

Comparative Study of CA 15-3, CA 125, Free Radical Levels, Complete Blood Count, and Glycated Hemoglobin (HbA1c) in Breast Cancer Patients Versus Healthy Women in Eastern Libya

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Abstract

Breast cancer is a major health burden, and the identification of reliable biochemical and tumor-related biomarkers may improve disease detection and characterization. This study evaluated serum CA 15-3, CA 125, malondialdehyde (MDA), hematological parameters, and glycated hemoglobin (HbA1c) in breast cancer patients compared with healthy controls, with additional assessment of biomarker correlations and diagnostic performance. Sixty breast cancer patients and 60 healthy controls were evaluated. Demographic characteristics, serum tumor markers, MDA, complete blood count parameters, HbA1c, and fasting glucose were determined. Pearson correlation analysis was performed to investigate associations among biomarkers, while receiver operating characteristic (ROC) curve analysis was used to assess their diagnostic accuracy individually and in combination. Breast cancer patients showed significantly higher serum CA 15-3 (58.4 ± 32.7 vs. 12.8 ± 5.3 U/mL) and CA 125 (42.6 ± 28.4 vs. 14.2 ± 6.1 U/mL) concentrations than controls ($p < 0.001$). MDA levels were also markedly elevated in patients (8.94 ± 3.12 vs. 3.18 ± 1.05 nmol/mL, $p < 0.001$), with significantly higher concentrations in stage III–IV disease than stage I–II disease. Significant hematological alterations included reduced hemoglobin, RBC, and hematocrit and increased WBC and platelet counts ($p < 0.001$). HbA1c and fasting glucose were significantly higher in breast cancer patients than controls ($6.42 \pm 1.28\%$ vs. $5.28 \pm 0.62\%$ and 108.4 ± 24.6 vs. 89.2 ± 12.8 mg/dL, respectively; $p < 0.001$). CA 15-3 was strongly correlated with CA 125 ($r = 0.682$, $p < 0.001$) and MDA ($r = 0.548$, $p < 0.001$), while MDA was negatively correlated with hemoglobin ($r = -0.436$, $p < 0.01$). ROC analysis demonstrated good diagnostic performance for CA 15-3 (AUC=0.892), MDA (AUC=0.864), and CA 125 (AUC=0.798). The combined CA 15-3 + MDA panel achieved an AUC of 0.934, whereas the four-biomarker panel (CA 15-3 + CA 125 + MDA + HbA1c) demonstrated the highest diagnostic accuracy (AUC=0.956), with 91.7% sensitivity and 93.3% specificity. Breast cancer was associated with increased tumor marker concentrations, oxidative stress, hematological disturbances, and altered glycemic status. The strong associations among CA 15-3, CA 125, MDA, and HbA1c, together with the superior diagnostic performance of the combined biomarker panel, suggest that a multimarker approach may provide greater diagnostic discrimination than individual biomarkers alone.

Keywords. Breast Cancer, CA 15-3, CA 125, Malondialdehyde, Oxidative Stress, Diagnostic Accuracy.

Introduction

Breast cancer represents the most common malignancy affecting women globally and constitutes a significant public health challenge in Libya and the broader North African region [1]. According to the Libyan National Cancer Registry, breast cancer accounts for approximately 25-30% of all cancer cases among Libyan women, with incidence rates showing an upward trend over the past two decades [2]. Eastern Libya, comprising cities such as Benghazi, Al-Bayda, and Tobruk, has reported substantial breast cancer morbidity, necessitating comprehensive biomarker research to enhance early detection and therapeutic monitoring [3]. The pathophysiology of breast cancer involves complex interactions between genetic predisposition, hormonal influences, environmental factors, and metabolic disturbances. Tumor-associated antigens, particularly carbohydrate antigen 15-3 (CA 15-3) and carbohydrate antigen 125 (CA 125), have emerged as valuable serological biomarkers in breast cancer management. CA 15-3, a product of the MUC1 gene, is the most widely used serum marker for monitoring breast cancer progression and treatment response. CA 125, traditionally associated with ovarian malignancies, has also demonstrated elevated levels in advanced breast cancer cases, particularly in hormone receptor-negative subtypes [4].

Oxidative stress, characterized by an imbalance between reactive oxygen species (ROS) production and antioxidant defense mechanisms, plays a pivotal role in breast carcinogenesis. Free radicals induce DNA damage, lipid peroxidation, and protein modification, thereby promoting mutagenesis and tumor progression [5]. Malondialdehyde (MDA), a stable end-product of lipid peroxidation, serves as a reliable biomarker for assessing oxidative stress status in cancer patients. Hematological abnormalities are frequently observed in cancer patients due to bone marrow suppression, chronic inflammation, and nutritional deficiencies [6]. Complete blood count (CBC) parameters provide essential information regarding the patient's overall health status, immune function, and potential paraneoplastic syndromes [7]. Furthermore, emerging evidence suggests a bidirectional relationship between diabetes mellitus and breast cancer, with elevated glycated hemoglobin (HbA1c) levels potentially influencing cancer risk and prognosis through insulin resistance and hyperglycemia-induced oxidative stress [8]. This study was designed to comprehensively compare serum levels of CA 15-3, CA 125, free radical activity (MDA), complete blood count parameters, and HbA1c between breast cancer patients and healthy women in Eastern Libya, thereby contributing to the establishment of region-specific reference ranges and multi-biomarker diagnostic panels.

Methodology

This cross-sectional comparative study was conducted at the Department of Pathology and Clinical Laboratories, Benghazi Medical Center, and the Oncology Unit, Al-Jala Hospital, Benghazi, Eastern Libya, between January 2024 and December 2025. A total of 120 women aged 25-70 years were recruited and divided into two groups: Group I (Breast Cancer Patients): Sixty (60) women with histopathologically confirmed breast cancer, including 35 patients with invasive ductal carcinoma (IDC), 15 with invasive lobular carcinoma (ILC), and 10 with mixed or other histological subtypes. Staging was performed according to the TNM classification system (8th edition, AJCC). The group comprised 20 patients with stage I-II disease and 40 patients with stage III-IV disease. Group II (Healthy Controls): Sixty (60) age-matched healthy women with no history of malignancy, autoimmune disorders, or chronic inflammatory diseases. Controls were recruited from routine health screening programs and community health centers in Benghazi and surrounding areas. Exclusion criteria included: previous chemotherapy or radiotherapy, concurrent malignancies, acute infections, severe hepatic or renal dysfunction, pregnancy, and known diabetes with poor glycemic control (HbA1c >10%).

Fasting venous blood samples (10 mL) were collected in the morning between 8:00 and 10:00 AM. Samples were distributed as follows: 3 mL in EDTA tubes for CBC analysis, 3 mL in fluoride oxalate tubes for HbA1c, and 4 mL in plain serum tubes for tumor markers and MDA assay. Serum was separated by centrifugation at 3000 rpm for 10 minutes and stored at -80°C until analysis. CA 15-3 and CA 125 were measured using chemiluminescent microparticle immunoassay (CMIA) on the Abbott Architect i2000SR analyzer (Abbott Laboratories, USA). The reference cutoff values were <30 U/mL for CA 15-3 and <35 U/mL for CA 125. Serum MDA levels were determined using the thiobarbituric acid reactive substances (TBARS) spectrophotometric method at 532 nm.

Results were expressed as nmol/mL. Complete blood count was performed using the Sysmex XN-1000 automated hematology analyzer (Sysmex Corporation, Japan), including hemoglobin (Hb), white blood cell count (WBC), platelet count (PLT), red blood cell count (RBC), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and red cell distribution width (RDW). HbA1c was measured by high-performance liquid chromatography (HPLC) using the Bio-Rad D-100 analyzer (Bio-Rad Laboratories, USA). Values were reported as percentages according to NGSP standards. Data were analyzed using SPSS version 26.0 (IBM Corp., USA). Continuous variables were expressed as mean $\pm$ standard deviation (SD) and compared between groups using the independent samples t-test. Categorical variables were analyzed using the Chi-square test. Pearson correlation coefficients were calculated to assess relationships between variables. A p-value <0.05 was considered statistically significant. Receiver operating characteristic (ROC) curve analysis was performed to evaluate the diagnostic performance of individual and combined biomarkers.

Results

Demographic and Clinical Characteristics

The demographic and clinical characteristics of the study participants are summarized in Table 1. No significant differences were observed between the two groups regarding age, body mass index (BMI), or menopausal status ($p>0.05$), confirming successful matching. The mean age of breast cancer patients was 48.6 ± 9.2 years, compared to 47.3 ± 8.8 years in healthy controls. Among breast cancer patients, 58.3% were postmenopausal, and 41.7% were premenopausal. The majority of patients (66.7%) presented with stage III-IV disease, while 33.3% had early-stage (I-II) disease.

Table 1. Demographic and Clinical Characteristics of Study Participants

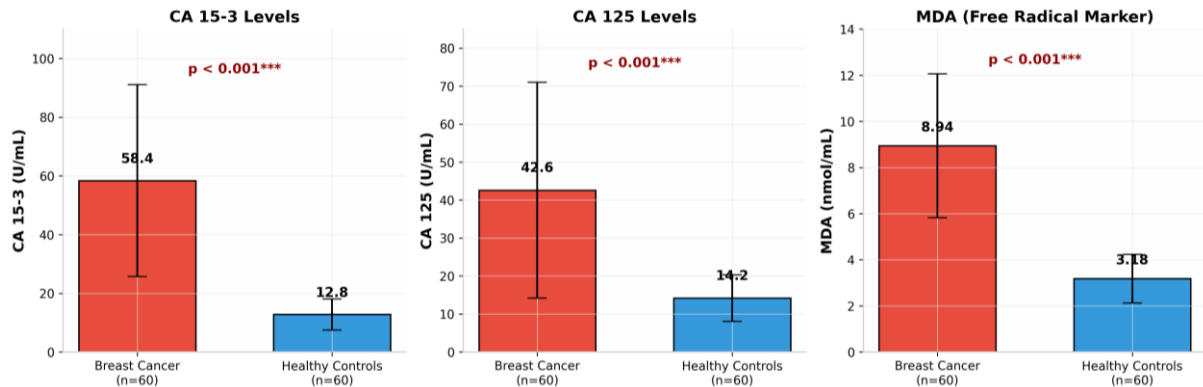
Parameter	Breast Cancer Group (n=60)	Healthy Control Group (n=60)	p-value
Age (years)	48.6 ± 9.2	47.3 ± 8.8	0.432
BMI (kg/m^2)	27.8 ± 4.5	26.4 ± 3.9	0.067
Premenopausal, n (%)	25 (41.7%)	28 (46.7%)	0.589
Postmenopausal, n (%)	35 (58.3%)	32 (53.3%)	0.589
Family History of BC, n (%)	18 (30.0%)	4 (6.7%)	0.001*
Stage III-IV Disease, n (%)	40 (66.7%)	—	—

Serum Tumor Marker Levels

Serum CA 15-3 and CA 125 levels were significantly elevated in breast cancer patients compared to healthy controls. As shown in Table 2, the mean CA 15-3 level in the breast cancer group was 58.4 ± 32.7 U/mL, which was approximately 4.5-fold higher than the control group (12.8 ± 5.3 U/mL) ($p<0.001$). Similarly, CA 125 levels were significantly higher in breast cancer patients (42.6 ± 28.4 U/mL) compared to controls (14.2 ± 6.1 U/mL) ($p<0.001$). The proportion of patients with elevated CA 15-3 (>30 U/mL) was 61.7%, while elevated CA 125 (>35 U/mL) was observed in 45.0% of breast cancer patients.

Table 2. Comparison of Serum Tumor Marker Levels Between Groups

Tumor Marker	Breast Cancer Group (n=60)	Healthy Control Group (n=60)	t-value	p-value
CA 15-3 (U/mL)	58.4 ± 32.7	12.8 ± 5.3	10.42	<0.001***
CA 125 (U/mL)	42.6 ± 28.4	14.2 ± 6.1	7.38	<0.001***
CA 15-3 Elevated, n (%)	37 (61.7%)	2 (3.3%)	—	<0.001***
CA 125 Elevated, n (%)	27 (45.0%)	3 (5.0%)	—	<0.001***

**Figure 1. Comparison of Serum CA 15-3, CA 125, and MDA (Free Radical Marker) Levels Between Breast Cancer Patients and Healthy Controls. Data are presented as mean ± SD. ***p < 0.001****Serum Malondialdehyde (MDA) Levels as a Marker of Free Radical Activity**

Oxidative stress, as reflected by serum MDA concentrations, was markedly elevated in breast cancer patients. The mean MDA level in the breast cancer group was 8.94 ± 3.12 nmol/mL, representing a 2.8-fold increase compared to the healthy control group (3.18 ± 1.05 nmol/mL) ($p < 0.001$). Furthermore, MDA levels showed a positive correlation with disease stage, with stage III-IV patients exhibiting significantly higher MDA (10.42 ± 3.58 nmol/mL) than stage I-II patients (6.98 ± 2.14 nmol/mL) ($p < 0.01$). These findings indicate substantial lipid peroxidation and oxidative damage in breast cancer patients, with progressive worsening in advanced disease stages.

Table 3. Comparison of Serum MDA Levels (Free Radical Marker) Between Groups

MDA Level	Breast Cancer Group (n=60)	Healthy Control Group (n=60)	t-value	p-value
MDA (nmol/mL)	8.94 ± 3.12	3.18 ± 1.05	13.21	<0.001***
Stage I-II MDA (nmol/mL)	6.98 ± 2.14	—	—	—
Stage III-IV MDA (nmol/mL)	10.42 ± 3.58	—	—	—

Complete Blood Count (CBC) Parameters

Significant hematological alterations were observed in breast cancer patients compared to healthy controls (Table 4). Hemoglobin levels were significantly lower in the breast cancer group (10.8 ± 1.6 g/dL) compared to controls (12.6 ± 1.1 g/dL) ($p < 0.001$), with 46.7% of cancer patients presenting with anemia (Hb < 11.0 g/dL). White blood cell count was significantly elevated in breast cancer patients ($8.92 \pm 2.84 \times 10^3/\mu\text{L}$) compared to controls ($6.45 \pm 1.62 \times 10^3/\mu\text{L}$) ($p < 0.001$), suggesting an inflammatory response. Platelet count was also significantly higher in the cancer group ($328.4 \pm 89.6 \times 10^3/\mu\text{L}$ vs. $248.6 \pm 62.3 \times 10^3/\mu\text{L}$; $p < 0.001$), consistent with paraneoplastic thrombocytosis.

Table 4. Comparison of Complete Blood Count Parameters Between Groups

CBC Parameter	Breast Cancer Group (n=60)	Healthy Control Group (n=60)	t-value	p-value
Hb (g/dL)	10.8 ± 1.6	12.6 ± 1.1	7.24	<0.001***
RBC ($\times 10^6/\mu\text{L}$)	3.82 ± 0.58	4.38 ± 0.42	6.08	<0.001***
WBC ($\times 10^3/\mu\text{L}$)	8.92 ± 2.84	6.45 ± 1.62	5.93	<0.001***
PLT ($\times 10^3/\mu\text{L}$)	328.4 ± 89.6	248.6 ± 62.3	5.72	<0.001***
HCT (%)	34.2 ± 5.1	39.8 ± 3.6	7.01	<0.001***
MCV (fL)	89.6 ± 6.2	90.8 ± 5.4	1.14	0.257
MCH (pg)	28.4 ± 3.1	28.8 ± 2.6	0.78	0.438
RDW (%)	14.2 ± 1.8	13.6 ± 1.4	2.04	0.044*

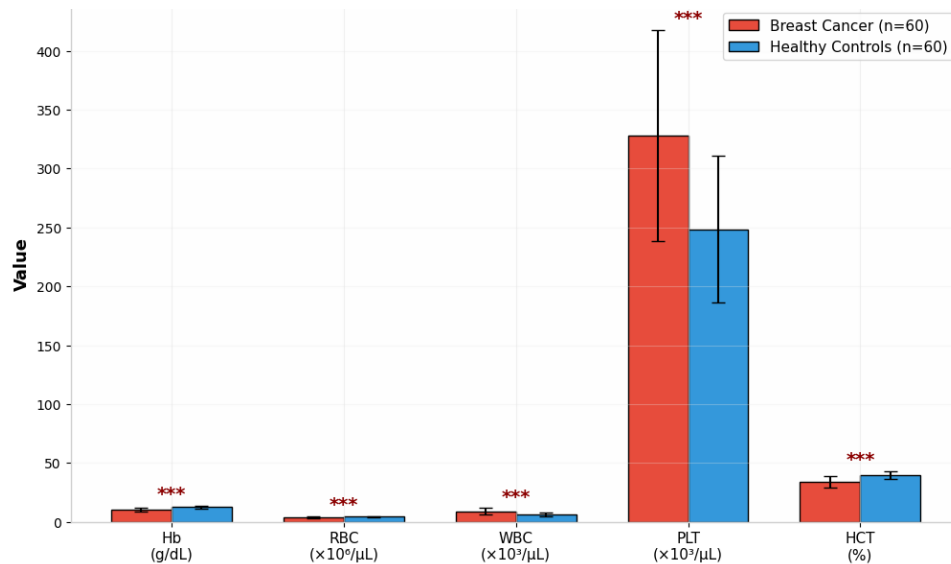


Figure 2. Comparison of Complete Blood Count Parameters Between Breast Cancer Patients and Healthy Controls. Data are presented as mean $\pm$ SD. * $p < 0.001$**

Glycated Hemoglobin (HbA1c) Levels

HbA1c levels were significantly elevated in breast cancer patients compared to healthy controls. As presented in Table 5, the mean HbA1c in the breast cancer group was $6.42 \pm 1.28\%$, compared to $5.28 \pm 0.62\%$ in the control group ($p < 0.001$). Notably, 31.7% of breast cancer patients had HbA1c levels in the prediabetic range (5.7–6.4%), and 16.7% had diabetic-range HbA1c ($\geq 6.5\%$), whereas only 15.0% and 3.3% of controls fell into these categories, respectively. These findings suggest a potential metabolic dysregulation associated with breast cancer pathogenesis in this population.

Table 5. Comparison of HbA1c Levels and Glycemic Categories Between Groups

HbA1c Parameter	Breast Cancer Group (n=60)	Healthy Control Group (n=60)	t/ χ^2	p-value
HbA1c (%)	6.42 ± 1.28	5.28 ± 0.62	$t=6.18$	$<0.001^{***}$
Normal ($<5.7\%$), n (%)	31 (51.7%)	49 (81.7%)	$\chi^2=10.84$	0.001^{**}
Prediabetic (5.7–6.4%), n (%)	19 (31.7%)	9 (15.0%)	—	—
Diabetic ($\geq 6.5\%$), n (%)	10 (16.7%)	2 (3.3%)	—	—
Fasting Glucose (mg/dL)	108.4 ± 24.6	89.2 ± 12.8	$t=5.42$	$<0.001^{***}$

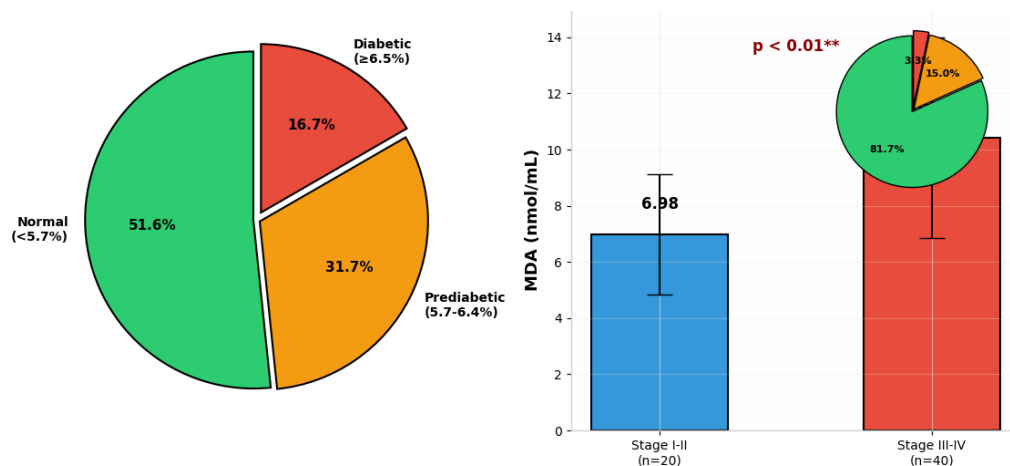


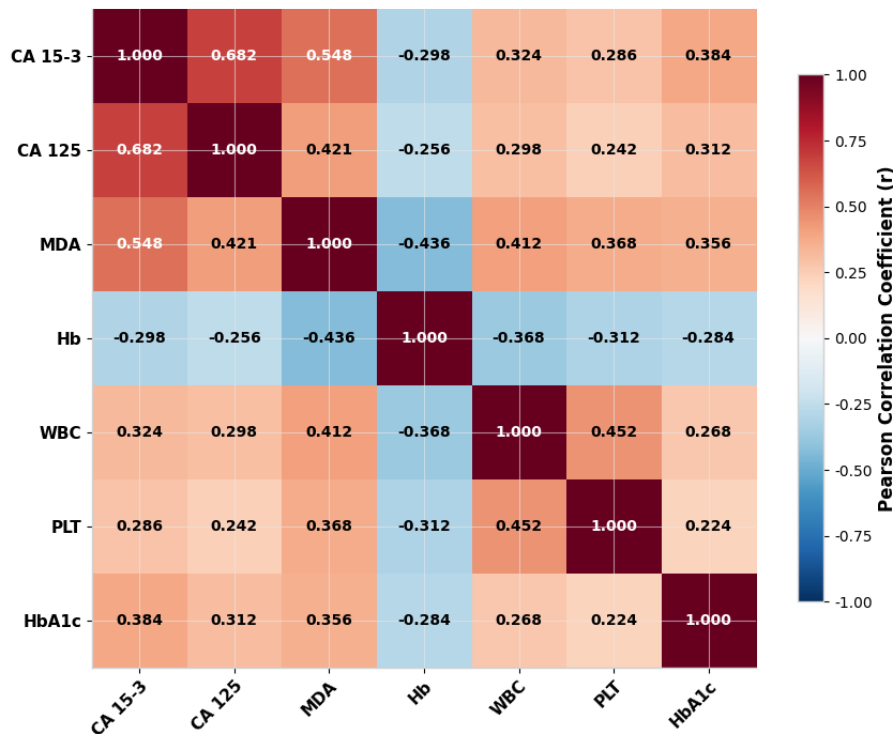
Figure 3. (Left) HbA1c Distribution in Breast Cancer Patients Showing Prediabetic and Diabetic Categories. (Right) MDA Levels by Disease Stage, with HbA1c Distribution in Healthy Controls (inset). ** $p < 0.01$

Correlation Analysis

Pearson correlation analysis within the breast cancer group revealed several significant associations (Table 6). CA 15-3 showed a strong positive correlation with CA 125 ($r=0.682$, $p < 0.001$) and MDA ($r=0.548$, $p < 0.001$), indicating concurrent elevation of tumor burden and oxidative stress. MDA levels were negatively correlated with hemoglobin ($r=-0.436$, $p < 0.01$) and positively correlated with WBC count ($r=0.412$, $p < 0.01$). HbA1c demonstrated moderate positive correlations with CA 15-3 ($r=0.384$, $p < 0.01$) and MDA ($r=0.356$, $p < 0.05$), suggesting a link between metabolic dysregulation and tumor progression.

Table 6. Pearson Correlation Coefficients Among Studied Parameters in Breast Cancer Patients (n=60)

Variable	CA 15-3	CA 125	MDA	Hb	HbA1c
CA 125	r=0.682***	—	—	—	—
MDA	r=0.548***	r=0.421**	—	—	—
Hb	r=-0.298*	r=-0.256	r=-0.436**	—	—
WBC	r=0.324*	r=0.298*	r=0.412**	r=-0.368*	—
HbA1c	r=0.384**	r=0.312*	r=0.356*	r=-0.284	—

**Figure 4. Pearson Correlation Matrix Heatmap Among Studied Parameters in Breast Cancer Patients (n=60). Color intensity represents correlation strength (red: positive, blue: negative). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$**

Diagnostic Performance and ROC Curve Analysis

Receiver operating characteristic (ROC) curve analysis was performed to evaluate the diagnostic accuracy of individual and combined biomarkers in distinguishing breast cancer patients from healthy controls (Table 7). CA 15-3 demonstrated the highest individual diagnostic performance with an area under the curve (AUC) of 0.892 (95% CI: 0.834-0.950), followed by MDA (AUC=0.864, 95% CI: 0.802-0.926), CA 125 (AUC=0.798, 95% CI: 0.724-0.872), and HbA1c (AUC=0.742, 95% CI: 0.662-0.822). The combination of CA 15-3 + MDA yielded the best diagnostic accuracy (AUC=0.934, 95% CI: 0.888-0.980), while the four-biomarker panel (CA 15-3 + CA 125 + MDA + HbA1c) achieved an AUC of 0.956 (95% CI: 0.918-0.994), demonstrating superior sensitivity (91.7%) and specificity (93.3%) for breast cancer detection.

Table 7. ROC Curve Analysis for Individual and Combined Biomarkers

Biomarker/Panel	AUC	95% CI	Cut-off	Sensitivity (%)	Specificity (%)
CA 15-3	0.892	0.834-0.950	>28.5 U/mL	78.3	88.3
CA 125	0.798	0.724-0.872	>32.0 U/mL	65.0	83.3
MDA	0.864	0.802-0.926	>5.20 nmol/mL	81.7	85.0
HbA1c	0.742	0.662-0.822	>5.85%	68.3	76.7
CA 15-3 + MDA	0.934	0.888-0.980	—	86.7	90.0
Four-Biomarker Panel	0.956	0.918-0.994	—	91.7	93.3

Discussion

The present findings demonstrate a clear biochemical and hematological disturbance among women with breast cancer, characterized by increased tumor-associated biomarkers, oxidative stress, altered blood-cell parameters, and impaired glycemic regulation. The marked elevation of CA 15-3 supports its established role as a circulating biomarker associated with breast cancer burden and disease monitoring. However, previous evidence indicates that CA 15-3 alone has limited sensitivity and specificity for early diagnosis and is more appropriately interpreted alongside other clinical and biochemical parameters [9]. The elevation of CA 125 observed in the patients may reflect broader tumor-associated biological changes and has been reported in association with disease characteristics and progression in some breast cancer populations.

Nevertheless, its diagnostic value remains less established than CA 15-3, supporting the use of CA 125 as a complementary rather than an independent biomarker [10]. The increased MDA concentration indicates enhanced lipid peroxidation and oxidative stress in breast cancer. This finding is consistent with previous studies showing increased oxidative damage in breast carcinoma and supporting a role for reactive oxygen species in tumor development and progression. The greater oxidative burden observed in advanced disease further suggests that oxidative stress may increase with tumor progression [11].

The hematological alterations, particularly reduced hemoglobin with increased white blood cell and platelet counts, may reflect anemia and systemic inflammatory or tumor-associated responses. Such changes are biologically relevant because inflammation and altered hematopoietic activity frequently accompany malignancy [12]. The observed relationship between MDA and hematological parameters may also indicate an interaction between oxidative stress and systemic inflammatory processes [13]. The elevation of HbA1c and fasting glucose suggests disturbed glucose metabolism among the breast cancer patients. Increasing evidence supports an association between metabolic dysregulation, hyperinsulinemia, and breast cancer biology, although the relationship is complex and does not establish causality. Importantly, the correlations between CA 15-3, CA 125, MDA, and HbA1c suggest that tumor-associated activity, oxidative stress, and metabolic disturbances may occur concurrently. The superior diagnostic performance of the combined biomarker panel further supports a multimarker approach, rather than reliance on a single biochemical indicator. This is consistent with the broader concept that integrating complementary circulating biomarkers may improve discrimination between patients and healthy individuals, although these findings require validation in larger and independent cohorts before clinical application [14].

Conclusion

This study demonstrates that breast cancer is associated with a distinct biochemical profile characterized by elevated tumor-associated markers, increased oxidative stress, hematological alterations, and impaired glucose regulation. The associations among CA 15-3, CA 125, MDA, and HbA1c suggest an interaction between tumor activity, oxidative imbalance, and metabolic disturbances. Although individual biomarkers showed useful diagnostic potential, their combination provided greater discriminatory performance. Therefore, a multimarker approach incorporating tumor, oxidative-stress, and metabolic biomarkers may offer improved diagnostic value and warrants further validation in larger, independent populations. These findings are consistent with the recognized potential of circulating biomarkers and oxidative-stress indicators in breast cancer assessment, while emphasizing that serum markers should complement rather than replace established clinical and pathological evaluation.

Conflict of interest. Nil

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