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Serum Adiponectin, Leptin, and Insulin Resistance Index as Independent Metabolic–Hormonal Determinants of Breast Cancer Risk and Tumour Stage

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Abstract

Background: The metabolic and hormonal axis that connects the dysfunction of adipose tissue with breast cancer has been identified as a very important pathobiological factor, Mostly in communities with high obesity rates like populations of southern Iraq. Adiponectin, which is an adipokine that has been found to inhibit cell growth and enhance insulin sensitivity, is negatively correlated with breast cancer risk However leptin, which is an inflammatory, angiogenic adipokine, helps the tumor grow and become more invasive. The Homeostatic Model Assessment of Insulin Resistance (HOMA-IR), which is derived from fasting blood glucose and insulin levels, measures systemic insulin resistance, which is a potent breast carcinogenesis activator through the IGF-1 pathway. The current research focuses on the clinical biochemistry profile of serum adiponectin, leptin, and HOMA-IR in breast cancer patients and assesses their potential as independent biomarkers for breast cancer diagnosis and tumor stage stratification. **Methods:** We carried out a prospective case control study in Maysan Oncology Centre Amarah Iraq, where we collected 80 samples in total. The case group (n = 40) consisted of females with breast cancer that was confirmed by histopathology. These patients were at any stage of breast cancer (AJCC 8th stage) and were sampled before getting any treatment. Control group (n = 40) were healthy women matched individually by age and menopausal status. We performed all biomarker tests on blood samples taken from a fasting state. The amount of serum adiponectin and leptin was determined using a sandwich enzyme-linked immunosorbent assay (ELISA). Fasting insulin was determined by an electrochemiluminescence immunoassay (ECLIA). HOMA-IR was calculated as [fasting glucose (mmol/L) fasting insulin (IU/mL)] / 22.5. **Results:** Serum adiponectin levels were Quite a bit lower in breast cancer patients (6.34 2.18 vs. 14.82 3.64 g/mL; $P < 0.001$; Cohen's $d = 2.84$). In contrast, these patients had much higher leptin (28.74 7.63 vs. 12.46 3.82 ng/mL; $P < 0.001$; Cohen's $d = 2.71$) and HOMA-IR (4.26 1.18 vs. 1.94 0.54; $P < 0.001$; Cohen's $d = 2.54$) levels. Stage-wise changes of all the three markers showed a consistent worsening trend from AJCC Stages IIV. Besides being independent predictors of breast cancer, they were also the three factors considered by multivariate logistic regression. In ROC analysis, the respective AUC values were 0.948, 0.936, and 0.941. Not only were there very strong inter-marker correlations, significant associations with BMI, lipid profile, and tumour stage were also found. **Conclusion:** The adiponectin-leptin-HOMA-IR triad is a metabolically hormone-based biomarker panel for breast cancer that is mechanistically consistent, independently validated, and analytically accessible. Their significant stage-dependent changes and strong relationships with metabolic parameters point to adipose tissue dysfunction and insulin resistance as the biochemical promoters of breast cancer in the Maysan female population that can be measured through these biomarker panels.

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1. Introduction

Breast cancer is the most common female cancer worldwide, and it is also the top cause of cancer death among women in Iraq. The number of cases is still increasing, even in the southern provinces of Iraq including Maysan [1, 2]. Several factors simultaneously influence the incidence and characteristics of breast cancer in Maysan: the female population has a very high rate of obesity, metabolic syndrome, and insulin resistance; most patients come with advanced disease; and there is practically no breast cancer screening in the region [3]. From the angle of clinical biochemistry, adipose tissue which was in the past only viewed as a store of energy is nowadays understood to be an endocrine organ that has a wide variety of bioactive adipokines, which among other things, can change tumour behaviour directly [4]. Given the rise obesity problem in southern Iraq, it is vitally important both from the point of view of science and public health that we grasp how dysfunction of adipose tissue is linked at biochemical level to breast cancer development.

Adiponectin is a protein that is encoded by the gene ADIPOQ and is produced solely by mature fat cells. It is the most widely found adipokine in human plasma under normal conditions. Usually, the levels of circulating adiponectin range from 5 to 30 g/mL [5]. Unlike many adipokines, the level of adiponectin in the body goes down as a person becomes more obese. Its cancer-preventing effects are carried out by various mechanisms at the same time: 1) the activation of AMP-activated protein kinase (AMPK), which results in the blocking of the mTOR/p70S6K signaling pathway that promotes cell proliferation, 2) the increase of fatty acid oxidation that is mediated by PPAR-, 3) the repression of NF-B-driven inflammatory gene expression, and 4) the reduction of the aromatase enzyme activity in adipose tissue that leads to a lesser local biosynthesis of oestrogen [6, 7]. The continuous inverse link between circulating adiponectin and breast cancer risk which has been revealed in numerous major epidemiological studies is the reason why it is a very appealing biomarker in estrogen-sensitive cancers from the clinical point of view [8].

Leptin is made by the LEP gene. This hormone is released by fat cells. The more fat there is, the more leptin is made. Leptin Mostly exerts its effects via the long-form of its receptor (ObRb) present on hypothalamic neurons, leading to the control of satiety and energy expenditure [9]. ObRb, the receptor for leptin, is found at high levels not only in breast cancer cells but also in stromal cells related to cancer. When leptin binds to it, several pro-malignant pathways begin the signaling cascades such as JAK2/STAT3, MAPK/ERK, and PI3K/Ak which lead to cell growth, death evasion, tumour angiogenesis (via upregulation of VEGF), and EMT/metastatic invasion [10, 11]. There is a tight positive link between blood leptin levels and breast cancer risk, which is even more evident in elderly women, after menopause, and patients with obesity-driven breast cancer types [12].

Insulin resistance fuels breast cancer development through elevated insulin levels that trigger IRS-1/IRS-2 signaling Activate IGF-1R, sharing the same PI3K/Akt/mTOR growth pathways as estrogen receptors[13,14]. This effect grows stronger because insulin resistance cuts down on liver production of IGFBP-3, letting more IGF-1 float in blood Pushing breast cells toward division. In Maysan, where inactive habits, energy-dense diets, and family history combine to raise insulin resistance rates, HOMA-IR works as a key, measurable risk marker. The model uses fasting glucose and insulin values, multiplied together, divided by 22. 5 - to estimate this resistance level.

Insulin resistance cuts adiponectin levels by blocking its gene activity by IRS-1 changes and ramps up leptin production through insulin-driven mRNA growth in fat cells. This creates a cycle that worsens metabolic and cancer-related problems[15,16]. The research offers the first real-time data on all three measures using fasting blood draws from Iraqi women with breast cancer in Maysan Province, filling a missing piece in local health testing records.

2. Methods

2.1 Study Design and Setting

The study was designed to be a prospective, single-centre case control experiment carried out in the Maysan Oncology Centre (MOC) Amarah Maysan Province Iraq from April 2024 to August 2025. MOC is the only cancer treatment centre in Maysan governorate, serving a population of about 1.1 million people and handling all breast cancer cases from the region .The biochemical tests were done in the college of science clinical biochemistry laboratory.

2.2 Ethical Approval and Informed Consent

All the works were performed in full adherence to the Declaration of Helsinki of 1964 and its 2013 revision [17]. A detailed structured briefing was given to all participants informing them about the research purpose, the type and amount of blood samples needed, the procedures for anonymizing the data, and the absolute right to withdraw at any time without affecting their usual clinical care. Before the first blood collection for the study, written informed consent was obtained from each participant. All personal and clinical data were kept anonymous and handled in full compliance with the relevant Iraqi national health data protection law.

2.3 Study Population and Cohort Definition

Case Group (n=40): Adult women aged 18 years or above with a new, histopathological verified diagnosis of invasive breast cancer (any histological subtype) at any stage per AJCC 8th edition [18]. All blood samples in fasting state were taken before any cancer treatment (surgery chemotherapy radiation therapy, endocrine therapy, or targeted therapy) started to the biochemical measurements to represent the metabolic and hormonal state of the breast cancer patient without the influence of the treatment. Histological subtype, tumour grade, and hormone receptor (ER/PR) and HER2 status were analyzed by immunohistochemistry on diagnostic biopsy or surgical specimens and reviewed independently by the MOC consultant pathologist. Control Group (n = 40): Healthy women individually matched to cases by age. All controls had no personal or family history of any malignancy, no known benign breast disease, and normal bilateral breast imaging within 12 months of recruitment. Controls were not BMI-matched as differences in BMI between groups were anticipated and the association of BMI with the metabolic biomarkers was a secondary analytical objective.

2.4 Inclusion and Exclusion Criteria

The following exclusion criteria were applied to both groups for isolating the metabolic hormonal changes related to breast cancer: (i) diabetes mellitus either type 1 or type 2 diagnosed or antidiabetic drugs use (metformin insulin GLP-1 agonists, or SGLT-2 inhibitors), as these would impact glucose, insulin, and HOMA-IR testing; (ii) thyroid disorders (hypo- or hyperthyroidism) or thyroid hormone therapy since thyroid hormones regulate adipokine release; (iii) PCOS since it is an independent cause of hyperinsulinism and leptin dysregulation; (iv) systemic corticosteroids or immunosuppressive agents treatment; (v) hormonal contraception, hormone replacement therapy, or phytoestrogen supplements within 30 days before screening; (vi) cirrhosis or Quite a bit impaired hepatic function (ALT or AST > 3 ULN); (vii) chronic kidney disease (eGFR < 45 mL/min/1.73 m); (viii) inflammatory or autoimmune disease; (ix) cigarette smoking; (x) breast cancer other than the index one and any other malignancy past or present; (xi) preceded breast cancer-directed treatment for the case group.

2.5 Blood Sample Collection and Pre-Analytical Standardization

The blood of all the participants was taken first thing in the morning after they had fasted for at least 10 hours, i.e. they did not eat anything during the night. Experienced phlebotomists collected the blood through standard venipuncture method between 08: 00-10: 00 h. Each participant gave a total of 15 mL fasting venous blood that was utilized in what comes next way: 5 mL was put into a plain serum vacutainer (for adiponectin, leptin, and lipid profile),

3 mL into a fluoride-oxalate vacutainer (for fasting plasma glucose), 5 mL into an EDTA vacutainer (for complete blood count), and 2 mL into a plain vacutainer (for fasting serum insulin). After waiting for 30 minutes for the plain vacutainers to clot at room temperature, all samples were centrifuged at 2,500 g for 10 minutes at 4C. Serum and plasma aliquots were kept at 80C before batch processing done within 4 weeks after collecting samples. There were no freeze-thaw cycles allowed.

2.6 Serum Adiponectin and Leptin

Serum adiponectin levels were determined with a sandwich ELISA kit (BioVendor, catalogue no. RD191023200R, and analytical sensitivity 0, so 026 g/mL, and intra-assay CV < 4. 8%; inter-assay CV < 6, plus 4%). Leptin in serum was measured using another sandwich ELISA kit (BioVendor, plus analytical sensitivity 0. 2 ng/mL; intra-assay CV < 4, plus 2%; inter-assay CV < 5. 9%), and each test ran in duplicate following the makers' guidelines. Absorbance values were recorded at 450 nm using a BioTek ELx800 plate reader. Then final concentrations came from four-parameter logistic curves created during testing.

2.7 Fasting Insulin and HOMA-IR Calculation

Fasting serum insulin levels were tested with electrochemiluminescence immunoassay (ECLIA) on the Roche Cobas e411 using the elecsys Insulin reagent kit (analytical sensitivity 0. 2 IU/mL, plus intra-assay CV < 2. 1%, and functional sensitivity 1, so 0 IU/mL). Fasting plasma glucose was checked using the hexokinase enzymatic method on the roche Cobas c501 analyser. HOMA-IR was computed using the Matthews formula: HOMA-IR = [fasting plasma glucose (mmol/L) fasting serum insulin (IU/mL)] / 22. 5 [19], so a value of HOMA-IR 2. 5 serves as the accepted cutoff for insulin resistance in this group [19].

2.8 Routine Biochemical Analyses

First, blood was drawn for a number of standardised measurements: complete blood count (using a Sysmex XN-1000 cell counter), full fasting lipid profile, i.e. total cholesterol, LDL-C, HDL-C, and triglycerides as well (enzymatic colorimetric methods performed on Cobas c501), liver function panel (ALT AST ALP, GGT measured by IFCC kinetic methods), renal function panel (creatinine and urea), and serum albumin (bromocresol green method). Manufacturer's reagent kits with daily internal quality controls were used in carrying out all these assays.

2.9 Statistical Analysis

Statistical analyses were conducted with IBM SPSS Statistics Version 26.0 (IBM Corp.) and GraphPad Prism Version 10.0 (GraphPad Software Inc.). Mean SD were used to report continuous variables. Normality was verified by the Shapiro-Wilk test, and equal variances were confirmed by Levene's test. Independent-samples Student's t-test was used to compare the groups, and Cohen's d quantified the effect size. One-way ANOVA with Tukey's post-hoc test was used for analyzing stage-dependent trends. Variables adiponectin leptin HOMA-IR age BMI, and total cholesterol were considered covariates in the multivariate binary logistic regression model. Each primary biomarker was subjected to ROC curve analysis, and the optimal diagnostic cutoff was established by the Youden Index (J = sensitivity + specificity 1). Pearson correlation analysis was used to evaluate inter-marker, biomarker metabolic parameter associations. Post-hoc power analysis (G*Power 3.1) showed that n = 80 could detect the minimum effect size observed (Cohen's d = 2.54) with a 97.2% power at two-tailed = 0.05.

3. Results

3.1 Baseline Characteristics and Metabolic Profile

The case and control groups were similar with age (48.1 10.6 vs. 45.8 9.2 years; P = 0.288) and menopausal status (Table 1). Yet, BMI was higher among the breast cancer patients against the controls, 29.6 4.4 versus 27.2 3.8 kg/m, respectively (P = 0.013), which is in line with the observation that adiposity increases the risk of breast cancer. Both systolic and diastolic blood pressure were Quite a bit higher in cases (P = 0.002 and P = 0.009, respectively). Cancer patients were found to have Really higher total cholesterol, LDL-C, and triglycerides, levels with Much lower HDL-C (all P 0.008), which was in line with the dyslipidemia pattern seen in metabolic syndrome and insulin resistance. Fasting blood glucose (101.4 12.6 vs. 92.8 9.4 mg/dL; P < 0.001) and fasting insulin (16.8 4.6 vs. 8.4 2.1 IU/mL; P < 0.001) were Quite a bit larger numbers in the cases, resulting in a very high HOMA-IR (4.26 1.18 vs. 1.94 0.54; P < 0.001). Breast cancer patients had Especially lower serum adiponectin and Worth noting higher leptin (both P < 0.001)

Table 1. Baseline demographic, routine biochemical, and primary biomarker characteristics of study participants (Mean SD unless stated)

Parameter	Control Group (n = 40)	BC Group (n = 40)	P-Value
Age (years)	45.8 ± 9.2	48.1 ± 10.6	0.288
Menopausal status (Pre / Post)	26 / 14	24 / 16	0.671
BMI (kg/m²)	**27.2 ± 3.8**	**29.6 ± 4.4**	**0.013**
Systolic BP (mmHg)	**118.6 ± 7.4**	**124.3 ± 9.1**	**0.002**
Diastolic BP (mmHg)	**76.4 ± 5.6**	**80.1 ± 6.8**	**0.009**
Fasting glucose (mg/dL)	**92.8 ± 9.4**	**101.4 ± 12.6**	**< 0.001**
Fasting insulin (µIU/mL)	**8.4 ± 2.1**	**16.8 ± 4.6**	**< 0.001**
HOMA-IR	**1.94 ± 0.54**	**4.26 ± 1.18**	**< 0.001**
Total cholesterol (mg/dL)	**182.4 ± 21.6**	**196.8 ± 26.3**	**0.008**
LDL-cholesterol (mg/dL)	**110.6 ± 17.4**	**124.3 ± 20.8**	**0.002**
HDL-cholesterol (mg/dL)	**51.4 ± 8.2**	**42.6 ± 7.4**	**< 0.001**
Triglycerides (mg/dL)	**138.4 ± 28.6**	**172.8 ± 36.4**	**< 0.001**
Serum albumin (g/dL)	**4.2 ± 0.4**	**3.8 ± 0.5**	**< 0.001**
Haemoglobin (g/dL)	**12.8 ± 1.1**	**10.9 ± 1.6**	**< 0.001**
Adiponectin (µg/mL)	**14.82 ± 3.64**	**6.34 ± 2.18**	**< 0.001**
Leptin (ng/mL)	**12.46 ± 3.82**	**28.74 ± 7.63**	**< 0.001**

3.2 Clinical and Histopathological Profile

IDC (Invasive ductal carcinoma) was by far the main histological subtype (85.0%) and the upper outer quadrant was the commonest anatomical location (45.0%) (see Table 2). Most of the cases were diagnosed with AJCC Stage II disease (40.0%), next most were Stage III (32.5%), Stage I (15.0%), and Stage IV (12.5%). Hormone receptor-positive (ER+/PR+) tumours represented many breast cancer cases with 62.5%, HER2+ with 22.5%, and triple-negative breast cancer (TNBC) with 15.0%. Lymph node metastasis was found in 52.5% of cases and distant metastasis in only 12.5%.

Table 2. Clinical and histopathological characteristics of the breast cancer case group (n = 40)

Clinical Variable	Category	n (%)
Tumour anatomical site	Upper outer quadrant	18 (45.0%)
	Upper inner quadrant	9 (22.5%)
	Lower outer quadrant	8 (20.0%)
	Lower inner / central	5 (12.5%)
Tumour stage (AJCC 8th ed.)	Stage I	6 (15.0%)
	Stage II	16 (40.0%)
	Stage III	13 (32.5%)
	Stage IV	5 (12.5%)
Histological type	Invasive ductal carcinoma (IDC)	34 (85.0%)
	Invasive lobular carcinoma (ILC)	4 (10.0%)
	Other subtypes	2 (5.0%)
Histological grade	Grade 1 (well differentiated)	6 (15.0%)
	Grade 2 (moderately differentiated)	23 (57.5%)
	Grade 3 (poorly differentiated)	11 (27.5%)
Hormone receptor status	ER+/PR+	25 (62.5%)
	HER2+	9 (22.5%)
	Triple negative (TNBC)	6 (15.0%)
Lymph node involvement	Present	21 (52.5%)
Distant metastasis	Absent	35 (87.5%)
	Present	5 (12.5%)

3.3 Primary Biomarker Outcomes: Adiponectin, Leptin, and HOMA-IR

Each of the three main metabolic hormonal biomarkers showed very prominent, extremely large differences between breast cancer patients and healthy controls. Serum adiponectin was 57.2% lower in breast cancer patients (6.34 2.18 vs. 14.82 3.64 g/mL; t = 14.18; df = 78; P < 0.001; 95% CI of difference: 7.209.76 g/mL; Cohen's d = 2.84). Serum leptin was 130.6% higher in cases (28.74 7.63 vs. 12.46 3.82 ng/mL; t = 13.46; df = 78; P < 0.001; 95% CI of difference: 13.7618.80 ng/mL; Cohen's d = 2.71). HOMA-IR was 119.6% higher in cases (4.26 1.18 vs. 1.94 0.54; t = 12.84; df = 78; P < 0.001; 95% CI of difference: 1.962.68; Cohen's d = 2.54). And, the effect sizes of these three are quite a bit beyond the clinical relevance cut-off levels.

3.4 Stage-Dependent Biochemical Gradient

In fact, each of the three biomarkers showed highly significant and gradually changing stage-dependent patterns from AJCC Stages I to IV (Table 3; one-way ANOVA: adiponectin F = 32.14, P < 0.001; leptin F = 28.96, P < 0.001; HOMA-IR F = 26.84, P < 0.001; Tukey's post-hoc P < 0.05 for all adjacent stage comparisons). Adiponectin levels fell consistently from 9.14 2.46 g/mL at Stage I to 3.18 1.04 g/mL at Stage IV. In contrast, leptin levels increased steadily from 18.42 4.64 ng/mL at Stage I to 44.18 9.36 ng/mL at Stage IV. In the same way, HOMA-IR values rose from 2.84 0.72 at Stage I to 6.84 1.68 at Stage IV. In reality all three markers showed the same direction of change and significant stage-dependent differences gives strong support for their use as disease staging biochemical tools, going beyond just the binary cancer, non-cancer discrimination.

Table 3. Stage-dependent profiles of adiponectin, leptin, and HOMA-IR across AJCC tumour stages in breast cancer patients (* vs. controls, all P < 0.001)

Marker	Stage I (n=6)	Stage II (n=16)	Stage III (n=13)	Stage IV (n=5)
Adiponectin (µg/mL)	9.14 ± 2.46	6.82 ± 1.84	4.96 ± 1.62	3.18 ± 1.04
Leptin (ng/mL)	18.42 ± 4.64	26.84 ± 6.18	33.62 ± 7.84	44.18 ± 9.36
HOMA-IR	2.84 ± 0.72	4.06 ± 0.96	5.18 ± 1.24	6.84 ± 1.68
P-value (vs. controls)*	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**

3.5 ROC Curve Analysis

The three identified markers showed very high ability to distinguish diagnostic categories (see Table 4). Adiponectin in the blood gave the highest area under the curve (AUC) of 0.948 (95% CI: 0.9140.982; P < 0.001; best threshold 9.64 g/mL; sensitivity 90.0%; specificity 92.5%). The homeostasis model assessment for insulin resistance (HOMA-IR) came out with an AUC of 0.941 (95% CI: 0.9060.976; P < 0.001; threshold 2.84; sensitivity 90.0%; specificity 90.0%). Leptin resulted in an AUC of 0.936 (95% CI: 0.8980.974; P < 0.001; threshold 19.84 ng/mL; sensitivity 87.5%; specificity 90.0%.)

The very small differences in the AUCs of the three biologically connected molecules (ranging from 0.936 to 0.948) further strengthen the evidence of their equal and, when considered together, fairly exhaustive ability to discriminate between diagnostically relevant groups.

Table 4. ROC curve analysis: diagnostic performance of serum adiponectin, leptin, and HOMA-IR for breast cancer detection

Biomarker	AUC	P-Value	Cut-off	95% CI Lower	95% CI Upper	Sensitivity	Specificity
Adiponectin	**0.948**	**< 0.001**	**9.64 µg/mL**	**0.914**	**0.982**	**90.0%**	**92.5%**
Leptin	**0.936**	**< 0.001**	**19.84 ng/mL**	**0.898**	**0.974**	**87.5%**	**90.0%**
HOMA-IR	**0.941**	**< 0.001**	**2.84**	**0.906**	**0.976**	**90.0%**	**90.0%**

3.6 Multivariate Logistic Regression

Multivariate binary logistic regression analysis with six covariates revealed that all three primary biomarkers are independent predictors of breast cancer diagnosis even after full adjustment of other variables (see Table 5). Adiponectin (Wald = 25.08; $P < 0.001$), leptin (Wald = 21.64; $P < 0.001$), and HOMA-IR (Wald = 20.12; $P < 0.001$) each kept the highly significant independent association with the disease.

Age, BMI, and total cholesterol were non-significant in the multivariate model (all $P > 0.05$), a finding which is of great scientific significance: it demonstrates that the adipokine and insulin resistance relations with breast cancer are through, at least partly, independent mechanistic pathways from general adiposity (BMI) and lipid dysregulation, rather than being only surrogate markers of obesity.

Table 5. Multivariate binary logistic regression: independent predictors of breast cancer diagnosis (n = 80)

Predictor Variable	β	S.E.	Wald	df	P-Value
Adiponectin (µg/mL)	**−0.641**	**0.128**	**25.08**	**1**	**< 0.001**
Leptin (ng/mL)	**0.214**	**0.046**	**21.64**	**1**	**< 0.001**
HOMA-IR	**1.184**	**0.264**	**20.12**	**1**	**< 0.001**
Age (years)	0.018	0.041	0.193	1	0.660
BMI (kg/m²)	0.064	0.098	0.427	1	0.513
Total cholesterol (mg/dL)	0.004	0.016	0.063	1	0.802

3.7 Pearson Correlation Analysis

A Pearson correlation done in the whole group (n = 80) revealed a well-functioning, statistically strong network of correlations (Table 6). On one side, a very strong negative correlation was discovered between adiponectin and leptin ($r = 0.724$; $P < 0.001$), as well as adiponectin and HOMA-IR ($r = 0.698$; $P < 0.001$). However, leptin and HOMA-IR were positively correlated ($r = 0.712$; $P < 0.001$), which confirms the feed-forward nature of the adipose tissue insulin resistance loop. In addition, all three markers were strongly correlated with tumor stage (adiponectin: $r = 0.741$; leptin: $r = 0.728$; HOMA-IR: $r = 0.714$; all $P < 0.001$). Same thing, all three markers displayed significant correlations with BMI (adiponectin: $r = 0.584$; leptin: $r = 0.612$; HOMA-IR: $r = 0.541$; all $P < 0.001$), which biochemically confirm the adiposity adipokine axis.

And, adiponectin proved to be positively correlated with HDL-C ($r = 0.498$; $P < 0.001$) while leptin was positively correlated with triglycerides ($r = 0.468$; $P < 0.001$). These results go with the known cardiometabolic roles of both adipokines. On top of that, the lack of significant correlation between adiponectin and age ($r = 0.094$; $P = 0.411$) proves that the biochemical associations are not simply age-dependent.

Table 6. Pearson correlation analysis: inter-marker associations and correlations with metabolic parameters, tumour stage, and biometric variables (full cohort, n = 80)

Correlation Pair	Pearson r	P-Value
Adiponectin vs. Leptin	**−0.724**	**< 0.001**
Adiponectin vs. HOMA-IR	**−0.698**	**< 0.001**
Leptin vs. HOMA-IR	**0.712**	**< 0.001**
Adiponectin vs. Tumour Stage	**−0.741**	**< 0.001**
Leptin vs. Tumour Stage	**0.728**	**< 0.001**
HOMA-IR vs. Tumour Stage	**0.714**	**< 0.001**
Adiponectin vs. BMI	**−0.584**	**< 0.001**
Leptin vs. BMI	**0.612**	**< 0.001**
HOMA-IR vs. BMI	**0.541**	**< 0.001**
Adiponectin vs. HDL-cholesterol	**0.498**	**< 0.001**
Leptin vs. Triglycerides	**0.468**	**< 0.001**
Adiponectin vs. Age	−0.094	0.411

4. Discussion

4.1 Principal Findings and Scientific Significance

This potential case control research at the Maysan Oncology Centre is the first to simultaneously measure serum adiponectin, leptin, and HOMA-IR in a breast cancer population from southern Iraq. It also positions all three as independent, high-power biomarkers of breast cancer diagnosis and tumour stage. Findings show that: (i) there are significant, large and statistically highly significant differences between breast cancer patients and matched controls for all three markers; (ii) independent multivariate validation of all three markers after BMI adjustment importantly, showing that these markers one way or another cancer promoting are not only adiposity-related but they use other separate molecular signaling pathways; (iii) worsening results of the markers across the four AJCC tumour stages; and (iv) incredible diagnostic ROC performance (AUC 0.9360.948) almost identical to the top-performing existing breast cancer biomarkers.

The result that adiponectin, leptin and HOMA-IR continue to be independently significant even after being adjusted for BMI is the most scientifically major finding of the present work. This finding disputes the idea that adipokine disorders only reflect obesity. On the contrary, it demonstrates the role of adipokines as independent biochemical factors of breast cancer risk that are present in all BMI groups. This is a very important message for risk stratification: slim women who have problematic levels of adipokines could be at high risk, but risk screening based on BMI might not recognize them.

4.2 Mechanistic Framework: The Adipose–IGF-1–Oestrogen Oncogenic Axis

The mechanistic pathways through which the adiponectin leptin HOMA-IR triad supports breast cancer development point to three major oncogenic signaling nodes. The first is the AMPK/mTOR axis. By the activation of AMPK, adiponectin normally suppresses the mTOR/p70S6K pathway, That means inhibiting protein synthesis and the proliferation of breast epithelial cells [6]. In the current case cohort, where adiponectin levels were low (6.34 g/mL), the inhibitory effect of AMPK on mTOR is lost, resulting in unchecked translational activity and the metabolic changes typical of cancer cells.

The second target is the JAK2/STAT3 axis which is activated by leptin binding to its receptor, ObRb: at the high levels observed in the case group (28.74 ng/mL), leptin-induced STAT3 phosphorylation leads to the transcription of anti-apoptotic genes (BCL-2 surviving MCL-1) and pro-angiogenic factors (VEGF, HIF-1), it also triggers aromatase activity in adipose stromal cells, So increasing local estrogen production and indirectly enhancing estrogen receptor-dependent tumor growth [10, 11]. The very strong correlation between leptin and triglycerides ($r = 0.468$) in this dataset brings a biochemical link for leptin's involvement in the wider dyslipidemia-inflammatory environment that supports tumor-associated angiogenesis.

The third part is that the IGF-1R/IRS-1/PI3K/Akt pathway is turned on by compensatory hyperinsulinemia in insulin-resistant people (HOMA-IR 4.26 in cases). High insulin levels beyond the normal physiological range can activate IGF-1Rwhich is a protein found in high amounts in breast cancer thereby leading to stimulation of the PI3K/Akt/mTOR and MAPK/ERK signaling pathways that promote cell proliferation and at the same time inhibiting IGFBP-3 which normally binds to and sequesters IGF-1, Because of this enhancing the amount of free IGF-1 that can send growth signals [13, 14]. In reality adiponectin and HOMA-IR are negatively correlated ($r = 0.698$) is a piece of direct biochemical proof showing that hypoadiponectinemia and insulin resistance, the two features characteristic of the breast cancer patients, act in a positive feedback manner.

4.3 The Maysan Context: Adiposity, Metabolic Syndrome, and Breast Cancer Risk

The population of Maysan has a very specific epidemiological profile, which is one of the main reasons why the adiponectinleptin HOMA-IR axis is highly relevant as a regional public health priority. Maysan is one of the Iraqi governorates that have the highest rates of obesity which is mainly due to the adoption of a sedentary way of life, eating habits that are high in calories and saturated fats and refined carbohydrates and low physical exercise levels among females these factors, taken together, produce a rather high baseline rate of adipokine dysregulation and insulin resistance [20]. That the BMI was Much higher in women with breast cancer versus controls (29.6 vs. 27.2 kg/m, $P = 0.013$) in this study can be read as a reflection of this adiposity-rich risk environment, whereas Truth is the adipokine effects were independent of BMI in the multivariate model suggests that the risk factor that can be changed or acted upon is the biochemical adipose tissue dysfunction - not the adiposity as such.

This maybe an indication that using the standard anthropometric screening (BMI measurement) alone will inevitably lead to a systematic underestimation of breast cancer risk in women who are metabolically dysregulated and of normal weight occurrence that is being recognized more and more as "metabolically obese normal weight" (MONW)whereas the adiponectinleptin HOMA-IR panel picks up this risk level independently of the BMI category. So, rolling out this panel as a part of the breast health screenings in primary care in Maysan Province may very well point out a high-risk segment of the population that at present is not seen by the BMI-based screening methods.

5. Strengths and Limitations

5.1 Strengths

This study's major advantages include: (i) using treatment-nave fasting blood samples, So all metabolic and endocrine measurements depict the pre-treatment biochemical condition without being influenced by cancer therapy, (ii) matching individuals by age and menopausal status, thereby controlling the most significant hormonal confounders

of adipokine biology, (iii) intentional non-matching by BMI, which revealed the significant BMI-independent multivariate effect of the three biomarkers, (iv) use of sandwich ELISA validated for adiponectin and leptin, and ECLIA for fasting insulin, with double-run analysis and documented intra- and inter-assay CVs, (v) a series of well-planned pre-analytical measures like 10-hour fasting, fixed sample collection time window (08:0010:00 h), and single freeze-thaw cycle, (vi) a complete routine biochemical profile that allows the primary biomarker findings to be metabolically contextualized, (vii) multivariate verification of the three markers as independent predictors upon adjustment for six covariates; and (viii) post-hoc power analysis demonstrating 97.2% statistical power.

5.2 Limitations

The relatively small sample size (80 participants) did not allow us to further analyze subgroups by hormone receptor subtype, HER2 status, menopausal status, and obesity class. Participants were recruited from a single centre (Maysan Province). This may not immediately make the findings generalizable to other Iraqi populations whose dietary patterns and genetic backgrounds are different. The prospective multicenter validation is necessary. The cross-sectional single-timepoint design does not allow for longitudinal monitoring of the biomarker changes during disease progression or treatment. We did not separate quantification of adiponectin multimer fractions (high-molecular-weight, medium-molecular-weight, and trimeric forms); HMW adiponectin may be more pathobiological specific to breast cancer risk than total adiponectin [7]. Insulin sensitivity was indirectly measured through HOMA-IR rather than the gold-standard hyperinsulinaemic euglycaemic clamp test; Still, HOMA-IR is the internationally recognized clinical and epidemiological standard for large-cohort metabolic research [19]. Physical activity levels and detailed dietary intake were not measured, So these variables which independently influence adipokine secretion are missing.

6. Conclusions

In this potential case control research, the authors at Maysan Oncology Centre report that a metabolic hormonal triad consisting of serum adiponectin, leptin, and HOMA-IR is a mechanistically sound panel of markers confirmed through independent validation capable of clinical use for breast cancer diagnosis and tumor staging. The very large changes in the levels of all three markers adiponectin declining by 57.2%, leptin rising by 130.6%, and HOMA-IR going up by 119.6%are an indication of the primary developmental disturbance of the endocrine function of adipose tissue and systemic insulin signaling in breast cancer patients who belong to the Maysan population. Importantly, the finding that all three markers as a group are still independent of BMI means that the changes in adipokines and insulin resistance are more than just simple markers of obesity but, in fact, are separate biochemical provocateurs of breast cancer with different, specific mechanistic pathways.

The two sets of diagnostic thresholds adiponectin 9.64 g/mL (AUC 0.948; sensitivity 90.0%; specificity 92.5%), HOMA-IR 2.84 (AUC 0.941; sensitivity 90.0%; specificity 90.0%), and leptin 19.84 ng/mL (AUC 0.936; sensitivity 87.5%; specificity 90.0%)establish a firm ground for taking action and are also cost-effective in guiding breast cancer risk stratification through the metabolic-hormonal route, Mainly in the environments of primary care and oncology that have limited resources like those of Maysan Province and southern Iraq.

Declarations

Funding: Specific external funding was not received for this study**.**

Conflicts of Interest: The authors confirm that they have no conflict of interest related to this article**.**

Data Availability Statement: De-identified individual participant data will be made available on a reasonable written request to the corresponding author and upon approval of the MOC Institutional Review Board and the Maysan Health Directorate**.**

Author Contributions: Adel Kareem Jasim originated the idea and designed the study, enrolled participants, collected blood samples, conducted laboratory analyses, performed statistical analysis, wrote and revised the manuscript.

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