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Elevated Serum Interleukin-40 Levels and Gene Expression as Novel Biomarkers in Iraqi Women with Breast Cancer

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ABSTRACT

Background: Breast cancer (BC) is a leading cause of cancer-related mortality and morbidity among women worldwide. Recent evidence highlights the role of inflammatory cytokines in cancer biology. This study aimed to evaluate the biomarker potential of interleukin-40 (IL-40) in BC by assessing its serum levels and gene expression in female Iraqi patients.

Methods: A case-control study included 100 patients with BC and 100 frequency-matched healthy controls (HCs) stratified by 5-year age intervals. Serum IL-40 levels were quantified using an enzyme-linked immunosorbent assay (ELISA), and *C17orf99* (the gene encoding IL-40) expression was analyzed using quantitative polymerase chain reaction (qPCR). Statistical analyses were performed to compare age, body mass index (BMI), and clinical and molecular parameters.

Results: Patients with BC exhibited a significantly higher BMI (31.60 vs 26.63 kg/m²) and elevated serum IL-40 levels (20.26 ± 6.90 vs 14.08 ± 6.12 ng/mL) compared with HCs. Gene expression analysis revealed a 3.7-fold upregulation of *IL40* in patients with BC. Receiver operating characteristic (ROC) curve analysis demonstrated moderate diagnostic accuracy (area under the curve [AUC], 0.756).

Conclusion: Elevated IL-40 levels and gene expression in patients with BC highlight their potential role in disease pathogenesis and utility as diagnostic biomarkers. The findings contribute to our understanding of BC pathogenesis. IL-40 can serve as a promising biomarker for risk assessment and early detection in women in Iraq.

INTRODUCTION

Breast cancer (BC) is the most commonly diagnosed malignancy in women worldwide, accounting for substantial morbidity and mortality rates in both developed and developing countries.[1] Despite significant advances in early detection and treatment strategies, BC remains a heterogeneous disease with diverse molecular subtypes and variable clinical outcomes.[2] The interplay between genetic predisposition, epigenetic regulation, and the tumor microenvironment is central to its pathogenesis.[3] Increasing evidence supports the role of immune regulation and chronic inflammation in the development and progression of BC. Cytokines, which are small regulatory proteins secreted by stromal and immune cells, have been shown to shape tumor behavior through mechanisms involving immune evasion, angiogenesis, cell proliferation, and metastasis.[4] However, the precise contribution of individual cytokines to breast carcinogenesis remains unclear.

Among Iraqi women, BC is the most common killer. In 2019, BC accounted for approximately one-third of all cancer diagnoses in Iraq, making it the leading cause of cancer-related death among Iraqi women. Among the top 10 malignancies in 2019, BC accounted for 22.58% of all cancer cases, with an age-standardized incidence rate of 35.95 per 100 000 women.[5]

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Clinicopathological features have a significant impact on patient prognosis and therapy choices. These features include tumor size, histological grade, lymph node involvement, hormone receptor (HR) status, and molecular subtype.[6,7] Furthermore, hematological parameters—including hemoglobin concentration, white blood cell count, platelet levels, and inflammatory markers—have been increasingly recognized as possible indicators of tumor burden, systemic response, and disease course.[8] To determine the therapy type and prognosis, we characterized and classified the HR status of BC, namely, progesterone receptor (PR), estrogen receptor (ER), and human epidermal growth factor receptor 2 (HER2).[9]

Interleukin-40 (IL-40), a recently identified cytokine encoded by the *C17orf99* gene on chromosome 17q25.3, has drawn attention for its potential immunomodulatory functions. First described by Catalan-Dibene *et al.*,[10] IL-40, which is primarily expressed in B cells, regulates IgA production, mucosal immunity, and gut microbiota homeostasis. It has also been implicated in various autoimmune and inflammatory diseases.[11] Despite emerging evidence on the immunoregulatory roles of IL-40 in inflammatory and autoimmune disorders, its contribution to BC pathogenesis and biomarker potential remains underexplored, particularly in populations with unique epidemiological and environmental risk factors, such as Iraqi women. This study aimed to evaluate the serum levels and gene expression profiles of IL-40 in Iraqi females diagnosed with BC compared with frequency-matched healthy controls (HCs) stratified by age. By integrating molecular and clinical data, this research seeks to elucidate the role of IL-40 in BC biology and assess its utility as a diagnostic or prognostic biomarker in a high-risk, understudied population.

METHODS

Study participants

This case-control study comprised 200 Iraqi women, including 100 patients diagnosed with BC aged 35 to 65 years and 100 HCs frequency-matched to cases in 5-year age strata (e.g., 35–39, 40–44) to balance age distributions. No further confounding variables were adjusted for in the matching process. Recruitment was conducted between November 2024 and February 2025. Blood samples were collected from all participants at the Oncology Teaching Hospital, Medical City, Baghdad, Iraq. Laboratory analyses were performed in the Department of Biotechnology, College of Science, University of Baghdad. Ethical approval was granted by the department's committee (CSEC/0225/0018). After data were collected via structured questionnaires under the supervision of a medical practitioner, written informed consent was obtained from all participants. Age and body mass index (BMI) were recorded for all participants. A range of clinicopathological parameters—including disease grade, tumor size (T stage), lymph node status (N stage), metastasis (M stage), histological type of breast carcinoma (invasive ductal carcinoma and invasive lobular carcinoma), and tumor locality (right or left breast)—were recorded during data collection from patients with BC, in addition to the immunohistochemical profile (ER, PR, or HER2).

Inclusion and exclusion criteria

Inclusion criteria comprised female Iraqi patients with BC whose diagnoses were confirmed by triple assessment (imaging, physical examination, and histology) and who had received prior chemotherapy, radiotherapy, or hormonal therapy. Exclusion criteria encompassed individuals with a history of other malignancies, chronic inflammatory disorders, or unconfirmed histological BC diagnosis. HCs were age-matched Iraqi women with no personal history of malignancy, autoimmune disease, chronic inflammatory conditions, or recent acute infections; all HCs underwent clinical screening to confirm the absence of active illness.

Blood sample collection

Venous blood samples (5 mL) were aseptically collected and aliquoted into gel and Eppendorf tubes for downstream processing. For RNA extraction, 0.25 mL of blood from Eppendorf tubes was homogenized with 750 µL of TransZol Up reagent (TransGen Biotech) and stored at −20 °C. Simultaneously, 2 mL of blood in gel tubes was separated into serum and stored at −20 °C until analysis. Serum IL-40 concentration was measured from patients with BC and HCs using a commercial sandwich enzyme-linked immunosorbent assay (ELISA) kit (BT-Lab, China; Cat. No. E4654Hu) according to the manufacturer's protocol, and the absorbance was measured at 450 nm to determine analyte levels. The separation process began by clotting blood at room temperature for 2 hours and continued with centrifugation at 3000 rpm (approximately 1400*g*) for 15 minutes.

RNA extraction and cDNA synthesis

Using the TransZol Up Plus RNA Kit (ER501-01; TransGen Biotech Co Ltd,China), total RNA was extracted from the samples following the manufacturer's procedure. A Nanodrop instrument (Thermo Fisher Scientific, USA) was used to assess the concentration and purity of RNA. The results showed very pure RNA with an A260/A280 ratio of 2.00. We then used the EasyScript One-Step gDNA

Removal and cDNA Synthesis SuperMix Kit to synthesize complementary DNA (cDNA) from the isolated RNA.

Quantitative polymerase chain reaction (qPCR)

For qPCR, the expression levels of *IL40* mRNA were analyzed using the Bioer Real-Time PCR System and the TransStart Top Green qPCR SuperMix. The reaction mixture, with a total volume of 20 µL, comprised 10 µL of 2× qPCR mix, 1 µL each of forward and reverse primers (10 µM), 6 µL of nuclease-free water, and 2 µL of cDNA. Glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) served as an endogenous reference gene for normalization. The primers for *IL40* included a forward primer (5′-AGCCCACCTATCACCAACAG-3′) and a reverse primer (5′-CCAGAACCAGTCCGATGTCT-3′), both 20 nucleotides in length, designed for a 123-bp amplicon. *GAPDH* primers, sourced from Al-Faisal and Alfartusi,[12] consisted of a forward primer (5′-GAAATCCCATCACCATCTTCCAGG-3′; 24 nt) and a reverse primer (5′-GAGCCCCAGCCTTCTCCATG-3′; 20 nt), targeting a 160-bp amplicon.

A 30-second enzyme activation phase was the first stage in the thermal cycling procedure. Then, we conducted 40 cycles of denaturation (at 94 °C for 5 seconds), annealing (at 58 °C for 15 seconds), and extension (at 72 °C for 20 seconds). To evaluate the specificity of amplification, the temperature was progressively increased from 55 °C to 95 °C as the last dissociation step. Data were standardized to *GAPDH* levels before gene expression levels were computed using the $2^{-\Delta\Delta Ct}$ method.

Statistical analysis

Statistical analyses were performed using GraphPad Prism (version 9.0.0) and the Statistical Package for the Social Sciences (SPSS, version 23.0). Frequency matching was employed solely to ensure equal age distributions between cases and controls; no additional confounding variables (e.g., BMI, menopausal status) were matched or adjusted for in the study design. Frequencies and percentages were used to display the categorical data. Significant relationships among categorical variables were evaluated using the χ^2 test, with significance values set at $P < 0.05$ and $P < 0.01$. The findings for both the case and control groups are presented as mean (SD) for continuous variables that followed a normal distribution. To evaluate the diagnostic performance of the selected parameters, we used a receiver operating characteristic (ROC) curve analysis to determine their sensitivity, specificity, and area under the curve (AUC).[13]

RESULTS

Table 1 displays the results of the analysis of age and BMI. The 2 groups did not vary significantly in terms of mean age, although the BC group was older than the HC group (50.80 [10.78] vs 48.50 [6.69] years, respectively). Alternatively, the BC group exhibited a significantly higher BMI compared with the HCs (31.60 [7.65] vs 26.63 [5.33] kg/m², respectively). The *P* value was highly significant ($P = 0.0007$), suggesting a potential association between higher BMI and the onset of BC.

A range of clinicopathological parameters were assessed to evaluate disease severity, inform prognosis, and guide personalized treatment strategies for patients with BC, thereby enhancing both survival rates and quality of care. As shown in Figure 1A, the majority of patients (n=84; 84.09%) were diagnosed with grade II tumors, which are considered intermediate grade and are typically associated with a more favorable prognosis. Tumor size, a critical determinant of cancer staging, is shown in Figure 1B. Most patients (n=54; 53.49%) presented with T2 tumors, reflecting moderate tumor growth. Lymph node involvement, a key indicator of cancer dissemination and a determinant of therapeutic approaches, is shown in Figure 1C. Regional lymph node metastasis (N1) involving 1 to 3 axillary lymph nodes was found in 45.45% of patients.

Table 1. Baseline Characteristics of Study Participants

General characteristics	BC (N=100) Mean (SD)	HC (N=100) Mean (SD)	*P* value
Age, y	50.80 (10.78)	48.50 (6.69)	0.234
BMI, kg/m²	31.60 (7.65)	26.63 (5.33)	0.0007
Medical History Profile			
Disease duration, y	2.81 (0.33)	…	…
Treatment period, y	2.79 (0.33)	…	…
Family history, No. (%)			
Yes	36 (36)	…	…
No	64 (64)	…	…

BC, breast cancer; BMI, body mass index; HC, healthy controls. Data were analyzed using an independent sample *t* test.

Metastatic status (M stage) is essential for determining whether the malignancy has extended beyond the breast and regional lymph nodes. As shown in Figure 1D, 81.82% of the patients were free of distant metastases (M0), suggesting the potential for curative treatment. The histological subtypes, which can significantly influence treatment decisions and prognosis, are shown in Figure 1E. Invasive ductal carcinoma (IDC), the most common form of BC, was diagnosed in 72.73% of the patients. Finally, tumor laterality is illustrated in Figure 1F. The left breast was affected slightly more frequently (n=42; 43.18%) than the right breast (n=39; 38.64%).

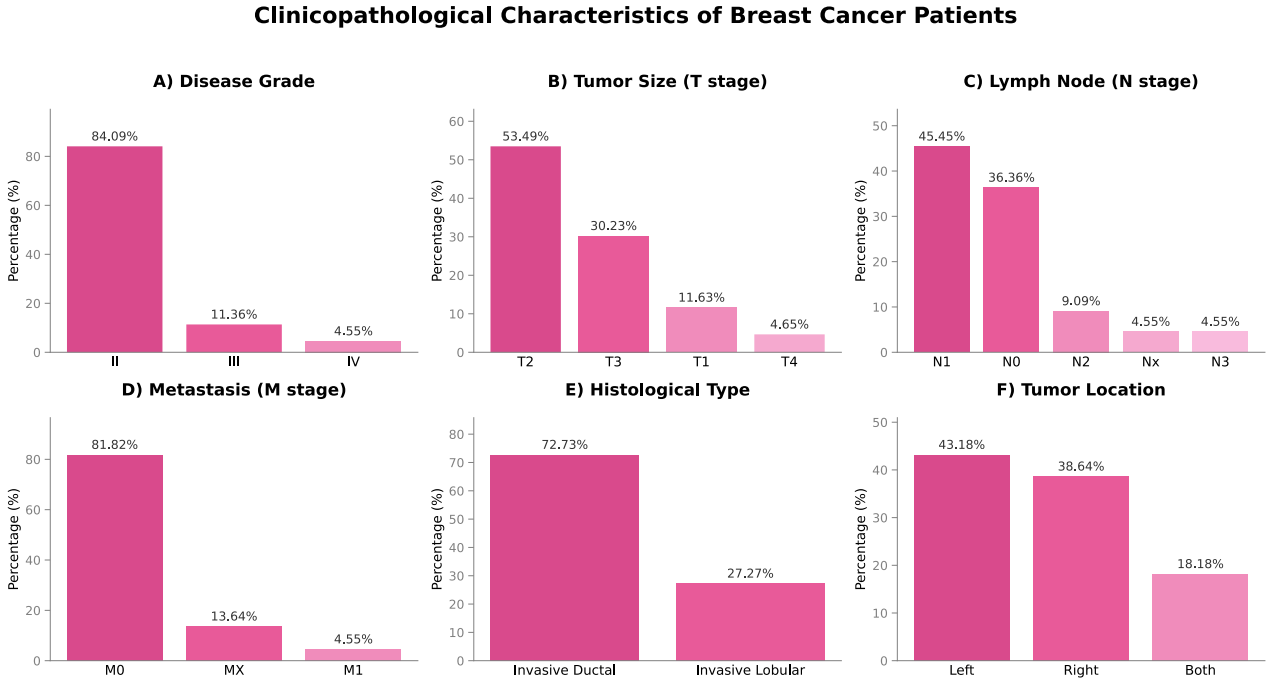

Figure 1. Frequency Distribution of Clinicopathological Characteristics in Patients with Breast Cancer. The column charts show the distribution of patients with breast cancer according to (A) disease grade, (B) tumor size (T stage), (C) lymph node status (N stage), (D) metastasis status (M stage), (E) histological type of breast carcinoma, and (F) tumor location.

The molecular classification of BC subtypes was determined based on the expression of key biomarkers, including HER2, PR, and ER (Table 2). Among the identified subtypes, Luminal A (ER^+/PR^+, $HER2^-$) was the most prevalent, accounting for 60% of the cases. This subtype is generally associated with a favorable prognosis and strong response to hormone therapy. The Luminal B subtype (ER^+/PR^+, $HER2^+$), which is characterized by a higher proliferative rate and potentially poorer outcomes than Luminal A, was observed in 12% of the cases. HER2-enriched BC (ER^-/PR^-, $HER2^+$) constituted 15% of the cohort. This subtype is typically more aggressive but can benefit from targeted HER2-directed therapies. Lastly, the triple-negative BC (TNBC) subtype (ER^-/PR^-, $HER2^-$), which lacks hormonal and HER2 expression and is often associated with poor prognosis and limited targeted treatment options, was identified in 13% of the cases.

Table 2. Classification of Breast Carcinoma by Immunohistochemical Profile

Subtype	ER	PR	HER2	Number (%)		*P* value
Luminal B (ER/PR^+, $HER2^+$)	Positive	Positive	Positive	5 (5)		0.0024
	Positive	Negative	Positive	5 (5)		
	Negative	Positive	Positive	2 (2)	Total: 12 (12)	
Luminal A (ER/PR^+, $HER2^-$)	Positive	Positive	Negative	50 (50)		0.55
	Positive	Negative	Negative	5 (5)		
	Negative	Positive	Negative	5 (5)	Total: 60 (60)	
$HER2^+$ (ER/PR^-, $HER2^+$)	Negative	Negative	Positive	15 (15)	15 (15)	0.0051
Triple negative (ER/PR^-, $HER2^-$)	Negative	Negative	Negative	13 (13)	13 (13)	<0.001

ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; PR, progesterone receptor.
The χ^2 test was used to compare the distribution of molecular subtypes based on immunohistochemical markers.

The serum levels of IL-40 were quantitatively assessed in female patients with BC and compared with those in HCs (Table 3). The analysis revealed that the mean serum IL-40 concentration in the BC group (n=100) was significantly elevated, with a mean (SD) of 20.26 (6.90) ng/mL, compared with a mean (SD) of 14.08 (6.12) ng/mL in the HC group.

Table 3. Comparison of IL-40 Serum Levels Among Study Groups

Parameter	BC Group (N=100)	HCs (N=100)	*P* value
IL-40, ng/mL	20.26 (6.90)	14.08 (6.12)	<0.0001

BC, breast cancer; HCs, healthy controls; IL-40, interleukin-40.
Data are presented as mean (SD). An independent sample *t* test was used for analysis.

ROC curve analysis was conducted to evaluate the diagnostic potential of serum IL-40 levels in distinguishing patients with BC from HCs, as illustrated in Figure 2. The analysis identified an optimal diagnostic threshold for serum IL-40 of 16.77 ng/mL (Table 4). At this cutoff value, IL-40 demonstrated a sensitivity of 62.79% and a specificity of 61.36% for distinguishing patients with BC from HCs. The Youden index, a measure of overall diagnostic effectiveness combining sensitivity and specificity, was 0.2415 at this optimal threshold. The analysis yielded an AUC of 0.75, suggesting moderate diagnostic accuracy for IL-40. The 95% CI was 0.65 to 0.85.

Table 4. Diagnostic Performance of Serum Interleukin-40 Levels in Discriminating Patients with Breast Cancer from Healthy Controls Using Receiver Operating Characteristic Curve Analysis

Threshold, ng/mL	Sensitivity, %	Specificity, %	Youden Index
14.00	78.49	41.38	0.1987
15.50	70.27	50.57	0.2084
16.77 (optimal)	62.79	61.36	0.2415
18.00	55.91	68.97	0.2488
19.50	48.38	75.86	0.2424

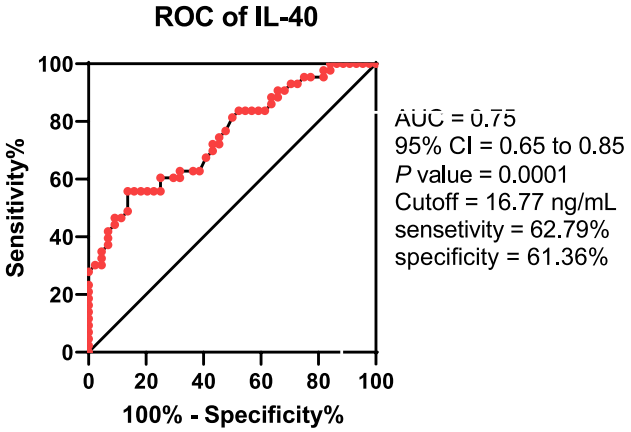

Figure 2. Receiver Operating Characteristic Curve for Serum Interleukin-40 Levels in Patients with Breast Cancer

Table 5 presents the comparative fold-change analysis of *C17orf99* gene expression between female patients with BC (n=100) and HCs (n=100). The findings revealed a significant upregulation of *IL40* in patients with BC, with a mean fold change of 3.71 compared with 1.03 in the control group, a variation that was highly statistically significant ($P = 0.0002$).

DISCUSSION

The analysis revealed no statistically significant difference in age between patients with BC and HCs, aligning with global trends where age does not consistently emerge as a differentiating factor in such comparisons. Previous studies have reported similar findings, underscoring that age alone may not distinctly separate patients with BC from healthy individuals in certain populations.[14] However, contrasting observations from other regions suggest that ethnic and geographical factors may influence the relationship between age and disease onset. For instance, research in Western populations has documented a higher mean age among patients with BC than among controls, highlighting the potential variability linked to demographic or environmental contexts.[15,16] These discrepancies emphasize the need to consider population-specific characteristics when evaluating age-related risk patterns.[17,18] The current findings, consistent with several international cohorts, reinforce the notion that age may play a more nuanced role in BC epidemiology, particularly in understudied populations such as Iraqi women.

Regarding BMI, a significantly higher BMI was observed in patients with BC than in HCs, suggesting a potential role of obesity in BC development. Atoum and Alparrey[19] similarly reported a higher BMI in patients with BC, although their control group had an

Table 5. Comparison of *C17orf99* Gene Expression Among Females with Breast Cancer and Healthy Controls

Parameter	BC (N=100)	HC (N=100)	*P* value
IL-40 fold change	3.71 (0.63)	1.03 (0.41)	0.0002

BC, breast cancer; HC, healthy controls; IL-40, interleukin-40.
Data are presented as mean (SD). An independent sample *t* test was used for analysis.

anomalously high BMI, which likely reflects data entry or reporting error. Despite this discrepancy, both studies underscored obesity as a significant modifiable risk factor. Although the mechanistic link between BMI and BC remains unclear, the association between elevated BMI and BC may reflect increased aromatase activity in adipose tissue, particularly in postmenopausal women. However, the menopausal status of our cohort was not stratified, limiting mechanistic interpretations. Environmental or lifestyle factors unique to Iraqi women, such as dietary patterns or physical inactivity, may also contribute to the results.[20] However, these mechanisms require further exploration by molecular and hormonal studies.

In terms of clinical parameters, the mean BC duration in patients was 2.81 (0.33) years, with a similar mean treatment duration of 2.80 (0.33) years. These values reflect trends reported by Gnant *et al.*,[21] whose trial of extended-release anastrozole found that 3208 of 3484 women remained progression-free after 2 years of treatment. Nevertheless, the optimal therapy duration remains a topic of debate. These complications can lead to reduced treatment adherence and early discontinuation, thereby compromising outcomes.[22] Interestingly, the current study found no significant association between family history and the incidence of BC. This is consistent with the findings of Ho *et al.*,[23] who reported that 84% of the patients denied a positive family history. Swahn *et al.*[24] found that responses were split, with 33.2% of patients with BC reporting no family history, 38.2% reporting a positive history, and 30.0% unsure. Although family history remains an important traditional risk factor, its predictive value may be limited in isolation.

The current study found that the majority of patients were diagnosed with grade II tumors, which is consistent with previous reports by Salman *et al.*[25] (76.7%) and Mohsin and Mohamad[26] (64.44%). A higher prevalence of grade I tumors was reported by Nayyef and Aziz,[27] which is indicative of earlier detection. In contrast, more advanced tumor grades were documented by Andrikopoulou *et al.*[28] and Nag *et al.*,[29] in which grade III tumors predominated, suggesting later-stage diagnoses in these cohorts. Regarding tumor size (T stage), T2 tumors were the most prevalent in the current study, which is consistent with the findings of some previous studies (61.7%).[25,29] Similarly, Nayyef and Aziz[27] reported that 74% of their cohort presented with T1 or T2 tumors. A slightly lower T2 rate was observed in the study by Ameen.[30] These findings suggest that a significant proportion of BC cases are detected at intermediate stages, which may facilitate more favorable therapeutic outcomes. In terms of nodal involvement (N stage), 45% of the patients were classified as N1, reflecting limited regional lymph node spread. This is comparable to the findings of a study by Nag *et al.*,[29] who reported the majority of patients with N1 cases, and the study by Nayyef and Aziz,[27] who reported a higher N1 frequency. However, some studies have indicated that the majority of patients had no lymph node involvement (N0),[25] suggesting a more favorable prognosis in that group. Regarding distant metastasis (M stage), the majority of patients had no evidence of metastasis (M0), corroborating the findings of some previous studies.[25,27,29] These data support the notion that many patients are diagnosed before metastatic spread, which is an important factor in treatment planning and prognosis. IDC was the predominant histological type, accounting for 72.73% of the cases, which is consistent with global trends. Higher IDC frequencies have been reported by some studies,[25,28,31] affirming that IDC is the most common BC subtype worldwide. Regarding tumor laterality, the left breast was more frequently affected, in line with the reports by a few studies.[25,27,28] However, Mohsin and Mohamad[26] noted a greater incidence in the right breast, possibly due to population-specific or sample size variations. Molecular classification revealed that Luminal A (ER^+/PR^+, $HER2^-$) was the most common molecular subtype. This aligns with various studies.[27,32] Previous research[29] reported an even greater prevalence of ER^+/PR^+, suggesting a good response to hormonal therapy. Other studies have reported variability, including a study by Ntirenganya *et al.*[33] The predominance of Luminal A tumors (60%) in our cohort contrasts with studies reporting Luminal B dominance, possibly due to genetic variations (e.g., *CYP19* polymorphisms) or earlier detection practices in Iraq, whereas Andrikopoulou *et al.*[28] identified Luminal B (ER^+/PR^+, $HER2^+$) as the dominant subtype in 39% of the cases.

A key finding of this study was the markedly elevated serum IL-40 levels observed in patients with BC compared with those in HCs, with a statistically significant difference between the groups. Comparative studies have reported elevated levels of

other interleukins in patients with BC. For example, Sermaxhaj *et al.*[34] found significantly greater IL-7 levels in patients with BC than in HCs, suggesting robust immune activation in malignancy. By contrast, Wang *et al.*[35] reported significantly lower IL-1β levels in patients with BC than in controls, reflecting the heterogeneity of immune responses associated with different cytokines. Further supporting the immunological relevance of IL-40, Jaber and Ad'hiah[36] found elevated IL-40 levels in patients with ankylosing spondylitis compared with controls, while Al Ghuraibawi *et al.*[37] reported similar results. In type 2 diabetes mellitus, Nussrat and Ad'hiah[38] observed significantly increased IL-40 levels, reinforcing the role of IL-40 in chronic inflammatory conditions.

The present study demonstrated statistically significant findings with a highly significant *P* value and a moderately narrow CI, indicating moderate diagnostic accuracy for IL-40 in Iraqi females with BC. At its optimal threshold, IL-40 showed moderate diagnostic accuracy (AUC), correctly identifying a moderate proportion of both cases and HCs. The AUC of 0.756 (95% CI, 0.6453–0.8484) suggests a moderate diagnostic utility for IL-40. Discrepancies with prior studies (e.g., Ahmed and Abed, 2024) may stem from differences in cohort size, ethnicity, or assay sensitivity, underscoring the need for standardized protocols for IL-40 measurement. These comparative outcomes highlight the variability in diagnostic efficacy across studies, underscoring the need for further validation of IL-40 clinical utility.[39]

The current study also demonstrated a significant upregulation of *C17orf99* gene expression in female patients with BC compared with that in HCs, with a mean fold change of 3.707 vs 1.026, respectively ($P = 0.0002$). IL-40, initially characterized as a B-cell–associated cytokine involved in humoral immune responses, has recently emerged as a potential immunomodulatory factor in various inflammatory and neoplastic conditions. Its overexpression in patients with BC, as revealed in this study, aligns with the growing evidence that cytokines contribute to tumor development by promoting chronic inflammation, angiogenesis, and immune evasion.[40] The application of the $2^{-\Delta\Delta Ct}$ method for relative quantification revealed a statistically robust fold-increase, further supporting the hypothesis that IL-40 may serve as a molecular biomarker. Notably, previous transcriptomic studies have implicated upregulated cytokine expression, including interleukins such as IL-6, IL-8, and IL-17, in breast tumor progression and poor prognosis.[41] Although IL-40 has not yet been fully characterized in the context of BC, its structural and functional similarities with other tumor-promoting cytokines warrant further investigation. The significantly elevated IL-40 expression observed in the present BC cohort may reflect the role of cytokines in shaping the pro-tumorigenic microenvironment. While our findings demonstrated elevated IL-40 levels and gene expression in patients with BC, the exact role of IL-40 in tumorigenesis remains unclear. Future studies should investigate cellular sources (e.g., tumor-infiltrating B cells) and their interactions with immune checkpoints. Moreover, recent bioinformatics analyses have identified IL-40 as a cytokine signature predictive of tumor immune subtypes and therapeutic response.[42] The constitutive production of IL-40 from lymphomatous B cells and its variable expression in distinct subtypes of lymphomas, as noted in previous reports in cell lines, imply that it promotes B-cell lymphomatous escape. The IL-4/STATs pathway, which is expectedly maintained by IL-40 production from lymphomatous naïve B cells, might potentially play a crucial role in the development of cancer, as shown in gene and networking studies.[43]

CONCLUSION

Elevated serum IL-40 levels and transcriptional upregulation were observed in Iraqi women with BC. While IL-40 shows promise as a diagnostic biomarker, further validation in longitudinal cohorts is required to assess its prognostic value and potential integration into risk stratification frameworks.

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CONFLICTS OF INTEREST

None of the authors reports any conflict of interest.

ETHICAL CONSIDERATIONS

This study was conducted in accordance with the ethical rules of medical research at the University of Baghdad, College of Science. Before sampling, the consent of the patient or their companion was taken. The study protocol, subject information, and approval form were reviewed and confirmed by the hematology unit and clinical chemistry unit of the laboratory in the Oncology Teaching Hospital in accordance with Document No. CSEC/0225/0018 to obtain this approval.

FUNDING

None.

DATA AVAILABILITY

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

AI DISCLOSURE

No AI- or AI-assisted technology was used in the writing or preparation of this document. All content was created solely by the author(s) without the assistance of artificial intelligence tools.

AUTHOR CONTRIBUTIONS

All authors contributed equally to conceptualizing the research, collecting data, data analysis, writing, editing, and review.

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