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Serum Adipocytokines (Visfatin and Resistin): New Biomarkers of Breast Carcinogenesis

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Abstract

Background: Recent epidemiological studies demonstrate that obesity is associated with an increased risk for breast cancer in women. Increased estrogen levels are suggested as one possible explanation, but this does not fully explain the relationship between obesity and breast cancer. One alternative explanation is secretion by adipocytes of metabolites, hormones and cytokines, collectively known as adipocytokines, which regulate physiological and pathological processes. Among these adipokines are visfatin and resistin. This study investigates whether visfatin or resistin in serum of breast cancer patients can be used as potential diagnostic and prognostic tools for breast cancer, taking into account clinicopathological features and anthropometric parameters.

Methods: Blood samples were collected from 70 breast cancer patients (35 obese and 35 non-obese) and 20 healthy females matched for age and body mass index as the control group. Serum visfatin levels were measured by enzyme linked immunosorbent assay and serum resistin levels were measured by radioimmunoassay. Inflammatory status was assessed by measuring C-reactive protein levels by an automated turbidimetric analyzer.

Results: We observed highly elevated serum resistin and visfatin levels in breast cancer patients compared to controls, independent of body mass index. Serum resistin and visfatin levels were likely to be associated with increased breast cancer risk and correlated with the inflammatory marker C-reactive protein.

Conclusion: Targeting resistin and visfatin inhibition can be an effective therapeutic strategy in breast cancer by downregulating the inflammatory microenvironment in breast tissue. Serum visfatin promises to be a novel biomarker of diagnostic and prognostic value. Larger prospective studies are required to confirm our findings.

Keywords: Breast cancer, Visfatin, Resistin, Prognosis

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Introduction

Breast cancer is the most common malignancy in women worldwide, and its prevalence is surprisingly increasing at a rapid rate. Therefore, it is critical to discover prognostic factors as well as therapeutic targets for breast cancer. Previous studies have clearly shown that obesity is correlated with the risk and prognosis of breast cancer.[1-3] Adipose tissue is not only a passive reservoir for energy storage but is now known to express and secrete a variety of metabolites, hormones, and cytokines, known as adipocytokines, which act at both the local and systemic levels.[4] It has been recently suggested that adipocytokines, such as TNF alpha, adiponectin, leptin and resistin are associated with the risk of cancer at various sites (e.g., breast, prostate gland, endometrium and colorectum).[5-7]

Visfatin is a recently described adipokine that is reported to be highly expressed by visceral adipose tissue (VAT) both in humans and mice. Visfatin, also known as pre-B cell colony–enhancing factor, is expressed in normal, inflamed, and tumor tissues.[8] It possesses NAD biosynthetic activity and regulates growth, apoptosis, and angiogenesis in mammalian cells.[9,10] Visfatin is also a cytokine-like molecule secreted from human peripheral blood lymphocytes considered essential for B-cell maturation and function.[11]

Visfatin is expressed in breast cancer tissue and MCF-7 breast cancer cells.[11] Kim et al.[12] have reported that visfatin regulated proliferation of MCF-7 human breast cancer cells. Exogenous administration of recombinant visfatin increased cell proliferation and DNA synthesis rate in MCF-7 cells. Visfatin also increased the expression of matrix metalloproteinase-2, matrix metalloproteinase-9, and vascular endothelial growth factor (VEGF) genes, which suggested that it might function in metastasis and angiogenesis of breast cancer.

Resistin is a 12.5 kDa protein originally found to be secreted by mouse adipocytes. Although adipocytes are a major source of resistin in rodents, resistin is mainly expressed by macrophages in humans. Compared with other adipose depots, VAT may serve as a primary source of resistin production. Resistin released by adipocytes is negligible compared to its release by non-fat cells of human adipose tissue. As an important adipose-derived hormone, resistin causes insulin resistance and inflammation.[13] Elevated levels of plasma resistin have been found in breast cancer[14] and high resistin expression in breast cancer tissue is associated with a more malignant clinicopathological status as well as poor patient survival.[15]

The aim of the present study was to investigate whether visfatin or resistin in serum of breast cancer patients could be used as potential diagnostic and prognostic tools for breast cancer, taking into account clinicopathological features and anthropometric parameters.

Patients and Methods

We enrolled 70 female patients newly diagnosed with breast cancer (35 obese and 35 non-obese) in this study as the patient groups. The control group comprised 20 women with normal mammography findings and no previous history of any kind of cancer (10 obese and 10 non-obese), matched for age and body mass index (BMI). Information regarding age, menopausal status, weight and height of all participants were recorded. Body mass index was calculated as weight in kilograms divided by the square of the height in meters (kg/m^2); a BMI of ≥30 kg/m^2 was used to define obesity. Exclusion criteria included breast cancer patients with inflammatory diseases such as rheumatoid arthritis, any viral infection, and metastatic patients at the time of diagnosis.

Patients were selected from those admitted to the Department of Cancer Management and Research, Medical Research Institute, Alexandria University in the time period between January to June 2012. A written consent for participating in the study was taken according to the Declaration of Helsinki and approved by the Ethical Committee of the Medical Research Institute.

Patients were subjected to preoperative evaluation that included history taking, clinical examination to detect the tumor site and the

Table 1. Patients' clinical characteristics.

	Obese (n=35)	Non-obese (n=35)	*P*-value
Age (years)			
Mean ± SD	50.10 ± 8.13	38 – 63	0.468
Range	49.76 ± 11.52	35 – 65	
Menopausal status			
Pre	15 (42.8%)	20 (57.1%)	0.473
Post	18 (51.4%)	17 (48.6%)	
Histological grade			
I-II	25 (71.4%)	10 (28.6 %)	0.403
III	28 (80%)	7 (20%)	
Clinical stage			
I-II	24 (68.6%)	11 (31.4%)	0.597
III	26 (74.3%)	9 (25.7%)	
ER status			
Positive	22 (62.9%)	13 (37.1%)	0.626
Negative	20 (57.1%)	15 (42.9%)	
PR status			
Positive	21(60%)	14 (40%)	0.470
Negative	18 (51.4%)	17 (48.6%)	
Her-2/neu expression			
Positive	13 (37.1%)	22 (62.9%)	0.615
Negative	11 (31.4%)	24 (68.6%)	
Vascular invasion			
Yes	26 (74.3%)	9 (25.7%)	1.000
No	26 (74.3%)	9 (25.7%)	
Tumor size (cm)			
≤3	12(34.28%)	23 (65.72%)	0.127
>3	15(42.86%)	20 (57.14%)	
Axillary lymph node involvement (%)			
Positive	55.26%	44.74%	0.287
Negative	45.37%	54.63%	

Abbreviations: SD: Standard deviation; ER: Estrogen receptor; PR: Progesterone receptor; Her-2: Human epidermal growth factor receptor 2.; *: Statistically significant at $P \leq 0.05$.

presence of enlarged axillary lymph nodes. Radiological investigations included mammogram, abdominal ultrasound and chest x-ray. Preoperative investigations also included fine needle aspiration cytology (FNAC) to diagnose the presence of malignancy. Patients were subjected to surgery (modified radical mastectomy or conservative surgery). Postoperative pathological evaluation of the tumor included type of tumor, grade, size of the tumor, numbers of axillary lymph nodes involved, and presence or absence of vascular invasion. Assessments of estrogen, progesterone receptors (ER, PR) and Her2/neu expression were also confirmed.

Blood sample collection

A total of 5 ml fasting venous blood was obtained from healthy controls and within a week before surgery for breast cancer patients. Blood samples were allowed to clot for 30 min before centrifugation; samples were centrifuged at 3000 rpm for 10 min to isolate sera. The serum was stored at -80°C until use for estimating the levels of resistin and visfatin. Circulating serum visfatin levels were measured by enzyme linked immunosorbent assay according to the manufacturer's instructions (Aviscera Bioscience, USA). Serum resistin levels were measured by radioimmunoassay according to the manufacturer's instructions (Phoenix Pharmaceuticals, California,

USA). Inflammatory status was assessed by measuring C-reactive protein levels by immunoturbidimetric test (HUMAN, Germany). Patients were followed up clinically, radiologically and by laboratory testing for 30 months to assure the prognostic value of the markers.

Statistical analyses

Statistical analyses were conducted using the statistical software package SPSS version 17 (SPSS Inc., Chicago, IL, USA). Differences between groups were assessed by the Mann Whitney U test for nonparametric variables. Multiple logistic regression analysis was used to assess the association between serum visfatin and resistin levels to breast cancer risk. The adjusted odds ratio (OR) and exact computation of 95% confidence intervals (95% CI) were calculated. Serum visfatin and resistin diagnostic performance was evaluated using ROC curve analysis. The Kaplan-Meier curve was used to calculate disease-free survival (DFS) and overall survival (OS) using log-rank test. We defined DFS as the time between surgery and the date of unfavorable outcome, whichever appeared first. Overall survival was defined as the time from surgery to death for any cause. Furthermore, hazard ratios (HRs) and 95% CIs computed from multivariate Cox regression models were used to determine the independent predictors of patient survival. Spearman's correlation coefficients were calculated to evaluate the association between relevant parameters. Statistical significance was set at $P \leq 0.05$.

Results

Patients' clinical characteristics

Table 1 shows the study patients' clinical characteristics. There was an insignificant difference between obese and non-obese breast cancer patients in terms of age, menopausal status, nuclear grade, stage, ER, PR, Her-2/neu status, tumor size, lymph node metastasis, and vascular invasion (Table 1).

Serum visfatin (ng/ml)

As seen in table 2, the median serum visfatin levels was highly elevated in breast cancer patients compared to controls ($P<0.001$). The median serum visfatin levels showed insignificant differences between the obese and non-obese control groups ($P=0.914$) or between the obese and non-obese breast cancer patients ($P=0.742$). We observed significantly higher median serum visfatin levels in both obese ($P<0.001$) and non-obese ($P<0.001$) breast cancer patients compared to the corresponding control values.

Serum resistin (pg/ml)

There were highly elevated median serum resistin levels in breast cancer patients compared to controls ($P<0.001$). The median serum resistin

Table 2. Serum visfatin and serum resistin levels in breast cancer patients compared to controls in all studied groups.

Visfatin	**Healthy controls (n=20)**	**All breast cancer patients (n=70)**	**Control groups**		**Breast cancer patient groups**	
			Obese (n=10)	**Non-obese (n=10)**	**Obese (n=35)**	**Non-obese (n=35)**
Range	0 - 4	0.2 - 63	0 – 4	0 – 3.5	0.4 – 63	0.2 – 55
Median	0.5	5	0.5	0.2	5	7
P	<0.001*					
P			0.914		0.742	
P			<0.001*	<0.001*		
Resistin						
Range	70-1400	250-2700	80-1400	70-1250	370-2500	250-2700
Median	780	1200	780	810	1150	1200
P	<0.001*					
P			0.940		0.697	
P			0.012*	0.003*		

*: Statistically significant at $P \leq 0.05$.

Table 3. Correlation between serum visfatin and serum resistin levels with clinicopathological characteristics.

	Visfatin (ng/ml)		Resistin (pg/ml)	
	rs	*P*-value	rs	*P*-value
Age (years)	0.203	0.126	0.088	0.511
Axillary lymph node involvement (%)	0.295	0.025*	0.380	0.034*
Histological grade	0.298	0.028*	0.251	0.047*
Tumor size	0.302	0.021*	0.025	0.851
Clinical stage	0.329	0.012*	0.057	0.673
BMI	0.181	0.174	0.018	0.895
C-reactive protein	0.310	0.036*	0.275	0.041*
Menopausal status	0.032	0.856	0.103	0.563
Resistin	0.124	0.355		

rs: Spearman coefficient.; *: Statistically significant at $P \leq 0.05$.; BMI: Body mass index

levels (pg/ml) showed insignificant differences between the obese and non-obese control groups (*P*=0.940) or between the obese and non-obese breast cancer patients (*P*=0.697). There were significantly higher median serum resistin levels in both obese (*P*=0.012) and non-obese (*P*=0.003) breast cancer patients compared to their corresponding control values.

Serum C-reactive protein (mg/l)

Serum C-reactive protein levels ranged from (0.15 - 1.5 mg/l) with a mean ± SD of 1.2 ± 0.8 mg/l in healthy controls; the range was 0.7 - 30.6 mg/l with a mean ± SD of 4.5 ± 1.3 mg/l in breast cancer patients. Serum C-reactive protein was highly elevated in breast cancer patients compared to controls (*P*<0.001).

Correlation between serum visfatin and serum resistin levels and patients' clinicopathological characteristics

According to tables 3 and 4, we observed that serum visfatin levels in breast cancer patients showed a significant positive correlation with clinicopathological variables that included axillary lymph node involvement (*P*=0.025), histological grade (*P*=0.028), tumor size (*P*=0.021), clinical stage (*P*=0.012), negative ER (*P*=0.043) and negative PR status (*P*=0.030), positive Her-2/neu expression (*P*=0.038), and C-reactive protein levels (*P*=0.036). There was no significant correlation observed between serum visfatin with serum resistin (*P*=0.355), age (*P*=0.126), menopausal status (*P*=0.856) and BMI (*P*=0.174).

We observed a significantly positive correlation between serum resistin levels in breast cancer patients to clinicopathological variables that included axillary lymph node involvement (*P*=0.0394), histological grade (*P*=0.047), negative ER status (*P*=0.033) and negative PR status (*P*=0.024), positive Her-2/neu expression (*P*=0.045), and C-reactive protein levels (*P*=0.041). There was no correlation with tumor size (*P*=0.851), clinical stage (*P*=0.673), age (*P*=0.511), menopausal status (*P*=0.563), and BMI (*P*=0.895).

Association of serum visfatin and resistin levels with risk of breast cancer

Table 5 shows the estimated OR for breast cancer risk according to serum visfatin and resistin levels using multiple logistic regression analysis. According to Table 5, the risk of breast cancer significantly increased in the higher serum visfatin group compared to the lower serum visfatin group after adjustments for age, BMI, menopausal status and serum resistin (OR: 28.866; 95% CI: 5.181-160.833). There was a significant increase risk for breast cancer in the higher serum resistin group compared to the lower serum resistin group; this significance remained after adjustments for age, BMI, menopausal status and serum visfatin (OR: 10.823; 95% CI: 2.710-43.220).

Comparison between serum visfatin and serum resistin levels as diagnostic markers for breast cancer

ROC curve analysis was used to compare the

diagnostic values of serum visfatin and resistin depending on the area under the curve (AUC). The higher AUC corresponded to the better diagnostic test. Serum visfatin showed significant AUC (0.932; $P<0.001$) with a sensitivity of 96.55% and a specificity of 95.0% at a cut-off value of 0.5 ng/ml. Serum resistin showed significant AUC (0.791; $P<0.001$) with a 62.07% sensitivity and 85% specificity at a cut-off value of 1050 pg/ml.

Prognostic values of serum visfatin (ng/ml) and resistin (pg/ml) in breast cancer patients

Disease-free survival (DFS) according to serum visfatin and serum resistin

Figure 1 shows that breast cancer patients with high serum visfatin (>0.5 ng/ml) levels had shorter median DFS (22 months) compared to patients with low serum visfatin (≤0.5 ng/ml) whose DFS was 28 months. The difference was statistically significant ($P<0.000$); the value of the log-rank test was 11.05.

According to figure 2, the median DFS time for breast cancer patients with high serum resistin (>1050 pg/ml) was 26 months. This value did not significantly differ ($P=0.402$) from the median DFS time for patients with low serum resistin (≤1050 pg/ml) levels, which was 24 months.

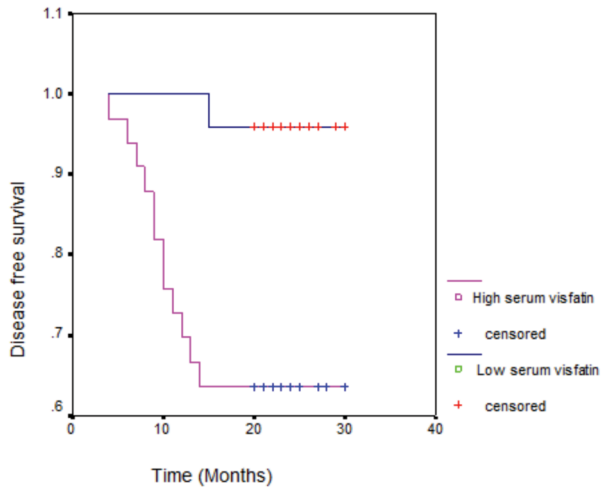

Figure 1. Disease free survival according to serum visfatin in 70 patients with breast cancer.

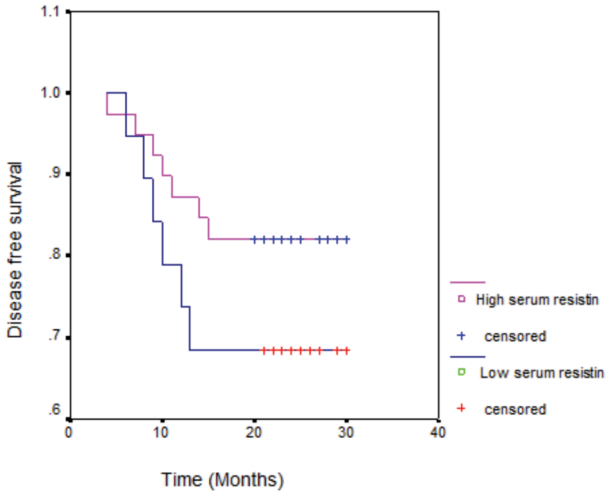

Figure 2. Disease free survival according to serum resistin in 70 patients with breast cancer.

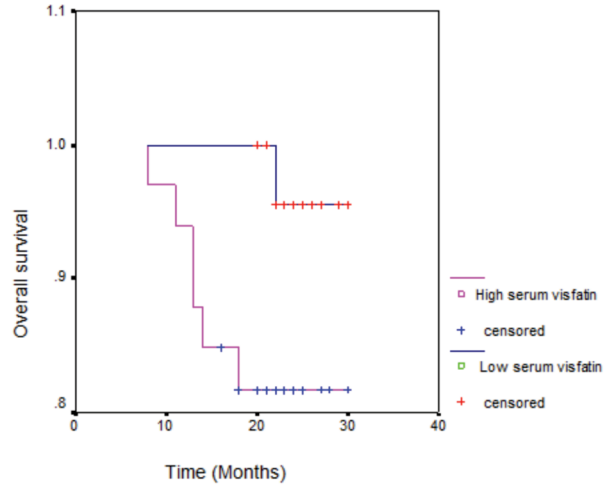

Figure 3. Overall survival according to serum visfatin in 70 patients with breast cancer.

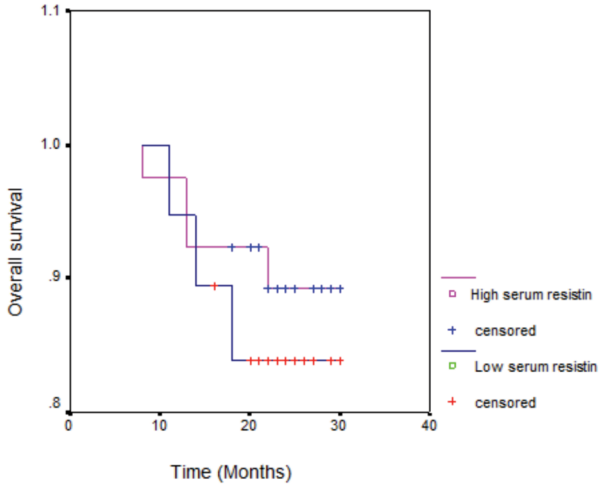

Figure 4. Overall survival according to serum resistin in 70 patients with breast cancer.

Overall survival (OS) according to serum visfatin and serum resistin

Figure 3 shows that breast cancer patients with high serum visfatin (>0.5 ng/ml) had shorter median OS time (23 months) compared to patients with low serum visfatin levels of ≤0.5 ng/ml (29 months). The difference was statistically significant (*P*=0.025) and the value of the log-rank test was 4.97.

As shown in figure 4, the median OS time for breast cancer patients with high serum resistin (>1050 pg/ml) was 28 months. This did not significantly differ (*P*=0.333) from the median OS time for patients with low serum resistin (≤1050 pg/ml) levels, which was 26.5 months.

Cox regression analyses for disease-free (DFS) and overall survival (OS) in breast cancer patients

We estimated the HRs by multivariate Cox regression analyses to find the independent predictors of DFS in breast cancer patients. In multivariate analysis, after adjustment for other factors, only clinical stage (HR: 8.361; 95% CI: 1.038 - 67.359; *P*=0.002) and serum visfatin (HR: 5.042; 95% CI: 0.355 - 31.450; *P*=0.006) were independent predictors of poor DFS. On the other hand, we found that age at presentation, ER and PR status, lymph node metastasis, tumor grade, BMI, Her-2/neu expression, C-reactive protein and serum resistin levels showed no association with breast cancer DFS (Table 6).

As shown in table 6, in multivariate analysis after adjustment for other factors, only clinical stage (HR: 11.505; 95% CI: 2.218 - 59.686; *P*=0.004) and serum visfatin (HR: 6.823: 95% CI: 0.616 - 44.620, *P*=0.011) were independent predictors of poor OS. On the other hand, we found that age at presentation, ER and PR status, lymph node metastasis, tumor grade, BMI, Her-2/neu expression, C-reactive protein, and serum resistin levels showed no associations with breast cancer OS.

Table 4. Association of serum visfatin and serum resistin levels with negative ER and PR status, and positive Her-2/neu expression in breast cancer patients.

	Visfatin Median (range)	***P*-value**
ER status		
Positive (n=42)	9.2 (0.2-18)	0.043*
Negative (n=28)	15 (5-63)	
PR status		
Positive (n=39)	7 (0.2-20)	0.030*
Negative (n=31)	13 (3.5-63)	
Her-2/neu expression		
Positive (n=24)	12 (4-63)	0.038*
Negative (n=46)	8.2 (0.2-22)	
	Resistin Median (range)	***P*-value**
ER status		
Positive (n=42)	920 (250-1450)	0.033*
Negative (n=28)	1260 (660-2700)	
PR status		
Positive (n=39)	850 (250-1700)	0.024*
Negative (n=31)	1350 (570-2700)	
Her-2/neu expression		
Positive (n=24)	1230 (770-2700)	0.045*
Negative (n=46)	950 (250-1570)	

*: Statistically significant at *P*≤0.05.; ER: Estrogen receptor; PR: Progesterone receptor; Her-2: Human epidermal growth factor receptor 2.

Discussion

Obesity and its metabolic complications have rapidly become major global health issues and are associated with an increased risk for cancer, particularly breast cancer in postmenopausal women. Adipose tissue is considered a genuine endocrine organ that secretes a variety of bioactive adipokines, such as leptin, adiponectin, resistin and nicotinamide phosphoribosyl-transferase/visfatin.[16] the main source of adipokines secretion is adipocytes but other cells and tissues can

Table 5. Analysis of multiple logistic regression for the risk of breast cancer according to serum visfatin and resistin levels.

	OR	**95% CI**	***P*-value**
Visfatin only	21.706	4.532 - 103.964	<0.001*
Visfatin + age+ menopausal status + BMI+ resistin	28.866	5.181 - 160.833	<0.001*
Resistin only	9.273	2.435 - 35.310	<0.001*
Resistin + age+ menopausal status + BMI+ visfatin	10.823	2.710 - 43.220	0.001*

OR: Odds ratio; CI: Confidence interval; BMI: Body mass index.; *: Statistically significant at *P*≤0.05.

Table 6. Multivariate Cox regression for disease-free (DFS) and overall survival (OS) in breast cancer patients.

Variables	DFS			OS		
	HR	95% CI	*P*-value	HR	95% CI	*P*-value
Age (≥50 vs. <50)	2.795	0.793 - 9.847	0.110	5.827	0.927 - 36.627	0.076
ER (positive vs. negative)	0.752	0.461 - 2.215	0.391	0.622	0.257 - 1.506	0.293
PR (positive vs. negative)	0.589	0.206 - 1.689	0.325	0.246	0.053 - 1.137	0.073
LN (positive vs. negative)	1.004	0.845 - 1.192	0.965	0.991	0.756 - 1.229	0.948
Grade (III vs. II and I)	0.721	0.097 - 5.349	0.749	0.474	0.030 - 6.557	0.597
Stage (III vs. II and I)	8.361	1.038 - 67.359	0.002*	11.505	2.218 - 59.686	0.004*
BMI (≥30 vs.<30)	1.063	0.677 - 1.668	0.790	2.100	0.583 - 7.567	0.256
Her-2 (positive vs. negative)	1.158	0.182 - 7.354	0.877	1.066	0.574 - 1.982	0.839
C-RP (positive vs. negative)	2.377	0.501 - 11.274	0.276	1.981	0.173 - 22.710	0.583
Visfatin (high vs. low)	5.042	0.355 - 31.450	0.006*	6.823	0.616 - 44.620	0.011*
Resistin (high vs. low)	0.484	0.144 - 1.623	0.240	0.501	0.091 - 2.754	0.426

HR: Hazard ratio; CI: Confidence interval; ER: Estrogen receptor; PR: Progesterone receptor; LN: Lymph nodes, BMI:; Body mass index; Her-2: Human epidermal growth factor receptor 2; C-RP: C-reactive protein.; * Statistically significant at $P \leq 0.05$.

produce a number of these adipokines.[17] Direct evidence is growing rapidly that supports the stimulatory and/or inhibitory role of adipokines in the process of development and progression of breast cancer. Recent studies support a role for adipokines as novel risk factors and potential diagnostic and prognostic biomarkers in breast cancer.[18]

The present study revealed highly elevated serum visfatin levels in breast cancer patients compared to controls. The median values of serum visfatin levels showed an insignificant difference between obese and non-obese breast cancer patients. The same was true for the control group. On the other hand, there were significantly higher median serum visfatin levels in both obese and non-obese breast cancer patients compared to the corresponding control values, which suggested that other major sources of this adipokine rather than adipose tissue might also be responsible for its high serum concentration in breast cancer patients.

In line with our results, Dalamaga et al.[19] found significantly higher mean serum visfatin levels in breast cancer patients compared to controls. Nakajima et al.[20] reported that visfatin levels in gastric cancer patients were significantly higher compared to controls and that visfatin might be a good biomarker of colorectal malignant potential, independent from BMI. Chang et al.[21] found no difference in visfatin mRNA levels between VAT and subcutaneous adipose tissues (SAT); neither were associated with measures of obesity. Visfatin mRNA levels were strongly correlated with proinflammatory gene expression that included CD68 and tumor necrosis factor-alpha gene in both VAT and SAT. This study agreed with our study which observed an insignificant correlation between serum visfatin levels and BMI.

This adipokine is not only an adipocyte-specific protein. Human peripheral blood lymphocytes have been the first reported to express this protein.[22] Neutrophils can also produce visfatin in response to inflammatory stimuli which inhibits apoptosis under inflammatory conditions.[23] Other sources of visfatin gene expression include peripheral blood mononuclear cells (PBMCs) and peripheral blood granulocytes (PBG) in synovial tissue of rheumatoid arthritis patients. Visfatin has been reported to be highly synthesized in human osteoarthritis chondrocytes.[22] These reports show the link between visfatin and inflammatory status. Ghaemmaghami et al.[16] have shown that HCT-116 colorectal cancer cells secrete and express visfatin protein endogenously, which may apply its possible carcinogenic effects by the autocrine manner. However lymphocytes, neutrophils, and other immune system cells that exist in the stroma compartment of cancerous tissue secrete this adipokine as an inflammatory phase protein due to the inflammatory status of colorectal cancer. Therefore, immune cell visfatin may affect colorectal cancer cells in a paracrine manner. Since inflammation may be present to a certain extent in cancer, immune cells in cancerous

tissue can also be the source of visfatin expression which leads to their high serum levels. Notably, although the adipose tissue function role is less than others, it is still considered the origin of this adipokine. Visfatin can affect cancer cells in an autocrine or paracrine and probably slightly in an endocrine (produced by adipocytes) manner.

The present study showed that serum visfatin had a significant positive correlation with positive axillary lymph node metastasis, histological grade, tumor size and clinical tumor stage. Breast cancer patients with negative ER, negative PR and positive Her-2/neu status were associated with significantly higher serum visfatin levels compared to those who were positive ER, positive PR and negative Her-2/neu. This suggested that high serum visfatin levels were associated with the aggressive malignant phenotype, with a poor prognosis.

Lee et al.[11] reported that high visfatin expression in breast cancer tissues was not correlated with age or BMI, but significantly correlated with tumor size, estrogen and progesterone receptor negativity, as an indicator for poor prognosis. Their results agreed with our findings. Dalamaga et al.[19] found that visfatin had a significant association with CA 15-3, hormone-receptor status, and lymph node invasion, but not with metabolic and anthropometric variables. The absence of estrogen and progesterone receptors was the strongest significant determinant of serum visfatin which agreed with our results.

Visfatin may play a role in mammary epithelium tumorigenesis via the following mechanisms. Visfatin represents an essential enzyme in energy metabolism, circadian clock and cell longevity through intracellular NAD generation.[24,25] Furthermore, the visfatin inhibitor APO866 has been shown to selectively inhibit tumor growth through depletion of intracellular NAD, the product catalyzed by visfatin, in murine gastric and bladder tumor models.[26] In addition, the addition of APO866 synergistically increases the caspase-dependent apoptotic activity of the TNF-related apoptosis-inducing ligand in leukemia cells by enhancing NAD depletion, mitochondrial transmembrane potential dissipation, and ATP depletion.[27-29] Visfatin is a proliferative, anti-apoptotic factor. It has been shown to stimulate the proliferation DNA synthesis rate of MCF-7 human BC cells.[24] Visfatin may play a role in breast cancer development by enhancing the cell proliferation rate through stimulation of cell cycle progression. Visfatin may play a pro-angiogenic, invasive and metastatic role. It was shown to promote angiogenesis via activation of the MAPK ERK-dependent pathway through endothelial fibroblast growth factor-2 and enhance VEGF via MAPK and PI3K/Akt signaling pathways. By increasing the expression of MMP-2 and 9, and VEGF genes, visfatin may contribute to angiogenesis and metastasis in breast cancer.[24,25]

Our results revealed that the risk of breast cancer significantly increased in the highest serum visfatin group compared to the lowest group. This finding agreed with Dalamaga et al.[30] who found that women with the highest quartile of visfatin concentration presented significantly higher odds for postmenopausal breast cancer.

The current study indicated that serum resistin was highly elevated in breast cancer patients compared to controls. The median values of serum resistin levels showed insignificant differences between obese and non-obese control groups, as well as between obese and non-obese breast cancer patient groups. On the other hand, the median values of serum resistin levels in both obese and non-obese breast cancer patients were significantly higher than their corresponding control values. According to these results, a reasonable prediction was that adipocytes were not the main source of resistin secretion.

In agreement with our study, Sun et al.[31] found higher levels of serum resistin in breast cancer patients than controls. Hou et al.[1] reported significantly increased serum resistin levels in breast cancer patients compared to controls. Nakajima et al.[20] reported that resistin levels were significantly higher in colorectal cancer patients compared to controls, independent of BMI, and

these levels gradually increased with tumor stage progression.

It has been suggested that high resistin levels are related to cancer associated chronic inflammation. Recent data indicate that stimulation of macrophages *in vitro* with endotoxin or pro-inflammatory cytokines leads to a marked increase in resistin production and vice versa, resistin strongly up–regulates IL–6 and TNF–alpha production.[31,32] Several studies have observed that adipokine concentrations were not correlated with anthropometric measures of adiposity or weight loss in both control subjects and cancer patients.[31,33] Previous studies revealed that resistin was expressed not only from adipose tissue but also from monocytes and macrophages, and correlated directly with C-reactive protein. The role of resistin as another marker of inflammation has received growing interest, which supported the current findings.[4,15] Interestingly, it has been reported that in human PBMCs, macrophages and bone marrow cells were the main origin of resistin production while human adipocytes moderately expressed resistin.[22,34] Fain et al.[35] found that with the exception of leptin and adiponectin, over 90% of the adipokines released by adipose tissue cultured *in vitro* were produced by nonfat cells.

In terms of the correlation between serum resistin with clinicopathological characteristics, our study showed a significant positive correlation between serum resistin with percent of lymph node metastasis and nuclear grade; there was an insignificant correlation between serum resistin levels with age, tumor size, stage and BMI. Furthermore, breast cancer patients with negative ER, negative PR and positive Her-2/neu receptor had significantly higher serum resistin than those with positive ER, positive PR and negative Her-2/neu expression. Resistin might represent a breast cancer biomarker that reflected an aggressive form of the disease with poor prognosis. Kang et al.[4] found that tumor size, BMI and age did not have any significant relationship with plasma resistin levels which was consistent with our study while contradictory to our results, there was an insignificant difference in status of lymph node metastasis, or ER and PR receptor status between breast cancer patients with higher plasma resistin compared to those with lower plasma levels.

Hou et al.[1] found that serum resistin levels correlated with the tumor size but had no association with lymph node metastasis. High resistin expression correlated significantly with tumor grade which agreed with our results. Lee et al.[15] showed that high resistin expression in breast cancer tissues correlated significantly with tumor stage, tumor size, lymph node metastasis and ER status. Resistin was associated with a more malignant clinicopathological status as well as poor patient survival. Dalamaga et al.[36] found that resistin significantly associated with tumor and inflammatory markers, cancer stage, tumor size, grade and lymph node invasion; they observed no association with anthropometric, metabolic parameters and hormone receptor status.

Possible mechanisms that associate resistin with breast cancer pathogenesis may include the following: upregulation of proinflammatory cytokines via the NF-κB pathway, an important component of cancer-promoting machinery.[14] In addition, activation of signaling pathways plays an important role in inflammation and tumorigenesis. Resistin has been shown to phosphorylate both MAPKs (Erk or p38) and Akt, a downstream substrate of PI3K, in several cell lines.[37] Induction of the proangiogenic protein: VEGF and formation of endothelial cell tubes that contribute to metastasis and induction of expression of MMPs and reduction of MMPs tissue inhibitors that participate in tumor invasiveness and metastasis.[14]

Regarding the association of resistin with breast cancer risk, our study showed that the risk of the breast cancer significantly increased in the highest serum resistin group compared to the lowest group. This finding agreed with Kang et al.[4] who reported that high serum resistin might be associated with breast cancer risk.

In this study we assessed inflammatory status

by measuring serum C-reactive protein levels in breast cancer patients compared to healthy controls. We found highly elevated C-reactive protein levels in breast cancer patients compared to healthy controls, which showed that breast cancer is associated with higher inflammatory status. Asegaonkar et al.[38] found that C-reactive protein levels were highly elevated in breast cancer patients compared to controls, which agreed with our study. The current study showed a significant positive correlation between serum visfatin and serum resistin with serum C-reactive protein. This suggested a link between these adipocytokines and inflammation. Inflammatory cells in the tumor microenvironment might be another source of visfatin and resistin production.

Cancer and inflammation are linked with each other in a bidirectional way. Inflammation triggers tumor development and progression, whereas the tumor also induces an inflammatory microenvironment.[39] The microenvironment of solid tumors is often rich in inflammatory cells which appear as essential players in the tumorigenic process. A protective role of the immune system, especially in early stages of tumorigenesis, is evident and a link between immune cell-infiltration and better prognosis has been described for various types of cancers. On the other hand, the immune system is known to be able to promote cancer initiation and progression; the causal relationship between chronic inflammation within the local tissue environment and cancer has received increased attention in recent years, leading up to the concept of cancer-related inflammation as an emerging hallmark of cancer.[40]

In the present study, serum visfatin showed a significant AUC (0.932; $P<0.001$), sensitivity (96.55%) and specificity (95.0%) at a cut-off value of 0.5 ng/ml. AUC for serum visfatin was higher than the AUC for serum resistin; hence the serum visfatin level was a more sensitive, specific marker for the diagnosis of breast cancer patients compared to resistin. Dalamaga[41] reported that serum visfatin might be a novel risk factor in addition to a potential diagnostic and prognostic biomarker in postmenopausal breast cancer. Also the potential harmful effect on postmenopausal breast cancer risk due to vitamin B3 (nicotinic acid, a natural NAD precursor in the biosynthetic route leading to NAD) intake has been speculated for the first time.

The follow up was performed to answer the question of whether preoperational serum visfatin or serum resistin levels would be of prognostic significance in breast cancer patients. In this study, breast cancer patients with higher levels of serum visfatin had shorter median DFS and OS time compared to those with low serum visfatin, which suggested that high serum visfatin levels were associated with poor prognosis in patients with primary breast cancer. This result agreed with a previous report that higher visfatin expression in breast cancer tissue was associated with more malignant cancer behavior as well as poor patient survival.[11] In multivariate Cox regression models, only clinical stage and serum visfatin levels were independent predictors of poor DFS and OS. In line with our results, Li et al.[42] found that elevated preoperative serum visfatin levels were identified as independent predictors of mortality and an unfavorable outcome. Lee et al.[11] reported that high visfatin expression in breast cancer tissue was found to be independently associated with poor DFS and OS in multivariate Cox regression analysis.

This study supported the finding that serum visfatin might add prognostic information to that obtained from classical prognostic factors and of benefit in the detection of early recurrence in breast cancer patients. However, the present study's results indicated that preoperative serum levels of resistin failed to predict survival or relapse in breast cancer patients. In fact, these results contrasted findings reported by Lee et al.[15] as they demonstrated a significant increase in resistin expression in breast cancer tissues, which was an independent predictor of worse DFS and OS in breast cancer patients. These contradictions have led us to suggest that high levels of serum resistin cannot be used for monitoring the response to chemotherapy and surveillance.

Conclusion

From this study we might conclude the following:

1. Serum resistin and visfatin levels were highly elevated in breast cancer patients compared to controls and correlated with inflammatory marker C-reactive protein.

2. Serum resistin and visfatin levels might be considered risk factors for breast cancer, independent of BMI.

3. Serum visfatin levels showed promise as novel biomarkers of diagnostic and prognostic value.

4. Targeting resistin and visfatin inhibition could be an effective therapeutic strategy in breast cancer by downregulation of the inflammatory microenvironment in breast tissue.

5. Further mechanistic, larger prospective studies are required to confirm these findings and determine whether resistin and visfatin may play a role as breast cancer tumor markers.

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Conflict of Interest

No conflict of interest is declared.

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