THE ROLE OF SOME OBESITY-RELATED BIOCHEMICAL PARAMETERS IN THE INCIDENCE, DIAGNOSIS, AND PROGNOSIS OF POSTMENOPAUSAL BREAST CANCER

Taha I. Hewala
Departments of Radiation Sciences
Samia A. Ebeid
El-Sayed Saad
Applied Medical Chemistry
Nadia A. Abd El-moneim
Cancer Management and Research
Mohamed Samir
Experimental and Clinical Surgery, Medical Research Institute,
Alexandria University, Egypt

Abstract

Aim: To figure out the association of insulin resistance, serum resistin, insulin, SHBG, and free estradiol with the etiology, diagnosis, and the prognosis of postmenopausal breast cancer. **Subjects and Methods:** Serum levels of resistin, insulin, SHBG, free E2, glucose, and albumin were assayed in a case-control study of 40 obese postmenopausal breast cancer females and 40 apparently healthy obese postmenopausal controls. **Results:** Serum levels of resistin, insulin, and free E2 were significantly elevated in breast cancer patients (9.89±0.49, 23.68±2.95 and 9.34±3.02, respectively) compared with controls (8.24±0.63, 13.55±1.31 and 1.01±0.23, respectively). Insulin resistance (IR) was significantly greater in breast cancer patients (7.33±0.95) than controls (3.46±0.37). However, serum SHBG levels were significantly declined in breast cancer patients (42.93± 2.52) compared with controls (64.2±4.89). Serum free E2 had the greatest significant area under the ROC curve, followed by insulin resistance, insulin, SHBG, and resistin. The odds ratio of serum resistin was 4.33 (95% CI=1.69 – 11.06, P=0.002), insulin was 3.66 (95% CI=1.41 – 9.46, P=0.006), insulin resistance was 3.56 (95% CI=1.39 – 9.08, P=0.007), SHBG was 0.25 (95% CI=0.092-0.67, P=0.005), and free E2 was 5.21(95% CI=1.86 –14.52, P=0.002) in breast cancer patients. **CONCLUSIONS:** From this study, it

could be concluded that although insulin resistance, serum resistin, insulin, SHBG, and free E2 may have a role in the incidence and diagnosis of obese postmenopausal breast cancer females, these biochemical parameters cannot be used for the prognosis of these patients. Serum free E2 was the most superior diagnostic marker followed by insulin resistance, insulin, SHBG, and resistin.

Keywords: Breast cancer, resistin, insulin, insulin resistance, SHBG, free estradiol, incidence, diagnosis, prognosis

Introduction

Breast cancer is the most frequently diagnosed cancer and the leading cause of cancer death among females worldwide. [1] In Alexandria (Egypt) 2010, it accounted for about 47 % of all malignancies in females. Obesity is a worldwide epidemical that continues to grow at an alarming rate. [2] Moreover, obesity has been associated with the development of several malignancies, particularly hormone-dependent cancers such as ovarian, endometrial, and breast cancer. [3]

In addition, Adipose tissue has been recognized as an endocrine organ that plays a pivotal role in insulin resistance and glucose homeostasis by secreting signaling molecules known as adipocytokines such as resistin. The exact role of adipocytokines in glucose metabolism and their physiological functions in humans remains under investigation. However, adipocytokines which is directly synthesized in adipose tissue may influence mammary tumorigenesis by impacting both circulating and locally produced levels of the estrogen. [4] Resistin gained interest in correlation with cancer, and was strongly associated with tumors of gastrointestinal system and hematological system. [5]

Although insulin is involved primarily in the regulation of carbohydrates, lipids, and proteins metabolism, it also has a significant role as a growth factor. Thus, it stimulates cell mitosis and migration, and also inhibits apoptosis. These effects may actually be increased under conditions of insulin resistance and consequent impairment of insulin-regulated metabolic pathways. [6] Some studies have also demonstrated that elevated insulin levels and hyperinsulinemia are associated with poor prognosis in patients with breast cancer. Consequently, insulin resistance and alterations in levels of adipocytokines are associated with an increased risk of developing pre and postmenopausal breast cancer. [7]

SHBG has been found to function as an active regulator of the steroid-signaling system in target tissues. In breast cancer cells, SHBG through its specific membrane receptor (SHBG-R) and second messenger system (cyclic AMP and protein kinase A), has not only effectively inhibits

estradiol induced cell proliferation, but also controls progesterone receptor expression at both the mRNA and protein levels. In addition, it has influences its function. [8]

Estradiol has been tied to the development and progression of breast cancer, ovarian cancer, and endometrial cancer. Estradiol affects target tissues by interacting with two nuclear hormone receptors called estrogen receptor α (ERα) and estrogen receptor β (ERβ). One of the functions of these estrogen receptors is gene expression. Once the hormone binds to the estrogen receptors, the hormone-receptor complexes then binds to specific DNA sequences causing an increase in cell division and DNA replication. [9] However, this study was carried out to investigate the role of serum resistin, insulin, insulin resistance, SHBG, and free estradiol in the incidence, diagnosis, and prognosis of breast cancer in postmenopausal females.

Subjects and Methods

Subjects

Eighty postmenopausal obese females were enrolled in this case-control study. Females were divided into two groups: **Group I (Obese control group)** included 40 apparently healthy obese women clinically free from any disease. Thus, their mean age and BMI were 58.43±1.13 years and 35±1.2 kg/m2, respectively. They were chosen from the staff members of the Medical Research Institute of Alexandria University (Egypt) and their relatives. **Group II (Obese patient group)** included 40 obese females having breast carcinoma of clinical stage II or III (10]. However, their mean age and BMI were 56.13±1.26 years and 36±1.5 kg/m2, respectively.

This study was approved by the Ethical committee of the Medical Research Institute, Alexandria University, Egypt. Thus, informed consent was signed by each subject. All subjects were recruited from the Experimental and Clinical Surgery, and the Cancer Management and Research Departments, Medical Research Institute, University of Alexandria within the period from June 2011 to October 2011. Consequently, all patients had primary invasive breast carcinoma, with no clinical manifestation of infection, not receiving immunomodulating agent or blood transfusion for 3 weeks recently. Also, all patients and controls with diabetes, hypertension, hormone replacement therapy, and hypo or hyper-thyrodism were excluded from this study.

Methods

To all patients, the following investigations were done: full history recording, thorough clinical examination, routine laboratory investigations including complete blood count, bleeding and coagulation times, mammography of breast and ultrasonography of abdomen, radiological

investigations including x-ray chest, CT scan and bone scan when needed, and preoperative fine needle-aspiration cytology (FNAC) of the breast mass to establish the pathological diagnosis.

All patients had undergone modified radical mastectomy [11]. The clinicopathological data of tumor size, pathological grade, estrogen receptor (ER) status, progesterone receptor (PR) status, Her-2 expression, axillary lymph node involvement, and vascular invasion were collected from patients' data sheets. Each patient's clinical stage was determined by the oncologist according to the TNM staging system [12] [table I].

After modified radical mastectomy, all breast cancer patients received adjuvant combination chemotherapy (5-fluorouracil, adriamycin and cyclophosphamide [FAC]) [13] for six cycles. The patients were evaluated clinically in the laboratory, and radiologically after three and six cycles of chemotherapy to estimate the clinical response. They were followed up for 45 months of assessment of disease-free survival based on observation metastasis or local recurrence.

Table (I). The clinicopathological data of breast cancer patients.

Clinicopathological data		Number (n)	Percent (%)
Tumor Size (cm)	**≤5**	**30**	**75%**
	>5	**10**	**25%**
Patient's clinical Stage	**II**	**22**	**55%**
	III	**18**	**45%**
Tumor pathological grade	**II**	**27**	**67.5%**
	III	**13**	**32.5%**
Estrogen receptor status (ER)	**-ve**	**3**	**7.5%**
	+ve	**37**	**92.5%**
Progesterone receptor status (PR)	**-ve**	**10**	**25%**
	+ve	**30**	**75%**
Her-2 expression	**-ve**	**40**	**100%**
	+ve	**0**	**0%**
Axillary lymph node involvement	**-ve**	**4**	**10%**
	+ve	**36**	**90%**
Vascular invasion	**-ve**	**7**	**17.5%**
	+ve	**33**	**82.5%**

n: number of cases

Laboratory Assays

In the morning, 5 ml fasting venous blood samples were withdrawn from each subject participating in this study. Blood samples were allowed to clot for 30 minutes, and was centrifuged at 3000 rpm for 10 minutes to isolate serum. 10µl of serum were immediately used for assaying fasting glucose levels. The remaining serum was divided into aliquots and stored at -80^{o}C until it was used for measuring the levels of resistin, insulin, SHBG, albumin, and total E2. Therefore,

all of these laboratory investigations were carried out at the radiation sciences department, Medical Research Institute, Alexandria University, Egypt.

Determination of Serum Resistin Levels

Levels of resistin were determined using a ready-for-use enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer's protocol (BioVender, USA). Briefly, standards, quality controls, and samples were incubated in microplate wells pre-coated with polyclonal anti-human resistin antibody. After 60 minutes incubation and washing, biotin-labelled second polyclonal anti-human resistin antibody was added. After 60 minutes incubation and washing, streptavidin-HRP conjugate was added. Also, after 60 minutes incubation and washing, the substrate solution (TMB) was added. The reaction was stopped by the addition of acidic solution, and the absorbance of the resulting yellow product was measured at 450 nm. Serum resistin concentrations (ng/ml) were determined by referring to a standard curve.

Determination of Serum Fasting Glucose Levels

Levels of serum glucose (mg/dl) were determined using a ready-for-use colorimetric kit according to the manufacturer's protocol (Spinreact, Spain). Briefly, glucose is oxidized in the presence of glucose oxidase. The hydrogen peroxide formed reacts under catalysis of peroxidase with phenol and 4 – amino phenazone giving a red-violet quinoneimine dye. The intensity of the color is directly proportional to the glucose concentration in the samples. The absorbance of the sample and the standard were measured against the blank at 546 nm.

Determination of Serum Fasting Insulin Levels

Serum insulin levels were determined using a ready-for-use immunoradiometric assay (IRMA) kit according to the manufacturer's protocol (Izotop, Hangerian). Briefly, the 125I-labeled signal-antibody binds to an epitope of the insulin molecule spatially different from that recognized by the biotin capture-antibody. The two antibodies react simultaneously with the antigen present in standards or samples, leading to the formation of a capture antibody-antigen-signal antibody complex, also referred to as “sandwich”. During a 2-hour incubation period, immuno-complex is immobilized to the reactive surface of streptavidin-coated test tubes. Reaction mixture is then discarded, test tubes washed, and radioactivity is measured for 1 minute in a gamma counter (perkin Elmer, Finland). Thus, the concentration of antigen is directly proportional to the radioactivity measured in test tubes. By constructing a calibration curve, the unknown serum concentrations of insulin (μIU/ml) were determined.

Evaluation of Insulin Resistance Status

Serum fasting insulin levels together with serum fasting glucose levels were grouped into the homeostatic model assessment (HOMA) index to estimate insulin resistance status as follows:

HOMA-IR = [Fasting insulin (μIU/ml) x fasting glucose (mg/dl)] / 405 [14]

Determination of Serum SHBG Levels

Serum SHBG levels were determined using a ready-for-use IRMA kit according to the manufacturer's protocol (Izotop, Hangerian). The principle of the method was identical to that of insulin. By constructing a calibration curve, the unknown serum concentrations of SHBG (nmol/L) were determined.

Determination of Serum Albumin Levels

Serum albumin levels (g/dl) were determined using a ready-for-use colorimetric kit according to the manufacturer's protocol (Diamond, Egypt). Briefly, albumin, in the presence of bromeresol green at a slightly acidic pH, produces a color change from yellow-green to green. The intensity of the color formed is directly proportional to the albumin concentration in the samples. The absorbance of the sample was measured against reagent blank at 630 nm.

Determination of Serum Total Estradiol Levels

The levels of serum total E2 were determined using a ready-for-use radioimmunoassay (RIA) kit according to the manufacturer's protocol (Siemens, Germany). Briefly, 125I-labeled estradiol competes with estradiol in the sample for antibody molecules coated on the tube wall. After incubation, separation of bound from free radioactivity was achieved by decantation. The tube was then counted for 1 minute in a gamma counter (perkin Elmer, Finland). In addition, the levels (pg/ml) of total estradiol in the samples were determined by interpretation from a calibration curve.

Quantification of Serum Free Estradiol Levels

Serum free E2 levels were estimated using an equation based on the law of mass action. This is dependent on the total E2 concentration and the fraction of E2 bound to albumin and SHBG according to the following equation [15]:

$$E2 = [E2_F]\left(1 + K_E^A[A] + \frac{K_E^{SH}[SH]}{1 + K_E^{SH}[E2_F]}\right), \qquad (1.1)$$

Where [*E*2] is total E2 concentration, [*E*2*F*] is free E2 concentration, [*SH*] is SHBG concentration, [*A*] is albumin concentration, and *KE A* and *KE SH* are association constants for the binding of E2 to albumin and SHBG, respectively. Furthermore, we assumed the following values for the association constants: KE A = 6 x104 and KESH = 0.68 x 109. Then, we substituted it into equation 1.1. Serum free E2 levels were found by substitution in equation 1.1.

Statistical Analysis

Statistical analysis was performed using SPSS 11.5 software package. Quantitative data were represented as mean and standard error. The data were abnormally distributed. Thus, non-parametric tests were used. The non-parametric Mann-Whitney U-test was used for studying differences between obese control group and obese breast cancer group regarding serum resistin, insulin, insulin resistance, SHBG, and free estradiol. The non-parametric Spearman's correlation test was used for correlating studied serum parameters concentrations with clinicopathological data. In addition, the odd's ratio was used to determine whether the serum marker is risky or protective for breast cancer. The diagnostic value of serum resistin, insulin, insulin resistance, SHBG, and free estradiol were compared using the Receiver Operating Characteristic (ROC) curve analysis. The Kaplan-Meier disease-free survival curve was applied to study the role of each preoperative serum biomarker to predict the disease-free survival of breast cancer patients. Therefore, P-value < 0.05 was considered to be statistically significant.

Results

Anthropometric Measurements of Breast Cancer Patients and Controls

For the control group, the mean±SE age was 58.43±1.13 years, and the mean±SE BMI was 35±1.2 kg/m2. For the breast cancer group, the mean±SE age was 56.13±1.26 years, while the BMI was 36±1.5 kg/m2. Because the cases and controls were frequency matched for age and BMI, there was no significant difference in the distribution of age and BMI between cases and controls.

Serum Levels of Assayed Biochemical Parameters in Breast Cancer Patients and Controls

As shown in table II, the serum levels of resistin, insulin, and free E2 were significantly elevated in breast cancer patients (9.89±0.49, 23.68±2.95, 9.34±3.02, respectively) compared with controls (8.24±0.63, 13.55±1.31, 1.01±0.23, respectively). Insulin resistance (IR) was significantly greater in breast cancer patients (7.33±0.95) than in controls (3.46±0.37). However,

serum SHBG levels were significantly declined in breast cancer patients (42.93± 2.52) compared with controls (64.2±4.89).

Table (II). Mean±SE levels of assayed serum biochemical parameters in cases and controls.

Biochemical parameter	control group (n=40)	Breast cancer group (n=40)	P-value
Resistin (ng/ml)	**8.24±0.63**	**9.89±0.49**	**0.02***
Insulin (μIU/ml)	**13.55±1.31**	**23.68±2.95**	**0.003***
(HOMA-IR)	**3.46±0.37**	**7.33±0.95**	**0.001***
SHBG (nmol/L)	**64.20±4.89**	**42.93±2.52**	**0.003***
Free E2 (pg/ml)	**1.01±0.23**	**9.34±3.02**	**0.001***

SE: Standard Error n: Sample Size

*: Significance was compared with control group

Significance was considered at p-value <0.05

The Receiver Operating Characteristic (ROC) Curve Analysis for comparing the Diagnostic Value of the Assayed Biochemical Parameters among Breast Cancer Patients

The ROC curve analysis was used in the present study to evaluate and compare the diagnostic value of insulin resistance, serum resistin, insulin, SHBG, and free E2 depending on the area under the ROC curve (AUC). Consequently, the higher AUC corresponds to a better diagnostic test. As shown in table III and figures (1-3), serum free E2 showed a significant AUC (71.3%) (p=0.001), with sensitivity (60%) and specificity (82%) at a cut-off value (1.07pg/ml). Insulin resistance showed a significant AUC (70.8%) (p=0.001), with sensitivity (55%) and specificity (80%) at a cut-off value (4.9). Serum insulin showed a significant AUC (69.5%) (p=0.003), with sensitivity (55%) and specificity (75%) at a cut-off value (16.5 μIU /ml). Serum SHBG showed a significant AUC (69.2%) (p=0.003), with sensitivity (52.5%) and specificity (80%) at a cut-off value (56.55nmol /L). Serum resistin showed a significant AUC (65.1%) (p=0.02), with sensitivity (72.5%) and specificity (65%) at a cut-off value (8.35ng/ml).

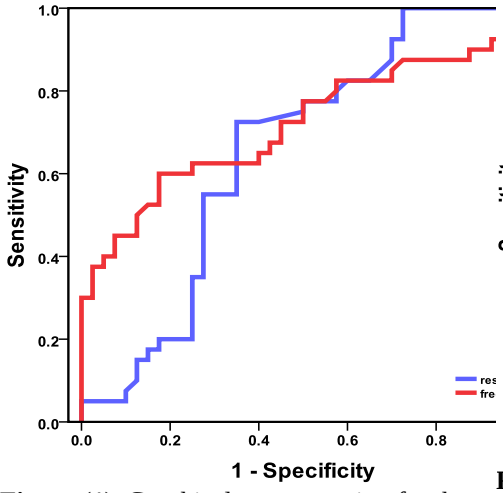

Figure (1). Graphical representation for the ROC curves of serum resistin and free E2.

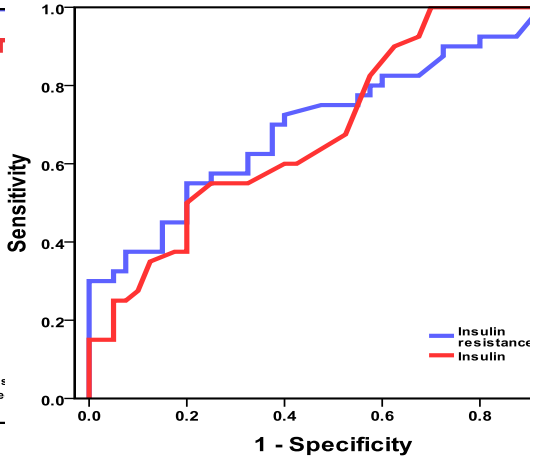

Figure (2). Graphical representation for the ROC curves of serum insulin and insulin resistance.

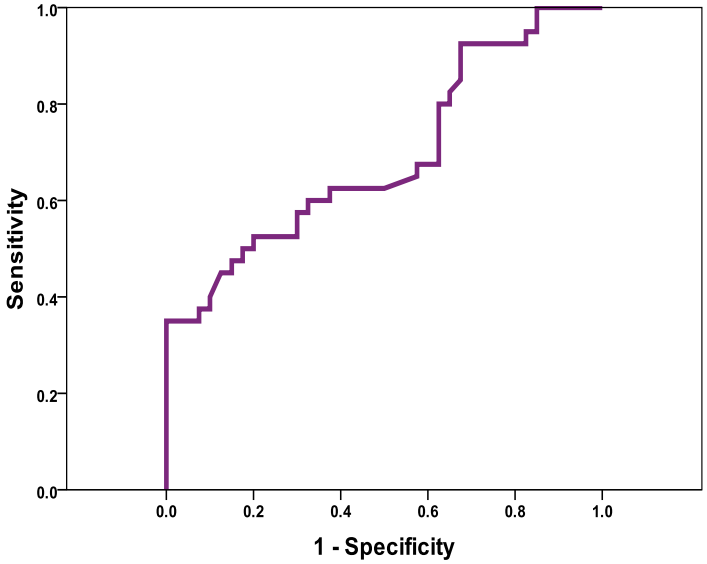

Figure (3). Graphical representation for the ROC curve of serum SHBG.

Table (III). The ROC curve-based characteristics for resistin, insulin, insulin resistance, SHBG, and free E2 in breast cancer group.

Serum biochemical parameter	AUC	p-value	Cut-off Value	Sensitivity %	Specificity %
Free E2 (pg/ml)	71.3	0.001*	1.07	60	82
Insulin resistance	70.8	0.001*	4.9	55	80
Insulin (µIU/ml)	69.5	0.003*	16.5	55	75
SHBG (nmol/L)	69.2	0.003*	56.55	52.5	80
Resistin (ng/ml)	65.1	0.02*	8.35	72.5	65

AUC: Area under the ROC curve *: Significance was considered at p-value <0.05

Correlation between the Studied Biochemical Parameters and Clinicopathological Data of Breast Cancer Patients before Surgery

None of the assayed biochemical parameters showed a significant correlation with any of the clinicopathological data of breast cancer patients (all P values >0.05).

The Association of Insulin Resistance, Serum Resistin, Insulin, SHBG, and Free Estradiol with Risk of Breast Cancer Incidence

According to table (IV), the odds ratio of serum resistin was 4.33 (95% CI=1.69 – 11.06, P=0.002), insulin was 3.66 (95% CI=1.41 – 9.46, P=0.006), insulin resistance was 3.56 (95% CI=1.39 – 9.08, P=0.007), SHBG was 0.25 (95% CI=0.092-0.67, P=0.005), and free E2 was 5.21(95% CI=1.86 – 14.52, P=0.002) in breast cancer patients.

Table (IV). The association of insulin resistance, serum resistin, insulin, SHBG, and free estradiol with risk of breast cancer incidence.

Biochemical parameter		Breast cancer group (n=40)	Control group (n=40)	Odds ratio (OR)	95% CI	p-value
Resistin (ng/ml)	< 8.35 ®	12	26	4.33	1.69 – 11.06	P=0.002 *
	≥ 8.35	28	14			
Insulin (µIU/ml)	< 16.5®	19	33	3.66	1.41 – 9.46	P= 0.006*
	≥ 16.5	21	7			
Insulin resistance	< 4.9®	17	29	3.56	1.39 – 9.08	P=0.007*
	≥ 4.9	23	11			
SHBG (nmol/L)	< 56.55	8	20	0.25	0.092 - 0.67	P=0.005*
	≥ 56.55®	32	20			
Free E2 (pg/ml)	< 1.07 ®	19	33	5.21	1.86 – 14.52	P=0.002 *
	≥ 1.07	21	7			

n= Sample Size; ® reference group; CI: Confidence Interval; *: Significance was considered at p-value<0.05.

Correlation between the Studied Biochemical Parameters and Disease-free Survival of Breast Cancer Patients before Surgery

In the present study, patients were followed up for 45 months after completing 6 cycles of chemotherapy to study the correlation of the assayed biochemical parameters and patients disease–free survival (DFS) based on observation of any metastasis or local recurrence. To study this correlation, the Kaplan-Meir disease-free survival (DFS) curves were constructed. As shown in Figures (4-8), Kaplan-Meir survival curves for breast cancer patients before surgery, revealed that the DFS of patients with elevated insulin resistance, serum resistin, insulin, and free E2 were non-significantly different from those with low levels of these biomarkers (P= 0.118, 0.872, 0.379 and 0.652, respectively). Also, the DFS of patients with low serum SHBG before surgery was non-significantly different from those with high levels of SHBG (P=0.389) (Table V).

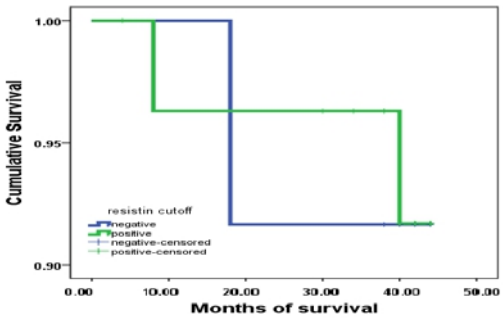

Figure (4). Kaplan- Meier DFS of breast cancer patients in relation to preoperative serum resistin levels.

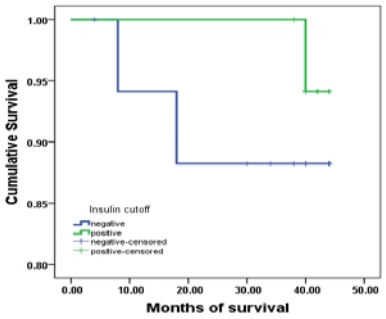

Figure (5). Kaplan- Meier DFS of breast cancer patients in relation to preoperative serum insulin levels.

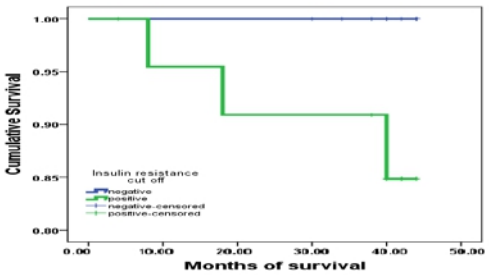

Figure (6). Kaplan- Meier DFS of breast cancer patients in relation to preoperative insulin resistance**.**

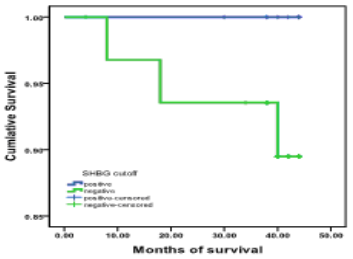

Figure (7). Kaplan- Meier DFS of breast cancer patients in relation to preoperative serum SHBG levels.

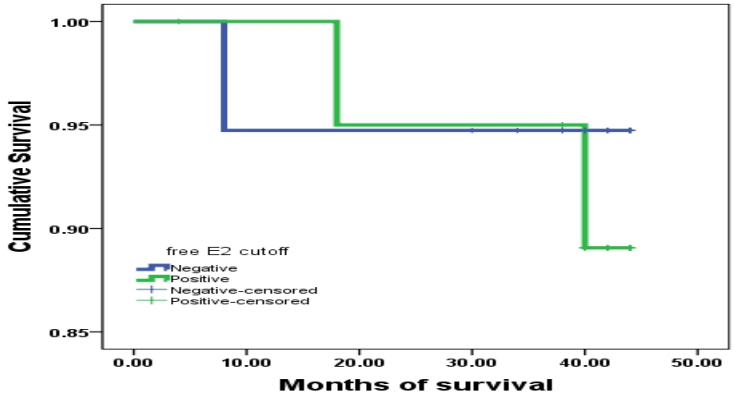

Figure (8). Kaplan- Meier DFS of breast cancer group in relation to preoperative serum free E2 levels.

Table (V). Correlation between preoperative insulin resistance, serum resistin, insulin, SHBG, and free E2 with DFS of breast cancer patients.

Biochemical parameters	Cut-off value	n	Metastasis		Non-metastasis		DFS (M± SE)	P-value
			No.	%	No.	%		
Resistin (ng/ml))	< 8.35 (-ve)	12	1	8.3	11	91.7	40.9±1.04	0.872
	≥ 8.35 (+ve)	28	2	7.1	26	92.9	41.24±0.66	
Insulin (µIU/ml)	<16.5 (-ve)	18	2	11.1	16	88.9	40.2±1.2	0.379
	≥16.5 (+ve)	22	1	4.5	21	95.5	41.9±0.1	
Insulin resistance	< 4.9 (-ve)	17	0	0	17	100	41.14±0.6	0.118
	≥ 4.9 (+ve)	23	3	13	20	87	40.05±0.22	
SHBG (nmol/L)	≥ 56.55 (-ve)	32	3	9.4	29	90.6	41.137±0.556	0.389
	< 56.55 (+ve)	8	0	0	8	100	40.047±0.95	
Free E2 (pg/ml)	< 1.07 (-ve)	19	1	5.3	18	94.7	41.05±0.9	0.652
	≥ 1.07 (+ve)	21	2	9.5	19	90.5	42.23±0.63	

DFS=Disease-free survival
n=Sample size
Significance was considered at p-value <0.05
M± SE: Mean± standard error

Discussion

Since the relationship of obesity with several forms of cancer has been known for a long time, researchers were trying to discuss the possible

role of adipocytokines in the regulation of carcinogenesis as another link between obesity and cancer. Resistin, an adipocytokine, recently gained interest in correlation with cancer and was strongly associated with tumors of gastrointestinal system and hematological system [16].

In the present study, there was a significant elevation in resistin levels in breast cancer patients compared to apparently normal controls. Our results were in agreement with Dalamaga et al [17] who found that hyperresistinemia is involved in the development of postmenopausal breast cancer. Also, it reflects changes during postmenopausal breast cancer progression and therefore could be used as a biomarker for postmenopausal breast cancer.

Consequently, resistin inhibition could be used as an effective therapeutic strategy in breast cancer. On the other hand, our results disagreed with Gaudet et al [18], who found non-significant difference between breast cancer patients and normal controls regarding serum resistin.

In the present study, serum resistin showed a significant odd's ratio of 4.33 (95% CI=1.69 – 11.06) which indicated that postmenopausal breast cancer females with serum resistin ≥ 8.35 ng/ml have 4 fold increased risk of getting breast cancer compared with those who have serum resistin < 8.35 ng/ml. Our results were in agreement with Dalamaga et al [17]. Di-Simone et al [19] found that resistin enhanced matrix metal-oproteinase 2 (MMP-2) mRNA expression and protein synthesis, significantly reduced TIMP-1 and TIMP-2 synthesis, and it increased trophoblast-like cell invasiveness. Additionally, resistin induced the production of vascular endothelium growth factor (VEGF), and stimulated the formation of endothelial cell tube *in vitro*. On the other hand, our results disagreed with that of Gaudet et al [18].

Because a previous study revealed that resistin is expressed not only from adipose tissue but also from monocytes and macrophages, and is correlated with C-reactive protein, soluble TNF-α, and IL-6 directly, the role of resistin as a marker of inflammation has received growing interest [20]. Chronic inflammation is known to be one of the causes of cancer development. Thus, the correlation between plasma resistin levels and breast cancer risk might be partly explained by inflammation [21]. These findings may strengthen and explain the risky role of resistin in carcinogenesis and progression of breast cancer. In view of this study, we can speculate that decreasing serum resistin levels may represent a therapeutic option for the reduction of breast cancer risk. This can be done via obesity control, increasing physical activity, and pharmacological intervention.

High insulin levels were most common in overweight women. Hyperinsulinemia induces proliferative tissue abnormalities because of the strong anabolic effect of insulin, which stimulates DNA synthesis and cell proliferation [22]. In the present study, serum insulin levels were

significantly elevated in breast cancer patients compared with apparently normal controls. Therefore, at the same time, serum insulin showed a significant odd's ratio of 3.66 (95% CI=1.41 – 9.46), which indicated that postmenopausal breast cancer females with serum insulin levels ≥16.5 µIU/ml have about 4 fold increased risk of having breast cancer compared with those who have serum insulin levels < 16.5 µIU/ml. Our results were in agreement with Chowdhury [23] who reported the involvement of high insulin levels in the promotional stage of breast tumorigenesis and progression to express the metastatic phenotype, rather than the initiation and neoplastic transformation of the breast epithelial cell. On the other hand, our results disagreed with Autier et al [24], who found non-significant difference between breast cancer patients and controls regarding serum insulin levels.

Insulin resistance is one of the most pronounced metabolic changes associated with obesity. Although insulin is most widely known for its metabolic effects, studies showing that insulin has mitogenic effects on both normal and malignant breast tissue provided the biologic basis for an association of hyperinsulinemia with breast cancer [7]. In the current study, we estimated insulin resistance status in both patients and controls using the homeostatic model assessment (HOMA) method [25]. The results showed that there was a significant increase in insulin resistance in breast cancer patients compared to apparently normal controls. Also, insulin resistance showed a significant odd's ratio of 3.56 (95% CI=1.39 – 9.08), which indicated that postmenopausal breast cancer females with insulin resistance ≥ 4.9 have about 4 fold increased risk of having breast cancer compared with those who have serum insulin resistance < 4.9. Our results confirmed the results of Al Awadhi et al [7] and Formica et al [26]. *Therefore, they found that* insulin resistance has causative and prognostic role in breast cancer development and progression in postmenopausal patients.

However, because obesity is often complicated with hyperinsulinemia and insulin could stimulate cellular mitosis, insulin resistance probably might be one of the mechanisms underlying the relationship between obesity and breast cancer risk. Various explanations have been proposed for the association of insulin and insulin resistance with breast cancer incidence in obese postmenopausal females as follows: **(a)** Chronic hyperinsulinemia in affected individuals may promote cancer as insulin can exert its oncogenic potential via abnormal stimulation of multiple cellular signaling cascades, enhancing growth factor-dependant cell proliferations, and /or by directly affecting cell metabolism. **(b)** Insulin increases bioactivity of IGF-1 by enhancing hepatic IGF-1 synthesis and by reducing hepatic protein production of the insulin–like growth factor binding protein-1(IGFB-P-1) and 2(IGFB-P-2) [27]. Therefore, although insulin can directly induce tumor growth, many of its mitogenic and antiapoptotic

effects are operating through the IGF-1 system as reported in individuals with high levels of circulating IGF-1, in which an increased risk of developing certain types of tumors, in particular breast cancer, has been documented [28]. **(c)** Insulin, by reducing SHBG levels, exerts a positive effect on estrogen bioavailability, therefore increasing the risk of breast cancer. **(d)** obesity, the most common cause of insulin resistance, is increasingly recognized as a low grade inflammatory state in which overproduction of certain molecules such as free fatty acids, IL6, adiponectin, TNF-α, plasminogen activator inhibitor-1, and monocyte chemoattractant protein (MCP-1) can play a role in malignant transformation and /or cancer progression. In this context, chronic hyperglycemia and increased oxidative stress may also contribute to increased cancer risk. However, these lines of evidence support the concept that a relationship exists between insulin resistance and cancer.

The s**ex hormone-binding globulin** (SHBG) **transport**s androgens and estradiol in blood and modulates their bioavailable fraction and access to target cells. Also, SHBG directly regulates the incidence of breast tissue [29]. In the current study, there was a significant decline in SHBG levels in breast cancer patients compared with apparently normal controls. Thus, serum SHBG showed a significant odd's ratio of 0.25 (95% CI=0.092- 0.67), which indicated that postmenopausal breast cancer females with serum SHBG levels < 56.55 nmol/ml have about 4 fold increased risk of having breast cancer compared with those who have serum SHBG levels ≥ 56.55 nmol/ml. Our results were in agreement with that of Woolcott et al [30].

The reduced levels of SHBG recorded in the current study is assumed to be due to higher circulating insulin concentration detected in obese breast cancer subjects, as basal secretion of SHBG by cultured human hepatoma cell line (HePG2) was greatly reduced by the physiological concentration of insulin [31]. Previous studies showed that *in vivo* diazoxide treatment, resulting in decreased insulin levels, produced a significant increase in SHBG [32]. Therefore, these intervention studies suggested that insulin negatively regulates hepatic production of SHBG.

Sex steroid hormones play a central role in the development of breast cancer. Collaborative analysis using individual data from prospective studies has demonstrated significant relationships between concentrations of endogenous sex hormones and breast cancer risk in postmenopausal women [33]. Sex hormones generally circulate bound to plasma proteins with only a very small fraction circulating unbound (between 1 and 5%). The majority of the protein binding to estradiol is provided by SHBG and albumin. Initially, it was hypothesized that it was only the free (unbound) fractions of sex hormones that were biologically active [15].

In the present study, there was a significant increase in the serum levels of free E2 in breast cancer patients compared with apparently normal controls. Serum free E2 showed a significant odd's ratio of 5.21(95% CI=1.86 – 14.52), which indicated that postmenopausal breast cancer females with serum free E2 levels ≥1.07 pg/ml have about 5 fold increased risk of having breast cancer compared with those who have serum free E2 levels <1.07 pg/ml. Our results confirmed those of Fourkala et al [34] who reported that the risk for breast cancer increased significantly with increasing concentrations of all sex hormones examined: total estradiol, free estradiol, non-sex hormone-binding globulin (SHBG)-bound estradiol (which comprises of free and albumin-bound estradiol), estrone, estrone sulfate, androstenedione, dehydroepiandrosterone, dehydroepian-drosterone sulfate, and testosterone. On the other hand, our results disagreed with Awio et al [35] who found non-significant difference between breast cancer and controls regarding serum free E2.

This estrogen excess is explained by over activity of aromatase cytochrome P450 enzyme which is expressed at high levels in white adipose tissue and is responsible for a key step in the biosynthesis of estrogen. Moreover, it is well known that SHBG through its specific membrane receptor (SHBG-R), cAMP and protein kinase A, inhibits the estradiol-induced cell proliferation. Accordingly, the decreased levels of SHBG observed in this study may be another contributing factor causing elevated levels of free estradiol [36]. It was reported that the effect of estradiol on breast cancer risk would be observed most strongly for the fraction of estradiol that is not tightly bound by SHBG. This is because this fraction of estradiol (which comprises of free and albumin bound estradiol) is readily able to enter cells, whereas SHBG-bound estradiol does not [37]. Our results supported this hypothesis.

In the present study, the receiver operating characteristic (ROC) curve analysis was used to compare the diagnostic accuracy of each biomarker based on the area under the curve (AUC) for each parameter. A marker having a greater AUC is a superior diagnostic marker to another marker having a smaller AUC. From the results of the present study, serum free E2 was the most superior diagnostic marker followed by insulin resistance, insulin, SHBG, and resistin. In the current study, all the AUCs were greater than 62%. Therefore, this means that each assayed biochemical parameter can be used as a diagnostic marker with an acceptable performance. Up to the best of our knowledge, this is the first study which evaluates and compares the diagnostic and values of insulin resistance status, serum levels of resistin, insulin, SHBG, and free E2 using the ROC curve analysis.

In the current study, insulin resistance status, serum levels of resistin, insulin, SHBG, and free E2 were non-significantly correlated with both clinicopathological features and disease-free survival of breast cancer patients. Therefore, this means that none of the assayed biochemical parameters can be used as a prognostic marker in obese postmenopausal breast cancer patients. Our result disagrees with the results of Gennari et al [38] and Assiri et al [39]. This contradiction between our results and previous ones may be due to the small sample size, short time follow up, and the conditions of the studies including the methodological differences.

Conclusion

From the current study, it could be concluded that: **(a)** all of these biochemical parameters can have a role in the etiology of postmenopausal breast cancer; **(b)** all of these biochemical parameters can be used to diagnose postmenopausal breast cancer patients with free E2 as the most superior diagnostic marker followed by insulin resistance, insulin, SHBG, and resistin; and **(c)** none of these biochemical parameters has a respectable prognostic role in postmenopausal breast cancer patients.

Reference:

Horner MJ, Ries LAG, Krapcho M, et al. (2009). SEER Cancer Statistics Review 1975-2006. National Cancer Institute. Bethesda.

Alexandria Cancer Registry Annual Report. (2011). Medical Research Institute, Alexandria University, Egypt.

Manulu MS, Sutanegara IN. (2006). Pathogenesis and pharmacologic treatment of obesity: the role of energy regulatory mechanism. Acta Med Indones 38:42-50.

Kershaw EE, Flier JS. (2004) Adipose tissue as an endocrine organ. J Clin Endocrinol Metab 89:2548–56.

Pamuk GE, Demir M, Harmandar F, et al. (2006). Leptin and resistin levels in serum of patients with hematologic malignancies: correlation with clinical characteristics. Exp Oncol 28: 241-44.

Rose DP, Vona-Davis L. (2012). The cellular and molecular mechanisms by which insulin influences breast cancer risk and progression. Endocr Relat Cancer 19:225-41.

Al Awadhi SA, Al Khaldi RM, Al Rammah T, et al. (2012). Associations of adipokines & insulin resistance with sex steroids in patients with breast cancer. Indian J Med Res 135: 500–5.

Vermeulen A, Verdonck L, Kaufman JM. (1999). A critical evaluation of simple methods for the estimation of free testosterone in serum. J Clin Endocrinol Metab 84: 3666- 72.

Thomas G, Strom A, Lindberg K, et al. (2011). Estrogen receptor beta decreases survival of p53-defective cancer cells after DNA damage by impairing G2/ M checkpoint signaling. Breast Cancer Research and Treatment 127:417–27.
Burstein HJ, Harris JR, Morrow M. Malignant tumors of the breast (2008). In: De Vita VT, Lawrence TS, Rosenberg SA (eds). Cancer: principles and practice of oncology; 8th ed. Philadeliphiia: Lippincott Williams & Wilkins (pub.), chapter 43, pp. 1606-1654.
Rintoul RF. Operations on the breast. (1986). In: Farquhaerisons text book of operative surgery; 7th ed. Churchill living stone (pub.), pp. 270-281.
Haskell CM, Lowitz BB, Casciato AD. Breast cancer (1985). In: Casciato AD and Lowitz BB; eds. Manual of clinical oncology; 2nd ed. Little and Brown Company, Boston, Toronto (pub.), pp. 150-65.
Abeloff MD, Lichter AS, Niederhuber JE, Pierce LJ and Aziz DC (1995). Breast. In: Abeloff MD, Armitage JO, Licher AS, and Niederhuber JE; eds. Clinical Oncology. Churchil livingstone Inc. (Pub.), Chapter 73, pp 1617-714.
Tara MW, Levy JC, Matthews DR. (2004). Use and Abuse of HOMA Modeling. Diabetes Care; 27:1487–95.
Sodergard R, Backstrom T, Shanbhag V, et al. (1982). Calculation of free and bound fractions of testosterone and 17 ß- estradiol to human plasma proteins at body temperature. J. Steroid Biochem; 16: 801–10.
Steppan CM, Bailey ST, Bhat S, et al. (2001). The hormone resistin links obesity to diabetes. Nature; 409: 307-12.
Dalamaga M, Karmaniolas K, Papadavid E, et al. (2013). Hyperresistinemia is associated with postmenopausal breast cancer. Menopause 20: 243-362.
Gaudet MM, Falk RT, Gierach LG, et al. (2010). Do adipokines underlie the association between known risk factors and breast cancer among a cohort of U.S. women? Cancer Epidemiol; 34: 580–6.
Di Simone N, Di Nicuolo F, Sanguinetti M, et al. (2006). Resistin regulates human choriocarcinoma cell invasive behaviour and endothelial cell angiogenic processes. J Endocrinol 189: 691-9.
Reilly MP, Lehrke M, Wolfe ML, et al. (2005). Resistin is an inflammatory marker of atherosclerosis in human. Cirulation 111: 932-9.
Shacter E, Weitzman SA. (2002). Chronic inflammation and cancer. Oncology 16: 217-26.
Giovannucci E, Harlan DM, Archer MC (2010). Diabetes and cancer. Diabetes Care 33:1674-85.
Chowdhury TA. (2010). Diabetes and Cancer. Q J Med 103:905–15.
Autier P, Koechlin A, Boniol M, et al. (2013). Serum insulin and C-peptide concentration and breast cancer. Cancer Causes Control 24: 873-83.

Yang X, Edgerton SM, Kosanke SD, et al. (2003). Hormonal and dietary modulation of mammary carcinogenesis in mouse mammary tumor virus-c-erbB-2 transgenic mice. Cancer Res 63: 2425-33.
Formica V , Tesauro M, Cardillo C, et al. (2012). Insulinemia and the risk of breast cancer and its relapse. Diabetes Obes Metab 14:1073–80.
Pollak M. (2008). Insulin and insulin-like growth factor signaling in neoplasia. Nature Reviews Cancer 8: 915- 28.
Vigneri V, Frasca F, Sciacca L, et al. (2009). Diabetes and cancer. Endocr Relat Cancer; 16:1103–23.
Hammond GL. (1995). Potential functions of plasma steroid-binding proteins. Trends Endocrinol Metab 6:298 – 340.
Woolcott CG, Shvetsov YB, Stanczyk FZ, et al. (2010). Plasma sex hormone concentrations and breast cancer risk in an ethnically diverse population of postmenopausal women. Endocr Relat Cancer 17: 125–34.
Janković D, Wolf P, Anderwald CH, et al. (2012). Prevalence of endocrine disorders in morbidly obese patients and the effects of bariatric surgery on endocrine and metabolic parameters. Obes Surg 22:62-9.
Kupelian V, Hayes FJ, Link CL, et al. (2008). Inverse association of testosterone and the metabolic syndrome in men is consistent across race and ethnic groups. J Clin Endocrinol Metab 93:3403-10.
Thomas HV, Key TJ, Allen DS, et al. (1997). Prospective study of endogenous serum hormone concentrations and breast cancer risk in postmenopausal women. Br J Cancer 76: 401–5.
Fourkala1 EO, Zaikin A, Burnell M, et al. (2012). Association of serum sex steroid receptor bioactivity and sex steroid hormones with breast cancer risk in postmenopausal women. Endocr Relat Cancer; 19: 137–47.
Awio JP, Galukande M, Kituuka O, et al. (2012). High serum estradiol confers no risk for breast cancer. Pan Afr Med J 12: 23.
Vermeulen A, Kaufman JM, Goemaere S, et al. (2002). Estradiol in elderly men. Aging Male 5:98-102.
Siiteri PK, Hammond GL, Nisker JA. (1981). Increased availability of serum estrogens in breast cancer: a new hypothesis. In: Pike MC, Siiteri PK, Welsch CW, ed. Banbury Report 8: Hormones and breast cancer. Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press (pub), pp. 87–101.
Gennari A, Puntoni M, Nanni O, et al. (2014). Impact of insulin resistance (IR) on the prognosis of metastatic breast cancer (MBC) patients treated with first-line chemotherapy (CT). J Clin Oncol 32:5s.
Assiri AM, Kamel HF, Hassanien MF. Resistin, Visfatin, Adiponectin, and Leptin: Risk of Breast Cancer in Pre- and Postmenopausal Saudi Females and Their Possible Diagnostic and Predictive Implications as Novel Biomarkers. Disease Markers. doi.org/10.1155/2015/253519.