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Serum Chemerin, Osteopontin, IL-3 and IFN-γ as Diagnostic Biomarkers for Breast Cancer in Iraqi Women: A Comparative Cross-Sectional Study

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Abstract Breast cancer remains the leading causes of cancer-related mortality among Iraqi women, with rising incidence and younger age at diagnosis compared to Western population. Early detection is critical for improving survival outcomes, yet current diagnostic practices primarily depend on invasive biopsy procedures that are costly, uncomfortable for patient and impractical for routine monitoring, highlighting the urgent need for exploration non-invasive method as an alternative. This study evaluated the diagnostic potential of serum chemerin, osteopontin, interleukin-3 (IL-3) and interferon-gamma (IFN-γ) as novel biomarkers for breast cancer in Iraqi women. A cross-sectional study was design and recruited 160 Iraqi women (aged 25-48 years) divided into four groups: healthy controls, newly diagnosed breast cancer patients, benign tumour patients and mastectomy patients (n = 40 each group). Serum biomarker levels of osteopontin, chemerin, IL-3 and IFN-γ were quantified using ELISA and correlation with clinicopathological parameters were analysed. Receiver Operating Characteristics (ROC) curves evaluated the diagnostic accuracy. Breast cancer patients exhibited significantly elevated serum concentration of chemerin (median: 6.65 vs 2.31 ng/mL), IL-3 (709.20 vs 328.95 pg./mL) and IFN-γ (645.58 vs 202.67 pg./mL) compared to healthy controls ($p<0.001$). However, osteopontin was lower in cancer patients (4.63 vs 9.30 ng/mL, $p<0.01$). All biomarkers demonstrated outstanding diagnostic performance achieving area under the curve (AUC) values of 1.00, which signifies both 100% sensitivity and specificity for distinguishing cancer from control. Combined biomarker panels maintained perfect discrimination. These finding suggest that chemerin, osteopontin, IL-3 and IFN-γ, may represent as promising non-invasive biomarkers for the early detection of breast cancer in Iraqi women.

Key Words Osteopontin, Chemerin, IL-3, IFN- γ, Breast Cancer

INTRODUCTION

Breast cancer represents the most frequently diagnosed malignancy and the leading cause of cancer death among women worldwide, where approximately 2.3 million new cases were recorded and estimated 685,000 deaths were reported in 2020[1]. In Iraq, breast cancer accounts for approximately about one-third of female cancers, with alarming epidemiological trends. Age-standardized incidence rates (ASIR) in Iraq were steadily increasing, from 26.6 per 100 000 in 2000 to 31.5 per 100 000 in 2009[2,3]. and even continued to increase with an Average Annual Percentage Change (AAPC) of +3.192% from year 2000 to 2019[2,3]. notably, the disease predominantly affects younger women in Iraq compared to western countries, with a significant proportion of cases diagnosed in women under 50 years of age [2]. The early identification of breast cancer greatly increases the survival rates of patients and reduces the extent of therapy that has to be used [4]. However, the traditional screening methods, particularly mammography, face inherent limitations in detecting small tumours and interpreting dense breast tissue, especially in younger women [4]. Furthermore, the tissue biopsy procedure, mandated following the identification of a suspected lesion in the breast, is both invasive and time-consuming and may fail to capture the complete heterogeneity of cancer [5].

A variety of molecular tests have been developed to enhance traditional clinicopathological prognostic factors

for breast cancer, such as lymph node metastasis, tumour size and tumour grade [6]. In recent years, growing attention has shifted toward the clinical value of circulation biomarkers, including protein, Autoantibodies, CTDNAs miRNAs and circRNAs, all of which possess potential to improve current diagnostic approaches [7,8].This has driven many studies to identify novel and minimally invasive biomarkers that can support early-stage diagnosis, prognostic assessment, treatment response monitoring and prediction of tumour progression in breast cancer patients[9,10,11].

Among the various circulating biomarkers been investigated, chemerin and osteopontin have emerges as promising candidates due to their roles in tumour microenvironment regulation and cancer progression. Chemerin is an adipokine involved in adipogenesis, metabolism and inflammation, has been implicated in various malignancy through its effects on angiogenesis, invasion dan metastasis [12]. Studies have reported changes in chemerin expression in breast cancer tissues and serum, suggesting its potential as diagnostic and prognostic marker [13]. Osteopontin, a secreted glycophospo protein, play crucial roles in cell adhesion, migration invasion and survival. Elevated osteopontin levels have been reported in several cancers including breast cancer, where it correlates with tumour aggressiveness behaviour, metastasis and poor prognosis [14].

Similarly, cytokines such as interleukin-3 (IL-3) and interferon gamma (IFN- γ) represent key roles in immune regulation and tumour immunology. IL-3, a hematopoietic growth factor, influences the immune cell proliferation, differentiation and survival [15]. Its role in cancer has been associated with the promotion of tumour growth and immune evasion mechanisms [16]. IFN- γ, a critical immunomodulatory cytokine produced primarily by T cells and natural killer cells, affects tumour immune surveillance, antigen presentation and antitumor immune responses [17]. While IFN- γ generally exhibits antitumor properties, paradoxical pro-tumorigenic effects have also been documented in certain cancer contexts [18].Previous studies have suggested the individual diagnostic potential of these markers in various cancers; however, their combined evaluation in breast cancer, particularly in the Iraqi population, remains largely unexplored [19,20].

The selection of chemerin, osteopontin, IL-3 and IFN- γ for combined analysis is based on their complementary roles in distinct aspects of breast cancer pathophysiology [21]. Despite extensive study, no adequately specific and less invasive marker has been identified to assist in the early identification, monitoring of disease progression and response to therapy in women with breast cancer. The combinatorial analysis of circulating biomarkers represents an innovative and hopeful approach, which may address the limitations associated with single biomarker assays [22,23]. While hundreds of individual biomarkers have been studied, there are still lacking about their performance in combination, especially when inflammation markers such as IL-3 and IFN- γ are paired with metabolic and structural markers like chemerin and osteopontin. This strategy also could improve early diagnosis and transform breast cancer screening and may result in better patient care and clinical results [24,25].

Therefore, this study aims to evaluate the diagnostic potential of serum chemerin, osteopontin, IL-3 and IFN- γ as biomarkers for breast cancer in Iraqi women through comparative cross-sectional analysis. The primary objective is to assess the utility of these biomarkers as an integrated diagnostic panel that could serve as a sensitive and specific non-invasive screening tool for breast cancer detection in the Iraqi woman, thereby addressing the critical gap in early diagnostic strategies for this high-risk, younger-onset population.

METHODS

Study Design and Sampling

This cross-sectional study recruited a total of 160 Iraqi women aged between 25 to 48 years who attended al-Amal National Oncology Hospital and al-Amamain al-Kadhimin Medical City Hospital in Baghdad, Iraq between September 2024 to February 2025. Participants were selected using a purposive sampling method, to ensure representation across breast conditions and disease stages.

Subjects were allocated to four groups of 40 each: (1) healthy controls with no history of breast disease, (2) newly diagnosed breast cancer patients, (3) women with histologically confirmed benign breast tumours and (4) woman who had undergone mastectomy for breast cancer. All breast cancer diagnoses were verified by an expert pathologist according to the World Health Organization (WHO) classification and staged using the TNM classification system. Tumour grading followed the Nottingham grading system [26,27].

Inclusion and Exclusion Criteria

The inclusion criteria for this study involve female participants aged between 25 and 50 years, histologically confirmed breast cancer or benign breast tumour via biopsy or fine needle Fine Needle Aspiration (FNA); apparently healthy women with no history of malignancies; willingness to provide informed consent. While the exclusion criteria eliminate individuals with other types of malignancies or systemic diseases such as diabetes mellitus, autoimmune disorders or chronic liver/kidney disease, those undergoing immunosuppressive therapy, chemotherapy or radiotherapy at the time of blood collection (except for post-mastectomy patients who have completed treatment), pregnant or lactating women and anyone with active infections or inflammatory conditions that could affect cytokine or lipid profile levels.

Sample Collection and Processing

Fasting venous blood six ml was drawn from each participant and separated into EDTA two ml and plain tubes four ml. Samples from plain tubes were allowed to clot for

30 minutes at room temperature, then centrifuged at 3500 rpm for five minutes. Serum obtained was aliquoted into two separated tubes for biomarker measurement and biochemical analysis, then stored at -80°c [28].

Biochemical Profiles

Serum lipid profiles (total cholesterol, triglycerides, HDL, LDL, VLDL) and fasting blood sugar were measured by enzymatic colorimetric assays on a chemical analyser (Cobas c311, Roche Diagnostics, Mannheim, Germany) [29].

Haematological Profiles

Full blood count was performed within two hours of collection using an automated haematology analyser (Sysmex XN-350, Kobe, Japan), measuring haemoglobin, red and white cell indices and platelet count.

Serum Concentration of Chemerin, Osteopontin, IL-3 and IFN- γ

Serum concentration of osteopontin, chemerin, IL-3 and IFN- γ were quantified in duplicate by commercial Elias kits (Elabscience, Texas, USA), according to manufacturer protocols. The absorbance was read at 450 nm using an automated microplate reader (Huma Reader HS, Wiesbaden, Germany).

Statistical Analysis

Data analyses were accomplished using Statistical Package for Social Science, version 28 (SPSS, Chicago, IL, USA). Data normality was assessed using Shapiro-Wilk tests. Non-normally distributed data were expressed as median (minimum-maximum) and compared using Kruskal-Wallis test with post-hoc pairwise comparisons (Bonferroni correction). Categorical data were compared using chi-square tests. Correlation was evaluated using Spearman's rank correlation coefficient (r). Receiver Operating Characteristics (ROC) analysis determined area under the curve (AUC), optimal cut-off value, sensitivity and specificity with 95% confidence intervals. A p-value <0.05 was considered statistically significant.

RESULTS

Demographic and Clinical Characteristics

The demographic and clinicopathological characteristics of study participant are presented in Tables 1 and 2. A total of 160 participants were included in the analysis comprising four groups, healthy control, newly diagnosed breast cancer patients, benign tumour patient and post-mastectomy patients, in which all the groups have equal number of participants (n = 40).

The median age was comparable across groups, with values of 36.50 years (range from 25-48) in controls, 37

Table 1: Clinicopathological characteristics of study groups

Parameter	Controls (N = 40)	Newly Diagnosed Breast Cancer (N = 40)	Benign Tumour (N = 40)	Post-Mastectomy (N = 40)	p value
Age (Years)					
Median (Min-Max)	36.50 (25-48)	37.00 (31-48)	36.00 (25-48)	35.00 (27-48)	0.12
BMI Groups, N (%)					
Underweight	0(0%)	0(0%)	0(0%)	0(0%)	0.73
Normal	26(65%)	26(65%)	26(65%)	22(55%)	
Overweight	14(35%)	14(35%)	14(35%)	18(45%)	
TNM Stage, N (%)					
Stage I	---	0(0%)	---	0(0 %)	—
Stage II	---	40(100%)	---	0(0 %)	
StageIII	---	0(0%)	---	35(87.5%)	
StageIV	---	0(0%)	---	5(12.5%)	
Histological Grade, N (%)					
Grade I	---	0(0%)	---	0(0%)	—
Grade II	---	40(100%)	---	37(92.5%)	
GradeIII	---	0(0%)	---	3(7.5%)	
Smoking Status, N (%)					
Non-Smoker	40(100%)	40(100%)	40(100%)	40(100%)	—
Smoker	0(0%)	0(0%)	0(0%)	0(0%)	

Table 2: Clinicopathological characteristics of study groups.

Parameter	Controls (n = 40)	Newly Diagnosed Breast Cancer (n = 40)	Benign tumour (n = 40)	Post-Mastectomy (n = 40)	p value
Alcohol Consumption, n (%)					
No	40(100%)	40(100%)	40(100%)	40(100%)	—
Yes	0(0%)	0(0%)	0(0%)	0(0%)	
Family History of Cancer, n (%)					
No	40(100%)	20(50.0%)	35(87.5%)	14(35.0%)	0.001
Yes	0(0%)	20(50.0%)	5(12.5%)	26(65.0%)	
Menopausal Status, n (%)					
Premenopausal	40(100%)	30(75.0%)	34(85.0%)	38(95.0%)	0.002
Postmenopausal	0(0%)	10(25.0%)	6(15.0%)	2(5.0%)	
Recurrent Status, n (%)					
No	---	---	---	37(92.5%)	—
Yes	---	---	---	3(7.5%)	

Table 3: Chemerin, Osteopontin, IL-3 and IFN- γ levels in patients with benign, breast cancer and control subjects.

Biomarker	Control (N = 40)	Breast cancer (N = 40)	Benign tumour (N = 40)	Post-mastectomy (N = 40)	p value
Chemerin (ng/mL), median (min-max)	2.31 (1.38-4.32) [a]	6.65 (6.10-7.47) [c]	5.72 (4.38-6.76) [b]	5.25 (2.14-6.80) [b]	0.001
Osteopontin (ng/mL), median (min-max)	9.30 (6.65-11.68) [a]	4.63 (3.71-5.46) [b]	4.60 (3.88-5.19) [b]	4.17 (3.10-5.38) [c]	0.001
IL-3 (pg/mL), median (min-max)	328.95 (176.20-633.02) [a]	709.20 (664.68-843.05) [b]	606.17 (510.59-724.81) [c]	468.85 (317.05-560.07) [d]	0.001
IFN-γ (pg/mL), median (min-max)	202.67 (180.62-246.85) [a]	645.58 (564.38-677.14) [b]	534.64 (382.34-619.02) [c]	336.80 (194.23-433.17) [d]	0.001

years (31-48) in newly diagnosed breast cancer patients, 36 years (25-48) in the benign group and 35 years (27-48) in the mastectomy group. No statistically significant difference (p = 0.12) in age was observed among the groups. This indicates good homogeneity among the groups and suggests that age did not act as a confounding factor in the present analysis. Likewise, Body Mass Index (BMI) was similar across groups (p = 0.73), with most participants classified as normal weight (55-65%) or overweight (35-45%). All participants were non-smokers and non-alcohol consumers, eliminating the influence of these potential confounding factors.

Concerning genetic and hormonal factors, the data showed a statistically significant difference in the family history of cancer (p = 0.001). Half of the breast cancer patients and 65% of post-mastectomy patients reported a positive family history, compared to much lower rates in the benign and control groups. This supports the hypothesis that hereditary predisposition plays a crucial role in breast cancer development. Similarly, menopausal status showed significant variation (p = 0.002), with a higher proportion of postmenopausal women in the breast cancer group, suggesting a link between hormonal changes and increased cancer risk tumour characteristics were valuated among the relevant clinical groups. All diagnosed breast cancer patients were classified as TNM stage II (100%). In the post-mastectomy group, most patients were at stage III (87.5%), with the remaining at stage IV (12.5%). Histologic grading showed that all diagnosed breast cancer patients had grade II tumour (100%). Among post-mastectomy patients, 92.5% were grade II and 7.5 were grade III. In addition, most mastectomy patients showed no recurrence (92.5%), whereas 7.5% had recurrent disease.

Values are presented as median (minimum-maximum) for continuous variables and as number (percentage) for categorical variables. Continuous variables were compared using the Kruskal-Walli's test. Categorical variables were analysed using the chi-square test or fisher's exact test, as appropriate. BMI categories were compared using fisher's exact test due to zero counts in the underweight category. Variables not applicable across all study groups or showing no variability were reported descriptively only. A p value <0.05 was considered statistically significant.

Serum Biomarker Concentrations

In order to evaluate immune and metabolic dysregulation linked to breast cancer, we measured serum concentrations of chemerin, osteopontin, IL-3 and IFN- γ across all study groups. These markers were chosen based on their established roles in immune regulation, inflammation and tumour microenvironment modulation. Significant differences were observed among groups for all four biomarkers (Table 3). Chemerin levels were markedly elevated in breast cancer patients (median: 6.65 ng/ml) compared to healthy controls (2.31 ng/ml, p<0.001), with intermediate levels in benign tumour patients (5.72 ng/ml) and post-mastectomy patients (5.25 ng/ml). The persistent elevation following mastectomy suggests ongoing inflammatory activity or potential residual disease burden. Surprisingly, osteopontin levels were significantly lower in breast cancer patients (4.63 ng/ml) and benign tumour patients (4.60 ng/ml) compared to healthy controls (9.30 ng/ml, p<0.001). Post-mastectomy patients showed the lowest levels (4.17 ng/ml), indicating sustained suppression after tumour removal. Both IL-3 and IFN-γ exhibited progressive elevations across varying pathological conditions. Specifically, IL-3 levels were recorded at 328.95 pg./ml in control groups, escalating to 606.17 pg./ml in benign tumours and reaching 709.20 pg./ml in breast cancer; however, a decrease was observed in post-mastectomy patients, where levels measured 468.85 pg./ml. Notably, all groups exhibited remarkable differences from one another (p<0.001). In a similar manner, IFN- γ levels were elevated in breast cancer patients at 645.58 pg./ml compared to controls, which stood at 202.67 pg./ml; intermediate levels of 534.64 pg./ml were identified in benign tumours, while there was a partial normalization observed in post-mastectomy patients at 336.80 pg./ml. These observed patterns indicate neoplasm-induced immune activation that shows partial resolution subsequent to surgical intervention.

In (Table2) data are presented as median and minimum-maximum value. Different letters within the same row indicate statistically significant differences between groups. Groups have the same letter means non-significant difference between them. Significance values have been adjusted by the Bonferroni correction for multiple ties.

Inter-Biomarker Correlation Across Disease State

This analysis evaluates the Spearman-rank correlations between chemerin, osteopontin, IL-3 and IFN- γ across the distinct clinical cohorts of healthy controls, patients with benign tumours, individuals with breast cancer and the post-mastectomy groups, to determine If the inter-relationships between these immune-metabolic biomarkers vary with disease state (Tables 4-7).

Table 4: Spearman rank correlation coefficients of chemerin, osteopontin, Il-3 and IFN- γ of control group.

Biomarker(s)	Correlation/p value	IL-3	IFN-γ	IL-3+ IFN-γ
Chemerin	*r*	0.24	0.12	0.26
	P	0.13	0.44	0.10
Osteopontin	*r*	0.009	0.71**	0.17
	P	0.95	0.001	0.27
Osteopontin + Chemerin	*r*	0.15	0.51**	0.28
	P	0.35	0.001	0.08

Table 5: Spearman rank correlation coefficients of chemerin, osteopontin, Il-3 and FN- γ of breast cancer group.

Biomarker(s)	Correlation/p value	IL-3	IFN-γ	IL-3+ IFN-γ
Chemerin	*r*	0.37*	0.08	0.44**
	P	0.01	0.60	0.004
Osteopontin	*r*	0.24	-0.10	0.007
	P	0.13	0.53	0.96
Osteopontin + Chemerin	*r*	0.41**	-0.05	0.24
	P	0.01	0.75	0.13

Table 6: Spearman Rank Correlation Coefficients of Chemerin, Osteopontin, IL-3 and IFN- γ of Benign Tumour Group.

Biomarker(s)	Correlation/p value	IL-3	IFN-γ	IL-3+ IFN-γ
Chemerin	*r*	-0.01	-0.13	-0.07
	P	0.92	0.39	0.64
Osteopontin	*r*	-0.11	-0.16	-0.19
	P	0.49	0.31	0.21
Osteopontin + Chemerin	*r*	-0.13	-0.20	-0.17
	P	0.41	0.19	0.28

Table 7: Spearman rank correlation coefficients of chemerin, osteopontin, IL-3 and IFN-γ of post-mastectomy group.

Biomarker(s)	Correlation/p value	IL-3	IFN-γ	IL-3+ IFN-γ
Chemerin	*r*	-0.16	-0.36*	-0.30
	P	0.30	0.02	0.05
Osteopontin	*r*	0.02	-0.13	-0.18
	P	0.88	0.39	0.25
Osteopontin + Chemerin	*r*	-0.10	-0.46**	-0.36*
	P	0.51	0.003	0.02

In the control group (Table 4), a strong positive correlation was observed between osteopontin and IFN- γ (r = 0.71, p = 0.001) and between the combined osteopontin + chemerin levels and IFN- γ (r = 0.51, p = 0.001), indicating that higher osteopontin levels were associated with elevated IFN-γ concentrations among healthy individuals. No significant correlations were found between chemerin and il-3 or between other cytokine pairs. In contrast, in the breast cancer group (Table 5), chemerin showed a moderate positive correlation with IL-3 (r = 0.37, p = 0.01) and a strong positive correlation with the combined IL-3 + IFN- γ (r = 0.44, p = 0.004). Similarly, osteopontin + chemerin demonstrated a significant positive correlation with IL-3 (r = 0.41, p = 0.01). These findings suggest a possible co-regulation between chemerin and IL-3 signalling pathways in the breast cancer group, which may not be evident in healthy controls.

In patients with benign tumours (Table 6), the data show no meaningful or statistically significant monotonic relationships, as all correlations were weak with coefficients close to zero (the strongest being only r = -0.20), all reported coefficients were negative suggesting only a very slight tendency for higher levels of chemerin or osteopontin to be associated with lower levels of the cytokines and none of these relationships were statistically significant as all p-values were well above the common threshold of 0.05. In the post-mastectomy group (Table 7), while most correlations were weak and non-significant, several specific, statistically significant inverse relationships were identified: a moderate negative correlation was found between chemerin and IFN-γ (r = -0.36, p = 0.02), a stronger negative correlation was observed between the combination of osteopontin + chemerin and IFN- γ (r = -0.46, p = 0.003) and a moderate negative correlation was found for the combination of osteopontin + chemerin with the combined IL-3+IFN- γ measure (r = -0.36, p = 0.02), indicating that higher levels of these biomarkers are significantly associated with lower levels of these specific immune cytokines.

Association between Biomarkers Expression with Clinicopathological Parameters

To investigate the potential clinical relevance of these immune-metabolic markers, this analysis further examines their correlations (Spearman-rank correlations) with key demographic and clinicopathological parameters, including age, BMI, family history, menopausal status, TNM stage, histological grade and recurrent status, across the control, benign, breast cancer and post-mastectomy groups, thereby assessing their utility as potential biomarkers for disease characteristics or progression (Tables 8-11). In the healthy control group In (Table 8), the correlation analysis revealed a very limited number of significant relationships. A single statistically significant correlation was identified, where IL-3 levels demonstrated a weak positive association with BMI (r = 0.32, p = 0.04). Furthermore, trends approaching significance were observed between increasing age and higher levels of both chemerin (r = 0.31, p = 0.05) and osteopontin (r = 0.29, p = 0.06). No other correlations with age or BMI were significant for the other biomarkers, indicating that in this healthy cohort, these demographic factors have a minimal and specific influence on the measured immune-metabolic profile.

In the breast cancer patient group in (Table 9), none of the correlations with the clinicopathological parameters reached statistical significance. However, several notable trends were observed. A moderate negative trend between chemerin and BMI approached significance (r = -0.29, p = 0.06), which contrasts with the lack of correlation seen in the control group. Additionally, osteopontin showed a trend toward a positive correlation with a family history of cancer (r = 0.27, p = 0.08) and IFN-γ levels tended to be higher in post-menopausal patients (r = 0.26, p = 0.10). The overall lack of strong, significant correlations suggests that in breast cancer patients, the levels of these biomarkers are likely influenced more by the disease pathology itself than by these basic demographic and historical factors.

In the benign tumour group in (Table 10), the correlation analysis revealed only one statistically significant relationship: a moderate positive correlation was observed between osteopontin levels and BMI (r = 0.37, p = 0.02). In contrast, no other significant correlations were found between

Table 8: Spearman rank correlation coefficients of chemerin, osteopontin, IL-3 and IFN- γ with age and BMI of control group.

Parameter	Correlation/p value	Chemerin	Osteopontin	IL-3	IFN-γ
Age	*r*	0.31	0.29	-0.05	0.04
	P	0.05	0.06	0.74	0.77
BMI	*r*	0.12	-0.03	0.32*	-0.01
	P	0.45	0.82	0.04	0.93

Table 9: Spearman rank correlation coefficients of chemerin, osteopontin, IL-3 and IFN- γ with clinicopathological parameters of breast cancer group.

Parameter	Correlation/p value	Chemerin	Osteopontin	IL-3	IFN-γ
Age	*r*	0.08	0.24	0.02	-0.14
	P	0.58	0.12	0.88	0.37
BMI	*r*	-0.29	-0.07	-0.02	-0.17
	P	0.06	0.63	0.85	0.27
Family history of cancer	*r*	0.12	0.27	-0.14	0.21
	P	0.44	0.08	0.37	0.18
Menopausal status	*r*	0.16	0.24	0.02	0.26
	P	0.30	0.12	0.90	0.10

Table 10: Spearman rank correlation coefficients of chemerin, osteopontin, IL-3 and IFN- γ with clinicopathological parameters of benign tumour group.

Parameter	Correlation/p value	Chemerin	Osteopontin	IL-3	IFN-γ
Age	*r*	-0.14	0.07	0.18	0.10
	P	0.36	0.65	0.26	0.52
BMI	*r*	0.11	0.37*	0.14	0.12
	P	0.48	0.02	0.38	0.44
Family history of cancer	*r*	-0.007	0.08	0.06	0.14
	P	0.96	0.58	0.70	0.38
Menopausal status	*r*	0.003	-0.04	-0.09	-0.21
	P	0.98	0.79	0.57	0.18

Table 11: Spearman rank correlation coefficients of chemerin, osteopontin, IL-3 and IFN- γ with clinicopathological parameters of post-mastectomy group.

Parameter	Correlation/p value	Chemerin	Osteopontin	IL-3	IFN-γ
Age	*r*	-0.09	-0.21	-0.01	0.02
	P	0.55	0.17	0.96	0.89
BMI	*r*	-0.10	-0.14	0.06	0.17
	P	0.52	0.38	0.68	0.27
TNM Stage	*r*	-0.01	0.14	0.12	0.08
	P	0.95	0.36	0.43	0.58
Histological Grade	*r*	-0.09	0.25	0.03	0.18
	P	0.56	0.10	0.82	0.25
Family history of cancer	*r*	0.20	0.47**	-0.09	-0.31*
	P	0.20	0.002	0.55	0.04
Menopausal status	*r*	0.09	0.33*	-0.15	-0.21
	P	0.54	0.03	0.32	0.17
Recurrent status	*r*	-0.004	0.16	-0.06	-0.16
	P	0.98	0.29	0.70	0.29

the biomarkers (chemerin, IL-3, IFN-γ) and the demographic parameters of age, BMI, family history of cancer or menopausal status. This pattern indicates that, aside from the specific link between body mass index and osteopontin, the levels of these immune-metabolic biomarkers in patients with benign tumours appear to be largely independent of the basic clinicopathological factors assessed in this analysis. In the post-mastectomy group in (Table 11), the correlation analysis revealed several statistically significant relationships, with osteopontin emerging as the most notably linked biomarker. Specifically, osteopontin showed a strong positive correlation with a family history of cancer (r = 0.47, p = 0.002) and a moderate positive correlation with menopausal status (r = 0.33, p = 0.03). Concurrently, IFN-γ demonstrated a significant negative correlation with a family history of cancer (r = -0.31, p = 0.04). In contrast, no significant correlations were found for any biomarker with age, BMI, TNM stage, histological grade or recurrent status, indicating that these specific disease progression and demographic parameters were not major determinants of the biomarker levels in this patient cohort following surgery.

Relationships with Haematological Profile

Complete blood count parameters provide insights into systemic inflammation and immune status. We evaluated correlations between biomarkers and haematological indices (Tables 12-15). In the control group in (Table 12), most correlations between chemerin, osteopontin, IL-3, IFN-γ and haematological parameters (WBC, HB, PCV, PLT) were weak and statistically non-significant. The correlation coefficients were generally close to zero, indicating no meaningful monotonic relationships. The only statistically significant finding was a moderate negative correlation between IL-3 and WBC count (r = -0.38, p = 0.01), suggesting that higher IL-3 levels were associated with slightly lower WBC values.

Table 12: Spearman Rank Correlation Coefficients of Chemerin, Osteopontin, IL-3 and IFN- γ With Blood Profile of Control Group.

Parameter	Correlation/p value	Chemerin	Osteopontin	IL-3	IFN-γ
WBC	r	-0.07	0.18	-0.38*	0.24
	P	0.64	0.24	0.01	0.13
HB	r	-0.03	0.12	0.06	0.06
	P	0.83	0.42	0.69	0.70
PCV	r	-0.02	0.18	0.13	0.22
	P	0.90	0.25	0.41	0.17
PLT	r	-0.08	0.16	-0.14	0.16
	P	0.62	0.32	0.36	0.31

Table 13: Spearman rank correlation coefficients of chemerin, osteopontin, IL-3 and IFN- γ with blood profile of breast cancer group.

Parameter	Correlation/p value	Chemerin	Osteopontin	IL-3	IFN-γ
WBC	r	0.17	0.09	0.18	-0.29
	P	0.27	0.56	0.25	0.06
HB	r	-0.001	-0.04	-0.09	-0.33*
	P	0.99	0.80	0.58	0.03
PCV	r	-0.007	0.01	0.05	-0.32*
	P	0.96	0.91	0.72	0.04
PLT	r	-0.19	0.22	0.39*	-0.26
	P	0.22	0.16	0.01	0.09

Table 14: Spearman rank correlation coefficients of chemerin, osteopontin, IL-3 and IFN- γ with blood profile of benign tumour group.

Parameter	Correlation/p value	Chemerin	Osteopontin	IL-3	IFN-γ
WBC	r	0.03	0.22	0.03	0.008
	P	0.82	0.16	0.82	0.96
HB	r	0.14	-0.09	-0.37*	-0.32*
	P	0.38	0.54	0.01	0.04
PCV	r	0.13	0.10	-0.41**	-0.46**
	P	0.40	0.50	0.008	0.003
PLT	r	0.04	0.31	0.20	0.13
	P	0.76	0.05	0.21	0.40

Table 15: Spearman rank correlation coefficients of chemerin, osteopontin, IL-3 and IFN- γ with blood profile parameters of post-mastectomy group.

Parameter	Correlation/p value	Chemerin	Osteopontin	IL-3	IFN-γ
WBC	r	0.06	0.02	-0.01	-0.27
	P	0.71	0.86	0.95	0.09
HB	r	-0.01	-0.17	-0.07	-0.14
	P	0.94	0.28	0.62	0.38
PCV	r	-0.11	-0.19	0.03	-0.07
	P	0.49	0.22	0.85	0.62
PLT	r	-0.02	0.05	0.01	0.10
	P	0.85	0.75	0.92	0.51

All other parameters, including haemoglobin (HB), packed cell volume (PCV) and platelet count (PLT), showed weak and non-significant correlations with chemerin, osteopontin, IL-3 and IFN-γ ($p > 0.05$). Overall, these results indicate that, in healthy individuals, circulating levels of these cytokines and adipokines are largely independent of routine haematological measures, except for a modest inverse relationship between IL-3 and WBC. In the breast cancer group in (Table 13), most correlations between chemerin, osteopontin, IL-3, IFN-γ and haematological parameters (WBC, HB, PCV, PLT) were weak to moderate in strength. Among these, a few relationships reached statistical significance. Specifically, IFN-γ showed a significant negative correlation with both haemoglobin (HB) ($r = -0.33$, $p = 0.03$) and packed cell volume (PCV) ($r = -0.32$, $p = 0.04$), suggesting that higher IFN-γ levels might be associated with reduced red blood cell indices in breast cancer patients.

Additionally, IL-3 exhibited a significant positive correlation with platelet count (PLT) ($r = 0.39$, $p = 0.01$), indicating that elevated IL-3 levels may be linked to increased platelet production or activation in this group. Other correlations, including those involving chemerin and osteopontin with WBC, HB, PCV and PLT, were weak and statistically non-significant (all $p > 0.05$). In patients with benign tumours in (Table 14), most correlations between chemerin, osteopontin, IL-3, IFN-γ and haematological parameters were weak and statistically non-significant. However, several notable negative correlations were observed. IL-3 showed significant inverse correlations with haemoglobin (HB) ($r = -0.37$, $p = 0.01$) and packed cell volume (PCV) ($r = -0.41$, $p = 0.008$), while IFN-γ was also negatively correlated with HB ($r = -0.32$, $p = 0.04$) and PCV ($r = -0.46$, $p = 0.003$). These findings suggest that higher levels of IL-3 and IFN-γ may be associated with reduced red blood cell indices in patients with benign tumours. Conversely, chemerin and osteopontin showed weak, non-significant correlations with all haematological parameters, in this group.

In the post-mastectomy group in (Table 15), all correlations between chemerin, osteopontin, IL-3, IFN-γ and

Table 16: Spearman rank correlation coefficients of chemerin, osteopontin, IL-3 and IFN- γ with biochemical profile parameters (lipid and glucose) of control group.

Parameter	Correlation/p value	Chemerin	Osteopontin	IL-3	IFN-γ
FBS	r	0.19	0.05	0.18	-0.17
	P	0.22	0.73	0.26	0.29
TC	r	-0.13	0.17	0.22	0.30
	P	0.40	0.28	0.16	0.05
TG	r	0.09	0.29	-0.19	0.12
	P	0.54	0.06	0.23	0.42
HDL	r	0.35*	0.28	-0.08	0.04
	P	0.02	0.07	0.60	0.76
VLDL	r	0.12	0.31*	-0.20	0.17
	P	0.46	0.04	0.20	0.28
LDL	r	-0.26	0.04	0.23	0.25
	P	0.10	0.76	0.14	0.12

Table 17: Spearman rank correlation coefficients of chemerin, osteopontin, IL-3 and IFN-γ with biochemical profile parameters (lipid and glucose) of breast cancer group.

Parameter	Correlation/p value	Chemerin	Osteopontin	IL-3	IFN-γ
FBS	r	0.33*	0.007	0.11	-0.26
	P	0.03	0.96	0.48	0.09
TC	r	-0.31*	0.09	0.01	-0.16
	P	0.047	0.58	0.91	0.32
TG	r	-0.15	-0.01	-0.06	-0.06
	P	0.33	0.92	0.69	0.68
HDL	r	0.16	-0.19	-0.11	0.12
	P	0.31	0.22	0.48	0.42
VLDL	r	-0.23	0.25	0.08	-0.05
	P	0.14	0.10	0.61	0.75
LDL	r	-0.23	-0.01	-0.003	-0.11
	P	0.14	0.93	0.98	0.49

Table 18: Spearman rank correlation coefficients of chemerin, osteopontin, IL-3 and IFN-γ with biochemical profile parameters (lipid and glucose) of benign group

Parameter	Correlation/p value	Chemerin	Osteopontin	IL-3	IFN-γ
FBS	r	-0.12	-0.08	-0.04	-0.14
	P	0.44	0.59	0.78	0.38
TC	r	-0.02	0.03	-0.20	-0.07
	P	0.86	0.81	0.21	0.63
TG	r	-0.13	-0.22	-0.06	-0.04
	P	0.42	0.15	0.68	0.77
HDL	r	0.07	0.25	-0.05	0.05
	P	0.65	0.12	0.71	0.75
VLDL	r	0.05	-0.28	-0.11	-0.13
	P	0.73	0.07	0.48	0.41
LDL	r	-0.04	0.10	-0.16	-0.05
	P	0.77	0.54	0.31	0.75

Table 19: Spearman rank correlation coefficients of chemerin, osteopontin, IL-3 and IFN-γ with biochemical profile parameters (lipid and glucose) of post-mastectomy group.

Parameter	Correlation/p value	Chemerin	Osteopontin	IL-3	IFN-γ
FBS	r	0.11	-0.20	0.01	0.09
	P	0.46	0.21	0.94	0.58
TC	r	-0.04	-0.37*	0.14	-0.35*
	P	0.79	0.01	0.37	0.02
TG	r	0.21	-0.13	-0.22	-0.35*
	P	0.18	0.39	0.17	0.02
HDL	r	-0.07	0.26	0.27	0.25
	P	0.63	0.10	0.09	0.11
VLDL	r	0.19	-0.14	-0.17	-0.34*
	P	0.22	0.36	0.28	0.03
LDL	r	-0.13	-0.35*	0.15	-0.36*
	P	0.39	0.02	0.34	0.02

haematological parameters (WBC, HB, PCV, PLT) were weak and statistically non-significant. The correlation coefficients were close to zero, indicating the absence of any meaningful monotonic relationships among these variables. None of the cytokines or adipokines showed significant associations with white blood cells, red blood cell indices (haemoglobin or PCV) or platelet count. The strongest observed trend was a weak negative, non-significant correlation between IFN-γ and WBC count (r = -0.27, p = 0.09).

Associations with Glucose Level and Lipid Profile

Lipid profile is a key laboratory test that measures blood levels of total cholesterol, low-density lipoprotein cholesterol (LDL-

C), high-density lipoprotein cholesterol (HDL-C) and triglycerides, providing essential information about cardiovascular and metabolic health [60]. To investigate the potential clinical relevance of chemerin, osteopontin, IL-3 and IFN-γ expression, this analysis further evaluates their correlations (Spearman-rank correlations) with major metabolic markers, including fasting glucose and lipid profile components (total cholesterol, LDL-C, HDL-C and triglycerides), across the control, benign, breast cancer and post-mastectomy groups, thereby determining their suitability as biomarkers reflecting metabolic dysregulation linked to disease development or progression (Tables 16-19).

In control group (Table 16), most correlations between chemerin, osteopontin, IL-3, IFN-γ and biochemical parameters (FBS, TC, TG, HDL, VLDL, LDL) were weak and statistically non-significant. However, a few significant associations were observed. chemerin showed a positive and significant correlation with HDL cholesterol ($r = 0.35$, $p = 0.02$), indicating that higher chemerin levels may be linked to increased HDL concentrations in healthy individuals. Osteopontin demonstrated a positive correlation with VLDL ($r = 0.31$, $p = 0.04$), suggesting a possible relationship with lipid transport or metabolism. All other relationships, including those involving IL-3 and IFN-γ, were weak and not statistically significant ($p > 0.05$).

In breast cancer group (Table 17), most correlations between chemerin, osteopontin, IL-3, IFN-γ and biochemical parameters were weak and statistically non-significant. However, two significant associations were observed. chemerin showed a positive correlation with fasting blood sugar (FBS) ($r = 0.33$, $p = 0.03$), suggesting that higher chemerin levels may be linked to elevated glucose levels in breast cancer patients. Conversely, chemerin exhibited a significant negative correlation with total cholesterol (TC) ($r = -0.31$, $p = 0.047$), indicating an inverse relationship between chemerin and lipid levels. In benign tumour group in (Table 18), all correlations between chemerin, osteopontin, IL-3, IFN-γ and biochemical parameters (FBS, TC, TG, HDL, VLDL, LDL) were weak and statistically non-significant. The correlation coefficients were generally close to zero, indicating the of any meaningful absence monotonic relationships among these variables. In the post-mastectomy group in (Table 19), several significant correlations were observed between osteopontin, IFN-γ and lipid profile parameters. osteopontin showed significant negative correlations with total cholesterol (TC, $r = -0.37$, $p = 0.01$), LDL ($r = -0.35$, $p = 0.02$) and trends with VLDL and triglycerides (TG), although not all reached statistical significance. Similarly, IFN-γ exhibited significant negative correlations with TC ($r = -0.35$, $p = 0.02$), TG ($r = -0.35$, $p = 0.02$), VLDL ($r = -0.34$, $p = 0.03$) and LDL ($r = -0.36$, $p = 0.02$).

Diagnostic Performance Analysis

ROC curve analysis evaluated the diagnostic accuracy of individual biomarkers and combined panels for distinguishing disease states from healthy controls, as shown in Tables 20-22.

Discriminating Breast Cancer from Controls

The ROC analysis in Table 20 demonstrates an exceptional diagnostic performance of the studied biomarkers, chemerin, osteopontin, IL-3 and IFN-γ, both individually and in combination, for predicting breast cancer. Each single biomarker exhibited an area under the curve (AUC) of 1.00, with a p-value of 0.001, indicating perfect discrimination between breast cancer patients and controls. Sensitivity and specificity were both 100% at their respective optimal cut-off values (chemerin: 5.21 ng/mL, osteopontin: 6.05 ng/mL, IL-3: 648.85 pg./mL, IFN-γ: 405.61 pg./mL), further confirming

Table 20: Area under the curve, cut-off value, sensitivity and specificity of ROC curve of single and combined markers of chemerin, osteopontin, IL-3, IFN-γ, predicting breast cancer.

Parameters	AUC	p value	Optimal Cut-off	95% CI	Sensitivity %	Specificity %
Single biomarkers						
Chemerin	1.00	0.001	5.21 ng/mL	1.00-1.00	100	100
Osteopontin	1.00	0.001	6.05 ng/mL	1.00-1.00	100	100
IL-3	1.00	0.001	648.85 pg./mL	1.00-1.00	100	100
IFN-γ	1.00	0.001	405.61 pg./mL	1.00-1.00	100	100
Combined biomarkers						
Model1: chemerin+ osteopontin	1.00	0.001	0.50 (prob.)	1.00-1.00	100	100
Model2: chemerin+ osteopontin+ IL-3	1.00	0.001	0.50 (prob.)	1.00-1.00	100	100
Model3: Chemerin+ Osteopontin+ IL-3+ IFN-γ	1.00	0.001	0.50 (prob.)	1.00-1.00	100	100

Table 21: Area under the curve, cut-off value, sensitivity and specificity of ROC curve of single and combined markers of chemerin, osteopontin, IL3, IFN-γ, predicting benign tumour.

Parameters	AUC	p value	Optimal Cut-off	95% CI	Sensitivity %	Specificity %
Single biomarkers						
Chemerin	1.00	0.001	4.28 ng/mL	1.00-1.00	100	100
Osteopontin	1.00	0.001	5.92 ng/mL	1.00-1.00	100	100
IL-3	0.97	0.001	524.80 pg./mL	0.92-1.00	94	94
IFN-γ	1.00	0.001	314.59 pg./mL	1.00-1.00	100	100
Combined biomarkers						
Model1: chemerin+ osteopontin	1.00	0.001	0.50 (prob.)	1.00-1.00	100	100
Model2: chemerin+ osteopontin+ IL-3	1.00	0.001	0.50 (prob.)	1.00-1.00	100	100
Model3: chemerin+ osteopontin+ IL-3+ IFN-γ	1.00	0.001	0.50 (prob.)	1.00-1.00	100	100

Table 22: Area Under the Curve, Cut-Off Value, Sensitivity and Specificity of ROC Curve of Single and Combined Markers of Chemerin, Osteopontin, IL-3, IFN- γ, Predicting Post-Mastectomy Breast Cancer.

Parameters	AUC	p value	Optimal Cut-off	95% CI	Sensitivity %	Specificity %
Single biomarkers						
Chemerin	0.95	0.001	4.13 ng/mL	0.91-1.00	90	90
Osteopontin	1.00	0.001	6.01 ng/mL	1.00-1.00	100	100
IL-3	0.88	0.001	370.79 pg./mL	0.80-0.96	85	85
IFN-γ	0.96	0.001	224.67 pg./mL	0.91-1.00	90	90
Combined biomarkers						
Model1: chemerin+ osteopontin	1.00	0.001	0.50 (prob.)	1.00-1.00	100	100
Model2: chemerin+ osteopontin+ IL-3	1.00	0.001	0.99 (prob.)	1.00-1.00	100	100
Model3: chemerin+ osteopontin+ IL-3+ IFN-γ	1.00	0.001	0.99 (prob.)	1.00-1.00	100	100

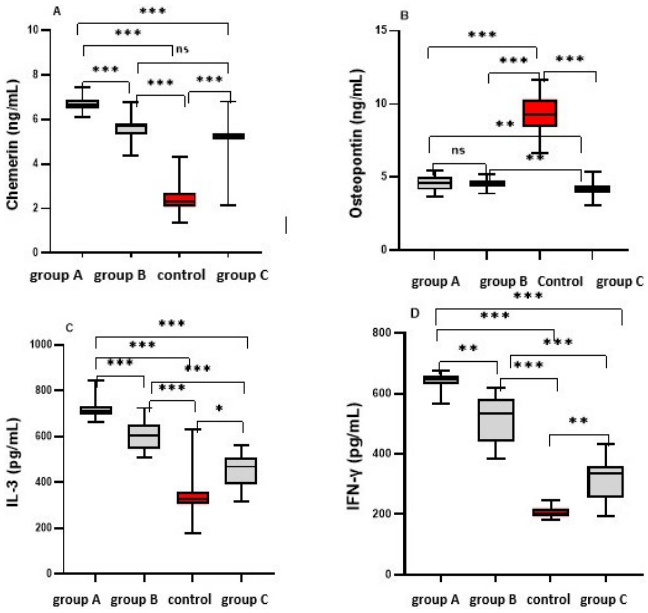

Figure 1 (A-D): Box and whisker plots of IQR with median to illustrate serum levels of (A) Chemerin, (B) Osteopontin, (C) IL-3 and (D) IFN-γ in patients with breast cancer (group A), Benign (group B), post-mastectomy (group C) and control

the strong diagnostic accuracy. The combined biomarker models (Model 1: chemerin + osteopontin, Model 2: chemerin + osteopontin + IL-3, Model 3: chemerin + osteopontin + IL-3 + IFN-γ) also achieved perfect AUC values (1.00) with 100% sensitivity and specificity, suggesting no added loss of diagnostic performance when combining these markers.

Discriminating Benign Tumours from Controls

Chemerin, osteopontin and IFN-γ achieved perfect discrimination (AUC = 1.00), while IL-3 showed slightly lower but still excellent performance (AUC = 0.97, 95% CI: 0.92-1.00) with 94% sensitivity and specificity at a cut-off of 524.80 pg./mL. Combined models achieved perfect discrimination (AUC = 1.00), demonstrating that multi-marker panels can compensate for individual marker limitations (Table 21).

Discriminating Post-Mastectomy from Controls

In Table 22, individual biomarker performance varied: osteopontin achieved perfect discrimination (AUC = 1.00), while chemerin (AUC = 0.95, 90% sensitivity/specificity) and IFN-γ (AUC = 0.96, 90% sensitivity/specificity) showed excellent performance. IL-3 demonstrated the lowest individual performance (AUC = 0.88, 85% sensitivity/specificity). Importantly, all combined biomarker models achieved perfect discrimination (AUC = 1.00, 100% sensitivity/specificity), highlighting the value of multi-marker approaches for post-treatment monitoring.

DISCUSSION

This study provides a comprehensive evaluation of the serum levels and interrelationships of chemerin, osteopontin, IL-3 and IFN-γ in Iraqi women across a spectrum of breast health, including healthy controls, patients with benign tumours, newly diagnosed breast cancer patients and post-mastectomy individuals. The results demonstrate a marked dysregulation of these biomarkers in breast cancer, reflecting their involvement in tumour-associated immune and inflammatory processes and suggest their potential utility as highly sensitive and specific diagnostic indicators in this population.

Women with benign breast tumours exhibited biomarker profiles intermediate between healthy controls and cancer patients, with important diagnostic implications. Chemerin (5.72 ng/mL) and IL-3 (606.17 pg./mL) showed elevations above control levels but below those in cancer patients, suggesting these markers reflect inflammatory processes common to both benign and malignant breast conditions rather than cancer-specific biology. This interpretation aligns with the established roles of chemerin in adipose tissue inflammation and IL-3 in general immune activation [30-32].

Critically, despite overlapping individual marker levels, benign tumour patients demonstrated fundamentally different biomarker relationships compared to cancer patients. The complete absence of significant inter-biomarker correlations in benign cases (Table 4) contrasts sharply with the coordinated dysregulation observed in cancer (Table 5), indicating qualitatively different pathological processes. This lack of integrated signalling networks in benign disease likely reflects localized inflammation without the systemic reprogramming characteristic of malignancy (Figure 1 (A-D)).

Furthermore, ROC analysis demonstrated that combined biomarker panels achieved perfect discrimination (AUC = 1.00, Table 8) between benign tumours and healthy

controls, despite intermediate individual marker levels. This finding has substantial clinical significance: these biomarkers can distinguish benign from malignant disease, potentially reducing false-positive rates a critical limitation of mammography, particularly in younger Iraqi women with dense breast tissue [5]. The single exception was osteopontin correlation with BMI in benign cases (r = 0.37, p = 0.02, Table 10), possibly reflecting adipose-associated inflammation rather than tumour biology, consistent with osteopontin expression in adipocytes [33]. This absence of correlation may reflect the non-malignant nature of benign tumours, where systemic immune and inflammatory dysregulation is less pronounced [21].

Serum chemerin was markedly elevated in breast cancer patients (6.65 ng/mL) compared to controls (2.31 ng/mL), aligning with previous reports of chemerin dysregulation in malignancy [34,45]. Chemerin, originally identified as an adipokine regulating metabolism and inflammation, exerts context-dependent effects in cancer [36]. In early disease stages, chemerin may recruit anti-tumour immune cells including natural killer cells and dendritic cells through its receptor CMKLR1, exerting tumour-suppressive effects. However, in established tumours, chronic chemerin signalling can promote a pro-tumorigenic microenvironment by recruiting tumour-associated macrophages, enhancing angiogenesis and facilitating immune evasion [37,38].

The persistent elevation in post-mastectomy patients (5.25 ng/mL), though lower than in active cancer, suggests ongoing metabolic-immune activation. This may reflect firstly, the residual inflammatory responses following surgery, presence of micro metastatic disease undetectable by conventional imaging or systemic metabolic dysfunction associated with obesity or insulin resistance, which are known chemerin-elevating conditions [39]. The correlation between chemerin and fasting glucose in cancer patients (r = 0.33, p = 0.03, Table 17) supports metabolic involvement.

Notably, chemerin's correlation pattern shifted dramatically in cancer: the emergence of significant associations with IL-3 (r = 0.37, p = 0.01) and combined IL-3+IFN-γ (r = 0.44, p = 0.004) in cancer patients (Table 5), absent in controls, suggests disease-specific metabolic-immune crosstalk. This coordinated dysregulation may represent a pathological signalling network where adipokine-driven inflammation synergizes with cytokine-mediated immune dysfunction to promote tumour progression. This hypothesis requires mechanistic validation but aligns with emerging concepts of immunometabolism in cancer [40].

The most unexpected finding was significantly lower serum osteopontin in cancer patients (4.63 ng/mL) and benign tumour patients (4.60 ng/mL) compared to healthy controls (9.30 ng/mL), with further decline post-mastectomy (4.17 ng/mL). This contrasts starkly with most literature reporting osteopontin overexpression in breast tumour tissue and elevated serum levels associated with poor prognosis [41,42]. However, our findings are not entirely without example, a previous study reported no significant difference in plasma osteopontin levels between early breast cancer patients and controls, indicating minimal prognostic impact [43].

Several mechanisms may explain this inconsistency. Firstly, population-specific genetic factors: Osteopontin is encoded by the SPP1 gene, which exhibits numerous Single Nucleotide Polymorphisms (SNPs) with functional consequences. Certain SPP1 genotypes influence circulating osteopontin levels independently of tissue expression [44]. Middle Eastern populations, including Iraqis, may harbour distinct SPP1 polymorphisms affecting serum levels. Population-specific genetic architecture could alter osteopontin secretion, stability or clearance, decoupling tissue expression from circulating concentrations. Secondly, the tissue compartmentalization**:** Osteopontin may be preferentially retained within the tumour microenvironment rather than released systemically. Cancer cells and tumour-associated stromal cells may sequester osteopontin locally, where it mediates cell adhesion, migration and survival through integrin and CD44 receptor interactions [45]. Local accumulation without systemic release would result in high tissue but low serum levels. This compartmentalization could be particularly pronounced in Iraqi women, who present at younger ages (median 37 years in our cancer group) with potentially distinct tumour microenvironment characteristics compared to Western populations where most osteopontin studies were conducted. Third is stage-dependent expression dynamics**:** Our post-mastectomy group predominantly comprised Stage III (87.5%) and Grade II (92.5%) tumours, indicating advanced disease at diagnosis (Tables 1 and 2). Osteopontin expression may follow non-linear kinetics across cancer progression. Some evidence suggests biphasic patterns where osteopontin increases in early disease but plateaus or decreases in advanced stages due to tumour necrosis, stromal remodelling or metabolic exhaustion [46]. Alternatively, specific breast cancer subtypes prevalent in our population may exhibit distinct osteopontin profiles. Lastly, the effect of post-translational modifications and isoforms**:** Osteopontin undergoes extensive post-translational modifications including phosphorylation, glycosylation and proteolytic cleavage, generating multiple isoforms with differing biological activities [47]. Standard ELISA assays may not equally detect all osteopontin variants. If Iraqi breast cancer patients generate primarily modified or cleaved forms with reduced immunoreactivity, this could result in apparently "low" levels despite normal or elevated production. Future studies employing mass spectrometry or isoform-specific assays could clarify this possibility.

Despite these possible mechanisms explaining lower absolute levels, osteopontin achieved perfect diagnostic discrimination (AUC = 1.00), confirming its clinical utility in this population. The inconsistency underlines the critical importance of population-specific biomarker validation rather than assuming universal applicability of findings from predominantly Western cohorts. Whether the decreased osteopontin represents a protective mechanism, a marker of specific tumour biology or simply population-specific

expression patterns requires further investigation with matched serum-tissue analyses.

IL-3 and IFN-γ both demonstrated significant elevation in breast cancer patients with partial normalization post-mastectomy, indicating tumour-driven immune activation. IL-3's role in breast cancer is emerging, with recent studies identifying its contribution to tumour angiogenesis, immune suppression and aggressive phenotypes, particularly in triple-negative breast cancer [48,49]. Our findings that IL-3 levels progressively increased from controls (328.95 pg./mL) to benign tumours (606.17 pg./mL) to cancer (709.20 pg./mL) before declining post-mastectomy (468.85 pg./mL) suggest IL-3 tracks disease burden. The correlation with platelet counts in cancer patients ($r = 0.39$, $p = 0.01$, Table 5B) aligns with IL-3's megakaryopoietic functions and may contribute to cancer-associated thrombocytosis, a known poor prognostic factor.

IFN-γ elevation (645.58 pg./mL in cancer vs. 202.67 pg./mL in controls) presents an apparent paradox given its conventional anti-tumour property. IFN-γ, produced by T cells and natural killer cells, enhances antigen presentation, activates macrophages and promotes Th1 immunity, all theoretically anti-tumorigenic [50]. However, chronic IFN-γ exposure induces complex adaptations including: upregulation of immune checkpoint molecules (PD-L1, IDO) facilitating immune escape, selection for IFN-γ-resistant tumour clones, paradoxical promotion of tumour cell proliferation and survival through JAK-STAT signalling, induction of immunosuppressive myeloid cells [51,52,53]. The elevated IFN-γ in our cancer patients likely represents a sustained but ineffective anti-tumour immune response that paradoxically contributes to tumour immune tolerance, consistent with findings by [54]. In estrogenic receptor-negative breast cancers. The negative correlations with haemoglobin and packed cell volume (Table 13) suggest IFN-γ may contribute to cancer-associated anaemia through inflammatory suppression of erythropoiesis, a recognized complication of chronic cytokine elevation [55].

The decline in both IL-3 and IFN-γ post-mastectomy, though not to control levels, indicates partial resolution of tumour-driven immune activation. Persistent elevation may reflect ongoing surveillance responses, subclinical inflammation or micro metastatic disease. These cytokines may therefore serve as monitoring biomarkers for disease recurrence or treatment response, a hypothesis requiring prospective validation. The big shift in biomarker correlation patterns across disease states provides insights into pathological mechanisms. In healthy controls, the strong osteopontin-IFN-γ correlation ($r = 0.71$, $p = 0.001$, Table 4) likely reflects coordinated immune surveillance, where osteopontin promotes Th1 differentiation and IFN-γ production maintains immunological homeostasis [45]. This physiological relationship completely disappeared in breast cancer, replaced by chemerin-IL-3 associations (Table 5), suggesting fundamental reprogramming from homeostatic immunity toward pathological metabolic-immune signalling.

The absence of any significant correlations in benign tumour patients (Table 6) distinguishes these lesions from malignancy at a systems level. Benign tumours may induce localized inflammatory responses without triggering coordinated systemic dysregulation, consistent with their limited growth potential and lack of metastatic capacity. This finding has diagnostic relevance: correlation network analysis could complement absolute biomarker levels in distinguishing benign from malignant disease.

The inverse correlations observed post-mastectomy (Table 7), particularly chemerin versus IFN-γ ($r = -0.36$, $p = 0.02$), represent an unexpected pattern warranting mechanistic investigation. This may be due to immune system rebalancing with restoration of regulatory circuits disrupted during active cancer; metabolic normalization reducing adipokine-driven inflammation; or compensatory counter-regulation where elevated chemerin suppresses certain immune responses [56]. Understanding these post-treatment dynamics could inform strategies to prevent recurrence.

The generally weak correlations between biomarkers and traditional clinicopathological parameters (age, BMI, tumour stage, grade) suggest these molecules primarily indicate disease presence rather than anatomical extent or differentiation status. Neither chemerin, osteopontin, IL-3, nor IFN-γ correlated with TNM stage or histological grade in post-mastectomy patients (Table 11), indicating they reflect systemic immune-metabolic dysregulation independent of tumour size or lymph node involvement. However, the significant associations between osteopontin and both family history ($r = 0.47$, $p = 0.002$) and menopausal status ($r = 0.33$, $p = 0.03$) in post-mastectomy patients (Table 11) suggest potential hereditary influences on osteopontin regulation. Family history associations could reflect inherited SPP1 polymorphisms or shared environmental factors affecting osteopontin expression. The menopausal association aligns with evidence that estrogenic influences osteopontin transcription [57]. though the predominance of premenopausal patients in our cohort complicates interpretation.

The lack of strong demographic associations enhances these biomarkers' clinical utility and they appear to specifically reflect disease biology rather than confounding factors like obesity or age, which often complicate interpretation of inflammatory markers. The metabolic correlations, particularly chemerin's positive association with fasting glucose and negative association with total cholesterol in cancer patients (Table 17), link these biomarkers to broader metabolic dysfunction. Chemerin is elevated in obesity, insulin resistance and metabolic syndrome [39]. conditions that increase breast cancer risk. The glucose association suggests chemerin may represent a mechanistic link between metabolic disease and cancer, supporting the "metabolic inflammation" hypothesis of cancer pathogenesis.

The negative correlations between IFN-γ and red blood cell indices in both cancer and benign tumour patients

(Tables 5B-C) highlight systemic inflammatory effects extending beyond the tumour itself. Chronic cytokine elevation suppresses erythropoiesis through multiple mechanisms including hepcidin upregulation, iron sequestration and direct bone marrow suppression [54,55]. This may contribute to cancer-related fatigue and reduced quality of life, suggesting potential therapeutic targets.

The perfect diagnostic performance (AUC = 1.00) of all four biomarkers for distinguishing cancer from controls represents both a strength and limitation. The exceptional accuracy reflects genuine biological separation: breast cancer induces dramatic immune-metabolic dysregulation measurable in peripheral blood. The clear-cut differences between groups (Table 2) enable complete discrimination in this cohort. However, several factors require consideration.

Sample size and overfitting: While our sample size (n = 40 per group) provides adequate statistical power, perfect AUC values suggest possible overfitting. Validation in larger, independent cohorts is essential. The slightly reduced performance of IL-3 for post-mastectomy discrimination (AUC = 0.88, Table 9) versus perfect performance for cancer discrimination (AUC = 1.00, Table 7) illustrates how performance may vary across contexts, supporting the need for extensive validation.

Population specificity: Iraqi women present with distinct breast cancer characteristics, younger age, advanced stage at diagnosis, high familial clustering, potentially enhancing biomarker separation compared to Western populations with more heterogeneous presentations. These cut-off values may not generalize to populations with different epidemiological profiles.

Clinical utility of combined panels: Interestingly, combined biomarker models (Models 2-3) did not improve upon individual marker performance when discrimination was already perfect (Tables 7-8). However, for post-mastectomy patients, multi-marker panels corrected IL-3's reduced individual performance (Table 9), demonstrating value in more challenging diagnostic contexts. In real-world clinical practice with broader patient populations, multi-marker approaches would likely enhance robustness and reduce false-positive rates [58,59].

CONCLUSIONS

This study demonstrates that serum chemerin, osteopontin, IL-3 and IFN-γ exhibit significant dysregulation in Iraqi women with breast cancer compared to healthy controls and benign tumour patients. The exceptional diagnostic performance (AUC = 1.00) of these biomarkers individually and in combination, achieving 100% sensitivity and specificity, establishes their potential as powerful non-invasive tools for breast cancer detection in this population. These results support the clinical translation of multi-biomarker panels for breast cancer screening and monitoring in Iraqi women, particularly valuable given the younger age at diagnosis and limitations of mammography in this population. However, validation in larger, prospective, multi-centre cohorts is essential before clinical implementation. Future research should clarify the mechanistic basis of these findings, evaluate longitudinal monitoring applications and assess cost-effectiveness for real-world deployment.

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Ethical Statement

This study was approved by the Nahrain University Ethics Committee (approval no: 30192 in 4/9/2024 and 110000 in 28/11/2024). All procedures were conducted in accordance with the Declaration of Helsinki (2013 revision) for research involving human participants. Written informed consent was obtained from all participants prior to sample collection and data recording.

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