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Research Article

Comparative Analysis of some Biomarkers and Heavy Metals in the Serum of Women with Breast Cancer

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Abstract

Background: Breast cancer (BC) is one of the most common malignancies among women worldwide. Biomarkers such as Interleukin-6 (IL-6), Cancer Antigen 15-3 (CA 15-3), C-reactive protein (CRP), and ferritin have been proposed as indicators of disease progression. Furthermore, chronic exposure to heavy metals such as lead, cadmium, and aluminum contributes to BC development.

Methods: A case-control study was comprised of 90 women, equally divided between BC patients and a control group of the same age. Serum levels of IL-6, CA 15-3, CRP, ferritin, and heavy metals (lead, cadmium, and aluminum) were measured. Statistical comparisons were made using t-tests, correlation analysis, odds ratios, and receiver operating characteristic (ROC) curve analysis.

Results: Breast cancer patients had significantly higher levels of IL-6 (34.87 ± 11.42 ng/ml), CA-15-3 (35.59 ± 10.79 ng/ml), CRP, and ferritin compared to the control group (p-value less than 0.001). A positive correlation was observed between IL-6 and CA 15-3 (R = 0.876) and CRP (R = 0.843). Heavy metal levels were also significantly higher in breast cancer patients than in the control group.

Conclusions: The study found an association between elevated inflammatory markers and heavy metal load in breast cancer patients. Both IL-6 and CA 15-3 demonstrated high diagnostic accuracy and could be used as non-invasive biomarkers. Furthermore, elevated levels of metals like lead (Pb), cadmium (Cd), and aluminum (Al) may indicate risk factors for breast cancer.

Keywords: Breast cancer, IL-6, CA 15-3, CRP, Ferritin, Heavy metals.

Introduction

Breast cancer (BC) is the second most common cancer diagnosed in women, after non-melanoma skin cancer. It is the leading cause of cancer mortality in women worldwide, surpassed only by lung cancer, with approximately 316,000 new diagnoses each year [1]. In the United States, it remains the leading cause of cancer mortality in women. The disease presents etiological and clinical diversity, with numerous recognized risk factors, especially those related to hormones, which differ by breast cancer subtype [2]. Survival rates have increased significantly in recent decades thanks to advances in mammographic screening and improved treatments. However, this progress has not been observed uniformly across ethnicities, races, or breast cancer subtypes, including triple-negative breast cancer [1].

Cancer antigen 15-3 (CA 15-3) is a widely investigated serum tumor marker, especially in advanced and metastatic breast cancer. CA 15-3 is a soluble variant of MUC1, a glycoprotein typically found in epithelial tissues but aberrantly overexpressed and glycosylated in breast cancer cells [3]. This atypical expression leads to increased CA 15-3 concentrations in the blood of patients with breast malignancies [4]. Interleukin-6 (IL-6) is a multifaceted cytokine implicated in inflammation and cancer advancement. In breast cancer, IL-6 serves a dual function: it promotes tumor advancement and affects the surrounding milieu [5]. Increased IL-6 levels are frequently correlated with unfavorable prognosis, including lymph node involvement, substantial tumor size, and advanced disease stage [6-7].

Ferritin is critical for iron homeostasis and is recognized to increase in response to inflammation, oxidative stress, and cancer [8]. Recent studies demonstrate markedly elevated serum ferritin levels in breast cancer patients relative to healthy persons, indicating its potential as a supplementary diagnostic marker [9-10]. In the initial phases of the

disease, increased ferritin levels may signify tumor-induced disruptions in iron metabolism, promoting cellular proliferation and tumor advancement via DNA damage from reactive oxygen species (ROS) and modified immunological responses [11].

C-reactive protein (CRP) is an acute-phase protein produced by the liver in response to systemic inflammation and has garnered significant interest in relation to breast cancer [12]. Imaging techniques, particularly mammography, remain the primary method for early detection [13-14]. Chronic inflammation is known to facilitate carcinogenesis by increasing genomic instability, angiogenesis, and immunosuppression [9]. Increased CRP concentrations have been observed in breast cancer patients compared to healthy individuals, which is associated with greater tumor grade, advanced stage, and poorer clinical outcomes [15].

Non-essential metals are elements that lack a recognized physiological function in the human body. Although not biologically necessary, they can be harmful by disrupting the balance and functionality of vital components. The term "heavy metals" is controversial; however, it generally refers to metallic and metalloid elements characterized [16-17]. Humans may be exposed to these metals through ingestion of contaminated food or water, inhalation of polluted air, or skin contact with contaminated materials in industrial, residential, or agricultural settings [18]. Long-term exposure to these metals leads to their accumulation in all body tissues. This bioaccumulation can cause various harmful effects in body tissues, such as enzyme inhibition, disruption of protein synthesis, and interference with nucleic acid activity [19]. The degree of toxicity and subsequent tissue damage depends on the properties of the metal, the route of exposure, and the amount consumed [20].

Aluminum (Al) is the third most abundant element and the predominant metal in the Earth's crust, constituting approximately 8% of its composition [21]. Its wide application spans multiple sectors, including food preservation, kitchen utensils, packaging, medicines, vaccine adjuvants, and automotive components [22]. Lead (Pb) is a hazardous heavy metal with no recognized vital biological function, widely present in the environment due to its widespread use in various industrial and domestic applications. Typical sources of exposure include contaminated water, batteries, gasoline, paints, canned foods, traditional remedies, plumbing, cosmetics, jewelry, cigarette smoke, and certain food products [23]. Once absorbed, lead is primarily bound to red blood cells and distributed to organs such as the liver and kidneys; however, approximately 90% of the lead body burden in adults is stored in bone [24]. Physiological states associated with increased bone turnover, such as pregnancy, menopause, breastfeeding, and aging, can mobilize stored lead back into the bloodstream, elevating blood lead levels [25].

Cadmium (Cd) can imitate estrogen by attaching to estrogen receptors, categorizing it as a metalloestrogen. This estrogen mimicking can stimulate the proliferation of estrogen-sensitive breast cancer cells, hence accelerating tumor growth [26]. Epidemiological studies indicate that prolonged exposure to cadmium, even at minimal levels, can disturb hormonal equilibrium [27]. A population-based study has shown a significant correlation between urinary cadmium levels and an elevated risk of breast cancer, particularly in postmenopausal women [28]. Furthermore, cadmium exposure is associated with epigenetic alterations such as DNA hypomethylation, potentially activating oncogenes implicated in breast carcinogenesis [29].

This study aims to conduct a comparative analysis of some biomarkers and heavy metals between women diagnosed with breast cancer and their healthy controls.

Materials and Methods

Patients

A case-control study design was adopted, with 90 participants participating. The study was conducted over a period extending from September 2024 to June 2025. Whole blood samples were collected from 45 breast cancer patients (aged 18–70) at the Breast Cancer Early Detection Unit at Al-Hussein Teaching Hospital, Karbala, Iraq. Personal, medical, and demographic data were collected through interviews and a structured questionnaire. A group of 45 apparently healthy women was selected from known volunteers at Al-Hussein Teaching Hospital. Blood samples were collected from the volunteers, and the participants had no history of any medical conditions. The ages of the participants were relatively similar across the study group.

Inclusion Criteria

Women presenting with classical signs of early breast cancer, such as a palpable lump, breast pain, nipple discharge, or skin changes, were included. Asymptomatic cases detected via routine screening or ultrasonography, especially in dense breast tissue, were also eligible. Only early-stage, non-metastatic lesions were considered.

Exclusion Criteria

Women previously or currently undergoing chemotherapy, as well as those diagnosed with benign breast tumors, were excluded from the study. Male breast cancer accounts for less than 1% of all cases and is therefore excluded from the scope of this report [30].

Assay

An enzyme-linked immunosorbent assay (ELISA) was used for detection of Interleukin-6 (IL-6), Cancer Antigen 15-3 (CA 15-3), and C-reactive protein (CRP).

Assay for CA 15-3

CA15-3 was determined using ELISA (BT LAB, China). A plate pre-coated with anti-CA15-3 antibodies was used. After sample addition, CA15-3 bound to the antibody, and then biotinylated antibodies and streptavidin-HRP were added. After washing, the color solution was added, and color developed proportionally to the amount of CA15-3. The absorbance was measured at 450 nm after the reaction was stopped. The standard curve range of CA15-3 is from 0.2 to 60 U/ml.

Assay for IL-16

Human interleukin 6 was determined using ELISA (BT LAB, China). A plate pre-coated with anti-human IL-6 antibodies was utilized. After the sample was added, the IL-6 in the sample bonded to the antibodies, and biotinylated antibodies and streptavidin-HRP were introduced. After washing, a colorimetric solution was added that changed color proportionally to the concentration of IL-6, and the absorbance was measured at 450 nm after the reaction was halted. The standard curve range of IL-6 is from 2 to 600 ng/L.

Assay for C-reactive protein

CRP-Q is a quantitative turbidimetric test for the measurement of C-reactive protein (CRP) in human serum or plasma. Latex particles coated with specific anti-human CRP antibodies are agglutinated when they react with samples containing CRP. The agglutination produces an absorbance change, which is dependent upon the CRP content of the patient sample. The CRP concentration can be determined by comparison to a calibration curve (GIESSE, Italy). The normal range of CRP is from 6 to 8 mg/L.

Assay for ferritin

Latex particles coated with specific anti-human ferritin antibodies are agglutinated when they react with samples containing ferritin. The latex particles agglutinate in proportion to the ferritin concentration in the sample and can be measured by turbidimetry (GIESSE, Italy). The normal range of ferritin is from 10 to 120 µg/L.

Measurement of metals in serum sample

Metal examination was conducted in the laboratories of the biochemistry department at the College of Medicine at Karbala University. by using Atomic Absorption Spectroscopy (SHIMADZU AA-6300/Japan) as an analytical technique, which is used for the analysis of trace amounts of metals in the entire range of natural and manmade materials such as geological samples, environmental samples, biological specimens, agricultural produce and soils, pharmaceuticals, foods, and drinking water. One of the main types of Atomic Absorption Spectroscopy (AAS) used in this research was the graphite furnace system (GF-AAS).

Ethical approval

The study obtained ethical approval from the College of Medicine at the University of Karbala at document NO 2446 on 24 June 2025, with additional approval from the administration of Al-Hussein Teaching Hospital, specifically from the Breast Cancer Early Detection Unit. Informed consent was obtained from each participant after a clear explanation of the study objectives and procedures.

Statistical analyses

Statistical analyses were conducted utilizing IBM SPSS Statistics for Windows, version 26.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics were utilized to encapsulate the data for each category. Continuous data were provided as means with standard deviations, whereas categorical variables were expressed as frequencies and associated percentages. The Kolmogorov-Smirnov test was utilized to evaluate the normality of the data distribution.

For data following a normal distribution, parametric inferential statistical methods were used. The two independent samples. The t-test was applied to compare continuous variables between two independent groups, whereas the one-way ANOVA test was used for comparisons across more than two groups. The Pearson correlation coefficient was used to assess relationships between biomarker levels.

To evaluate associations between variables, odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using non-conditional logistic regression analysis. Significant differences in categorical parameters were also assessed using appropriate statistical tests.

All hypothesis testing was conducted using two-tailed tests, with p-values less than 0.05 considered statistically significant. Additionally, receiver operating characteristic (ROC) curve analysis was

performed to determine the optimal cut-off values for biomarkers, maximizing both sensitivity and specificity in identifying critical cases.

Results

Table 1 describes the socio-demographic characteristics of the participants. It highlights that the age distribution is skewed, with the majority of patients falling in the 41–60-year age range, whereas the control group predominantly consists of those between 51 and 60 years old (Figure 1). The BMI data reveals a tendency toward higher body weight among patients, with more individuals categorized as overweight, while the control group had a higher proportion of individuals with overweight (Figure 2). Chronic diseases were entirely absent in the control group but were present in nearly 18% of the patients, with conditions such as hypertension, diabetes, or both being most common. Interestingly, all control participants reported not using antiperspirants, while almost 89% of patients did, suggesting a potential behavioral difference between groups.

Table 2 focuses on the comparison of key biomarkers between the control and patient groups. It demonstrates significantly elevated levels of IL6, CA15-3, CRP, and ferritin in the patient group, with all differences being statistically significant (p $2.41x10^{-25}$).

Table 1: Socio-demographic characteristics

Characteristics		Group	
		Control	Patients
Age groups (year)	20-30	5 (11.1%)	5 (11.1%)
	31-40	5 (11.1%)	3 (6.7%)
	41-50	10 (22.2%)	16 (35.6%)
	51-60	25 (55.6%)	21 (46.7%)
BMI (kg/m2)	Under weight	1 (2.2%)	0
	Normal weight	16 (35.6%)	16 (35.6%)
	Overweight	23 (51.1%)	22 (48.9%)
	Obese	5 (11.1%)	7 (15.6%)
Chronic disease	No	45 (100%)	37 (82.2%)
	Hypertension	0	1 (2.2%)
	Diabetes mellitus	0	2 (4.4%)
	Hypertension and Diabetes Mellitus	0	4 (8.9%)
	Rheumatoid	0	1 (2.2%)
D (antiperspirant)	No	45 (100.0%)	(11.1%) 5
	Yes	0	(88.9%) 40

BMI: Body mass index

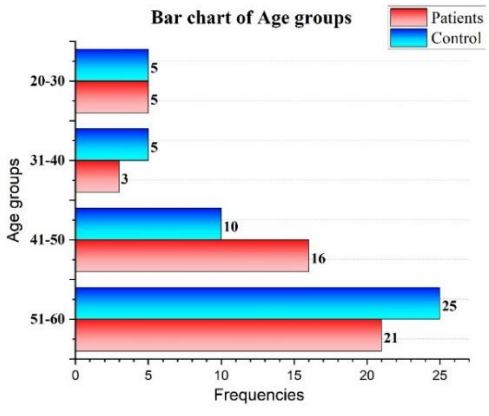

Figure 1: Bar chart of Age Groups

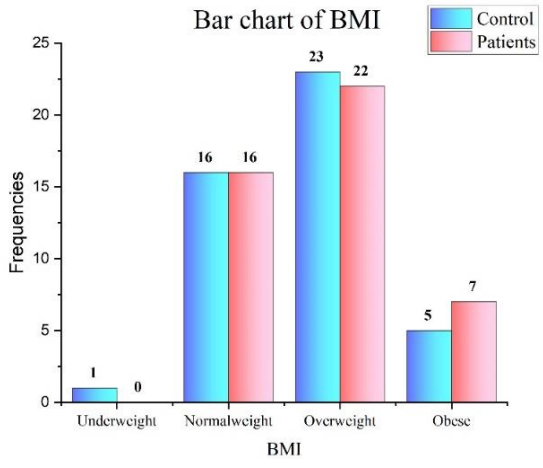

Figure 2: Bar chart of body mass index groups

Table 2: Comparison between studied groups according to IL-6 and CA15-3

Markers	Control (n=45) (mean ± SD)	Patients (n=45) (mean ± SD)	p-value
IL-6 (ng/ml)	9.33±2.462	34.871±11.428	$2.41x10^{-25}$
CA15-3 (ng/ml)	11.958±1.732	35.591±10.798	$4.83x10^{-25}$
C-Reactive Protein (ng/ml)	1.598±1.136	7.142±1.276	$3.40x10^{-37}$
Ferrtin (ng/ml)	42.733±14.428	131.911±30.332	$6.31x10^{-31}$

N: Number, SD: Standard deviation

The t-test is significant at a p-value less than 0.05.
In Table 3, the analysis extends to evaluating biomarker levels across different stages of breast cancer, including benign cases, stage 2, and stage 3. A progressive increase in both IL6 and CA15-3 levels is observed as the cancer stage advances. The statistical comparisons between benign and malignant stages, as well as between stages 2 and 3, all showed significant differences (Figures 3 and 4). Table 4 explores the variation in IL6 and CA15-3 levels across different age groups within the patient population. Results indicate that both markers increase with age, particularly peaking in the 51–60-year age group. The relationship between age and biomarker levels is statistically significant, which suggests that age may influence the tumor marker profile in breast cancer patients.
Table 5 examines how BMI influences biomarker levels. Obese patients exhibited the highest levels of IL6 and CA15-3, followed by overweight and then normal-weight individuals. While the increase in IL6 was statistically significant, the trend in CA15-3 levels approached significance, suggesting a possible link between higher body weight and elevated biomarker levels in breast cancer.

Table 3: Comparison between cancer stages according to IL-6 and CA 15-3 for patients with breast cancer

Marker	Stage			p-value		
	Benign (n=8) (mean ± SD)	Stage 2 (n=12) (mean ± SD)	Stage 3 (n=25) (mean ± SD)	B and 2	B and 3	2 and 3
IL-6 (ng/ml)	19.913±2.956	33.156±5.673	48.913±2.956	$5.42*10^{-7}$	$6.26*10^{-8}$	$2.39*10^{-7}$
CA 15-3 (ng/ml)	19.175±3.909	34.564±4.581	48.675±5.079	$1.21*10^{-9}$	$4.69*10^{-11}$	$5.34*10^{-10}$

N: Number, SD: Standard deviation, B: Benign, 2: Stage 2, 3: Stage 3
The t-test is significant at a p-value less than 0.05.

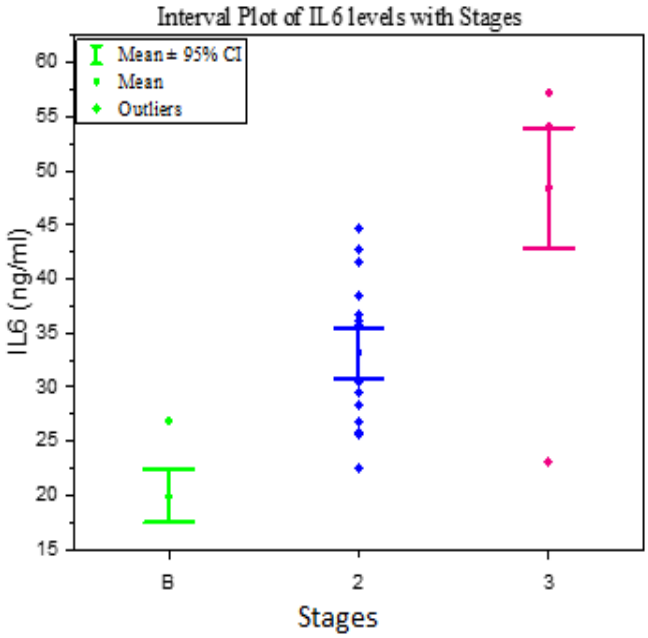

Figure 3: Interval Plot of IL-6 according to stages

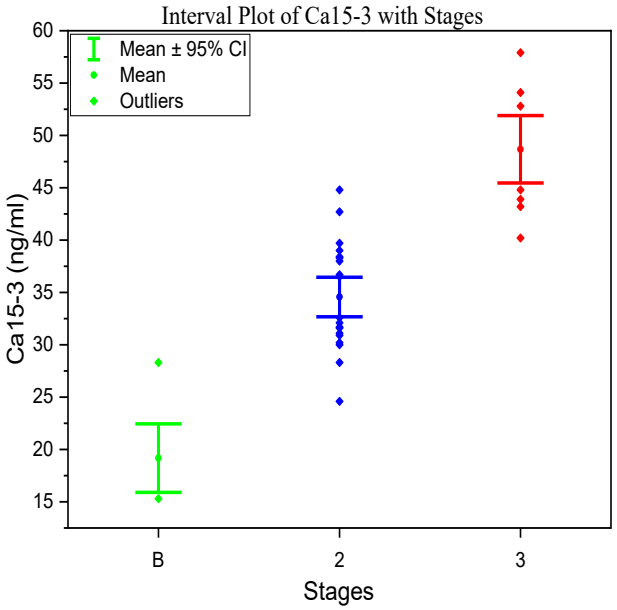

Figure 4: Interval plot of CA 15-3 according to stages

Table 4: Comparison between age according to IL-6 and CA 15-3 for patients with breast Cancer

Marker	Age (year)				p-value
	20-30 (n=5) (mean ± SD)	31-40 (n=3) (mean ± SD)	41-50 (n=16) (mean ± SD)	51-60 (n=21) (mean ± SD)	
IL-6 (ng/ml)	21.660±5.278	34.867±15.730	36.113±12.124	37.071±9.839	0.048
CA 15-3 (ng/ml)	24.540±10.434	34.033±13.205	37.206±10.991	37.214±9.537	0.101

N: Number, SD: Standard deviation, one-way ANOVA is significant at p-value less than 0.05.

Table 6 investigates whether the presence of chronic diseases among patients affects IL6 and CA15-3 levels. Although those with chronic conditions showed slightly higher marker levels, the differences were not statistically significant, indicating that chronic diseases may not be a major confounding factor in this context.

Table 7 presents correlation coefficients between IL6, CA15-3, and other inflammatory markers such as CRP and ferritin. Strong and statistically significant positive correlations are reported across all pairs, especially between IL6 and CA15-3, as well as between these markers and CRP or ferritin.

Table 8 provides the odds ratios for IL6 and CA15-3 with regard to breast cancer risk. Both markers showed statistically significant odds ratios above 1, indicating that higher levels of these markers are associated with increased odds of having breast cancer. The odds ratio for IL-6 was 1.331 (95% confidence interval: 1.172–1.512, $P < 0.001$), indicating that each one-fold increase in IL-6 levels was associated with a 33% increased risk of breast cancer. The odds ratio for CA 15-3 was 1.208 (95% confidence interval: 1.127–1.296, $P < 0.001$), indicating that an elevated tumor marker was associated with a 21% relative increased risk of developing the disease.

Table 9 presents the diagnostic performance of IL6 and CA15-3 through ROC analysis. Both markers demonstrated excellent discriminatory ability between patients and controls, with area under the curve (AUC) values exceeding 0.93 (Figures 5 and 6). Sensitivity and specificity values were also high (88.9% and 80.0%), confirming the reliability of these biomarkers in distinguishing between healthy individuals and those with breast cancer at defined cutoff values. The AUC values for both IL-6 and CA 15-3 were approximately 0.931 and 0.941, respectively, indicating high diagnostic accuracy. Both demonstrated a strong balance between sensitivity and specificity, with IL-6 being 88.9% sensitive at a cutoff of 13.51 ng/ml and CA 15-3 being 82.2% sensitive at a cutoff of 27.5 ng/ml, and both having a specificity of 80%.

Table 5: Comparison between body mass index (BMI) according to IL-6 and CA 15-3 for patients with breast cancer

	Body mass index (BMI)			
Marker	**Normal weight (n=7) (mean ± SD)**	**Overweight (n=22) (mean ± SD)**	**Obese (n=16) (mean ± SD)**	**p-value**
IL6 (ng/ml)	30.786±12.108	31.986±9.779	40.625±11.633	< 0.038
CA153 (ng/ml)	31.314±14.048	33.568±9.290	40.244±10.229	< 0.087

N: Number, SD: Standard deviation

One-way ANOVA is significant at a p-value less than 0.05

Table 6: Comparison between chronic disease according to IL-6 and CA 15-3 for patients with breast cancer

	Chronic disease		
Marker	**No (n=37) (mean ± SD)**	**Yes (n=8) (mean ± SD)**	**p-value**
IL-6 (ng/ml)	34.448±11.437	36.825±11.954	0.600
CA 15-3 (ng/ml)	35.395±10.556	36.500±12.599	0.796

N: Number, SD: Standard deviation

The t-test is significant at a p-value less than 0.05.

Table 7: Correlation coefficients of IL-6 and CA 15-3 with C-reactive protein and ferritin

Marker		**IL-6**	**CA 15-3**	**CRP**	**Ferritin**
IL-6	R		0.876	0.843	0.870
	p-value		$1.28*10^{-29}$	$2.02*10^{-25}$	$1.03*10^{-28}$
CA 15-3	R	0.876		0.837	0.840
	p-value	$1.28*10^{-29}$		$8.35*10^{-25}$	$4.16*10^{-25}$

CRP: C-reactive protein, R: Pearson correlation coefficient

Table 8: Odds ratio of IL-6 and CA 15-3 according to studied groups

Marker	**Odds Ratio**	**CI 95%**		**p-value**
		Lower	**Upper**	
IL-6	1.331	1.172	1.512	$1.10*10^{-5}$
CA 15-3	1.208	1.127	1.296	$1.12*10^{-7}$

CI: Confidence Interval, Association significant at p-value less than 0.05

Table 9: Sensitivity and specificity of IL-6 and CA 15-3 between control and patients' groups

Marker	**AUC**	**p-value**	**Cutoff value**	**Sensitivity**	**Specificity**
IL-6	0.931	$1.18*10^{-12}$	13.51 ng/ml	88.9%	80.0%
CA 15-3	0.941	$5.42*10^{-13}$	27.5 ng/ml	82.2%	80.0%

AUC: Area Under Curve

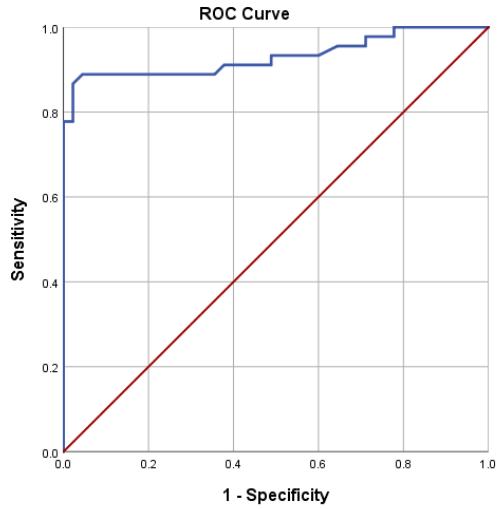

Figure 5: Receiver Operating Curve (ROC) of IL-6 between control and patients' groups

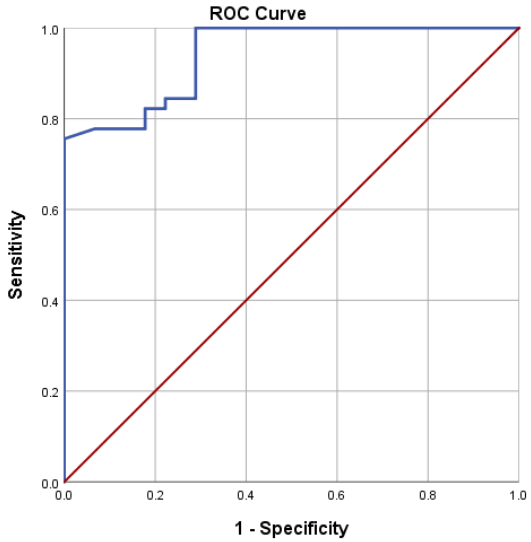

Figure 6: Receiver Operating Curve (ROC) of CA 15-3 between control and patients' groups

Table 10 shows there are significant differences between the control group and the patients' group at Al, Cd, and Pb, with a P-value less than 0.05. Lead concentrations increased from 1.306 ± 1.409 to 4.824 ± 7.372 µg/L (Figure 7), while aluminum values in the affected group were 30.122 ± 18.997 compared to 4.446 ± 4.667 in the healthy group (Figure 8). Cadmium showed the largest significant difference (29.728 ± 15.056 versus 3.235 ± 2.621), with a p-value < 0.001 (Figure 9).

Discussion

This study evaluated the levels of inflammatory markers, tumor antigens, and heavy metals in the serum of breast cancer patients compared to the control group. Marker results showed significantly elevated levels of interleukin-6 (IL-6), cancer antigen 15-3 (CA15-3), C-reactive protein (CRP), ferritin, and toxic heavy metals (lead, cadmium, and aluminum) in breast cancer patients. Age was also observed to play a role, as most breast cancer patients were between 41 and 60 years old. This is consistent with previous findings that the risk of breast cancer increases significantly with age due to cumulative exposure to hormones and genetic instability [1-2]. BMI analysis also revealed that breast cancer patients were more likely to be overweight or obese, suggesting that obesity increases the risk of breast cancer, possibly through estrogen production and chronic inflammation [6].

On the other hand, antiperspirants are products that reduce sweating by temporarily blocking sweat glands, usually using aluminum compounds, which may have a role, as 89% of patients used antiperspirants [3]. IL-6 and CA15-3 levels were significantly higher in patients compared to the control group in this study. IL-6 is a cytokine with multiple functions, including tumor proliferation, angiogenesis, and immune evasion [7, 9-10]. CA15-3, commonly used to monitor disease recurrence, showed significantly higher levels even in early-stage patients, suggesting the marker's potential as a diagnostic tool [14-15]. Both markers also showed a gradual increase from benign lesions to stage III malignancy, with significant differences across all stages ($p < 0.001$), supporting their application in disease staging [16]. Furthermore, IL-6 and CA15-3 levels were closely correlated, suggesting their mutual involvement in breast tumors. Both IL-6 and CA15-3 levels increased with advancing patient age, peaking in the 51–60 age group, likely due to the combined effect of inflammation and decreased immunomodulation [17-18, 20]. IL-6 levels significantly increased with BMI, reflecting the inflammatory nature of adipose tissue. CA15-3 also showed a similar trend; the p-value (0.087) indicates a lower but still significant association. Obesity can also promote tumor growth by disrupting cytokine balance and increasing estrogen levels [22-24]. Interestingly, chronic diseases such as hypertension or diabetes did not significantly affect marker levels, suggesting that the tumor-driven immune response takes priority over inflammation associated with comorbidities [25-26].

The IL-6 concentration in the patients of this study was significantly elevated. IL-6 is an important inflammatory cytokine in tumor progression, promoting proliferation, immune evasion, and distant metastasis in breast cancer cells [7]. During the study, the patients had a significantly higher mean CA 15-3 level than in the control group, indicating a significant increase in the risk of relapse and poor outcome.

Table 10: Significant differences between control group and the patients' group at Al, Cd, Pb, with a p-value less than 0.05

Metal concentration	Control (n=45) (mean ± SD)	Patients (n=45) (mean ± SD)	p-value
Pb	1.306±1.409	4.824±7.372	0.002
AL	4.446±4.667	30.122±18.997	$1.04*10^{-13}$
Cd	3.235±2.621	29.728±15.056	$1.76*10^{-19}$

Pb: Lead, Cd: Cadmium, AL: Aluminum

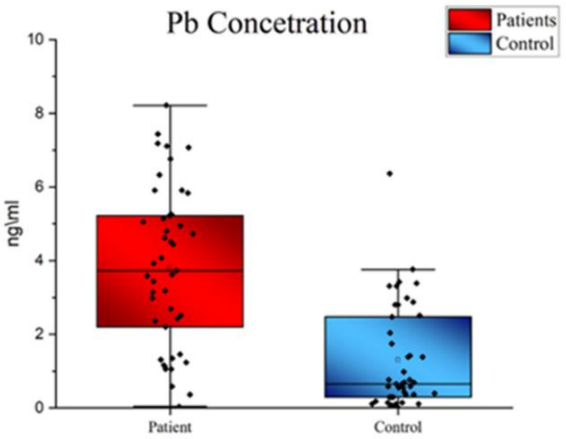

Figure 7: Lead concentration in patient and control

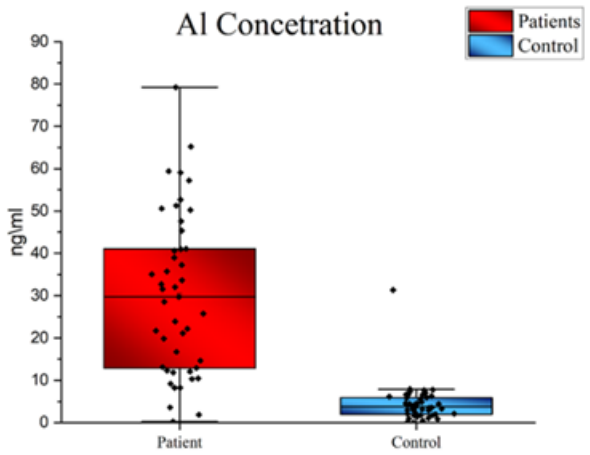

Figure 8: Aluminum concentration in patient and control

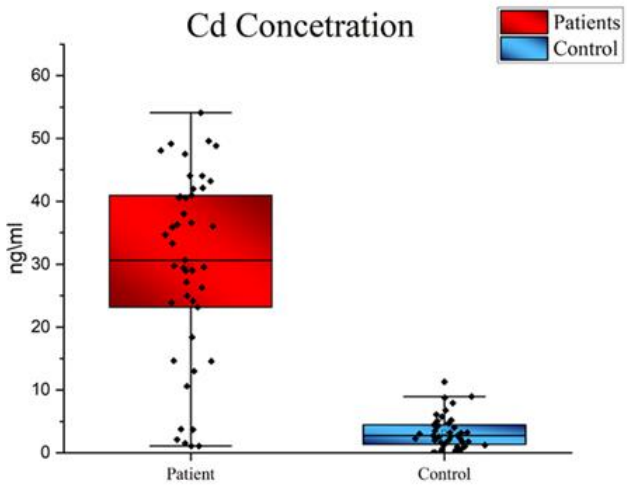

Figure 9: Cadmium concentration in patient and control

This marker is frequently used in breast cancer surveillance, and high levels have been associated with an increased risk of relapse and poor outcome [14]. Regarding ferritin, its levels were significantly higher in breast cancer patients than in the control group. Ferritin is the body's iron store and can be elevated in cases of inflammation or cell growth. Some studies have linked elevated ferritin to an increased risk of breast cancer [28-29, 31]. The interaction of the three elevated biomarkers (IL-6, CRP, and ferritin) suggests their potential use together to aid diagnosis, especially in the early stages of the disease. They were significantly elevated in breast cancer patients, indicating an active inflammatory state. Correlation analysis also indicated a significant and consistent positive correlation between these markers, particularly IL-6 and CA 15-3, suggesting a complex interaction between inflammation and tumor activity.

This study supports recent research indicating that the IL-6 marker stimulates an inflammatory response during tumor growth by activating the MUC1 protein signaling pathway, the primary source of the CA 15-3 antigen [11-14]. The correlation between IL-6 and CRP is consistent with the physiological role of IL-6 in stimulating the liver to

produce CRP, which measures the severity of systemic inflammation associated with cancer [32]. On the other hand, ferritin was closely correlated with both IL-6 and CA 15-3 markers, suggesting that disturbances in iron metabolism may play a role in the tumor environment, especially since iron is essential for the proliferation of rapidly proliferating cells such as cancer cells [33]. These findings support the concept that the inflammatory environment and metabolic activity play a role in the development of breast cancer. They also suggest that these markers can be used together not only for diagnosis but also to measure disease severity or response to treatment. Several studies have also shown that these markers have prognostic significance in the early stages of cancer [14, 34].

Regression analysis results showed a significant association between IL-6 and CA 15-3 in breast cancer patients. The results of this study are consistent with previous research showing that IL-6 is not only an inflammatory marker but also helps create a tumor-friendly microenvironment, which leads to enhanced tumor growth and immune escape [7, 35]. On the other hand, the CA 15-3 marker is a validated biomarker for tracking breast cancer progression, and several studies have found that elevated levels, even within normal limits, may be associated with an increased risk of recurrence or tumor spread [14]. The scientific importance of these two markers is not limited to diagnosis alone; they can also be used in risk prediction models or in building focused screening regimens for women at high risk, especially in resource-limited countries where routine mammography is challenging. These findings highlight the need to combine inflammatory and tumor markers in a comprehensive clinical assessment, as demonstrated by recent European recommendations to reduce reliance on imaging methods alone [36].

Receiver operating characteristic (ROC) curve analysis results showed that both IL-6 and CA 15-3 significantly discriminated between women with breast cancer and the control group. These results suggest that these markers could be used as useful non-invasive techniques for early detection or to complement the results of established screening procedures such as mammography. Recent research has confirmed these findings, with several studies demonstrating that IL-6 is not just an inflammatory marker but also an early indicator of cancer risk [31]. CA 15-3 has historically been used to monitor tumor growth, but its elevation in the early stages of the disease may serve as a diagnostic aid [32].

Finally, the results showed clear and very significant differences in the mean concentrations of heavy metals (lead, aluminum, and cadmium) between women with breast cancer and the control group, with the breast cancer group recording significantly higher levels. These findings suggest a potential link between the accumulation of toxic metals in the body and the development of breast cancer, as these elements are known to have endocrine disruptors, mimic estrogen, and cause DNA damage through oxidative stress [33, 37]. A recent European study within the EPIC project revealed that levels of several heavy metals, particularly cadmium, were higher in breast cancer patients, even after accounting for known risk factors [38]. Furthermore, a 2022 meta-analysis found that excessive levels of lead and cadmium in the blood or tissues were associated with a 30% increased risk of breast cancer [39].

Conclusions

This study found a correlation between elevated serum levels of hazardous heavy metals, lead (Pb), cadmium (Cd), and aluminum (Al), and breast cancer. Heavy metal levels were significantly higher in women with breast cancer than in healthy women, suggesting a potential environmental influence on the etiology of the disease. Furthermore, levels of inflammatory and tumor markers, such as interleukin-6 (IL-6), CA-15-3, CRP, and ferritin, were significantly elevated in breast cancer patients and showed strong correlations, supporting the relationship between systemic inflammation and tumor biology. Furthermore, both IL-6 and CA-15-3 demonstrated excellent diagnostic performance, with high sensitivity, specificity, and AUC values, and their likelihood ratios revealed significant predictive value for breast cancer risk. Combining heavy metal assessment with standard tumor markers may provide additional benefit in early diagnosis and risk stratification of breast cancer. Future breast cancer screening and diagnosis programs, particularly in environmentally exposed populations, are recommended to include assessment of serum heavy metals in addition to known tumor and inflammatory markers such as IL-6 and CA-15-3. Furthermore, larger-scale multicenter studies are needed to confirm the reported relationships and investigate the molecular processes through which heavy metals may influence tumor formation and progression. Public health policy should also consider environmental monitoring and

mitigation initiatives to reduce heavy metal exposure, especially in areas with significant industrial or agricultural pollution.

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