters cannot be completely excluded. Future studies should employ longitudinal designs with larger, ethnically diverse cohorts, incorporate quantitative viral load measurements, and assess the functional consequences of the rs16944 polymorphism on *IL-1 β* expression within the tumor microenvironment using tissue-based assays. Specifically, future studies should measure *IL-1 β* levels directly within tumor tissue and tumor-associated immune cells using immunohistochemistry (IHC) or flow cytometry, rather than relying solely on serum measurements.

In conclusion, the present study provides evidence that HCMV seropositivity and the *IL-1 β* rs16944 TT genotype are significantly associated with BC in an Iraqi patient cohort, whereas the paradoxical reduction in serum *IL-1 β* levels in cancer patients underscores the complexity of cytokine biology in the context of malignancy and its treatment. The integration of genetic susceptibility markers with viral infection status represents a promising

avenue for the development of multimodal biomarker panels for BC risk stratification, and further mechanistic studies are warranted to elucidate the precise molecular interplay between HCMV, *IL-1β* signalling, and breast carcinogenesis.

Author Contribution Statement

Conceptualization, AAM and AMA; methodology, AAM, AMA and HYS; validation, AAM, AMA and HYS; formal analysis, HYS; investigation, AAM, AMA and HYS; resources, AAM; data curation, HYS; writing – original draft preparation, AAM and AMA; writing – review and editing, all authors; visualization, HYS; supervision, AAM; project administration, AAM; funding acquisition, AAM and AMA.

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Ethical Approval

The Medical Research and Ethics Committee of the Faculty of Medicine, Jabir Ibn Hayyan University for Medical and Pharmaceutical Sciences authorized the study (Approval No.: [1326], dated [3/3/2025]). Written informed consent was obtained from all participants before inclusion in the study.

Use of Artificial Intelligence

Used artificial intelligence to improve sentence structure and clarity.

Availability of data and material

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

Conflict of Interest

No any conflict of interest to be declared

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