Bulletin of High Institute of Public Health
Vol. 41 No. 3 [2011]

Correlation of Ghrelin, Adiponectin and Leptin to BMI and Treatment Outcome in Breast Cancer Patients

Ebtsam RM. Zaher,[a] Ahmed Saad,[b] Mostafa A. Al-Naggar[c]

ABSTRACT

Background: Body mass index (BMI) is an independent prognostic factor for survival in breast cancer patients. Patients with higher BMI were found to have poorer cancer prognosis and lower survival rates. Many factors as ghrelin, adiponectin and leptin, have been implicated in obesity but their correlation with breast carcinogenesis and treatment outcome is still a debate. **Objective:** To identify the relation of ghrelin, adiponectin and leptin with BMI in breast cancer patients and their possible role in carcinogenesis and treatment outcome. **Subjects and Methods:** Sera from 80 breast cancer patients were analyzed. Ghrelin, adiponectin and leptin were assayed by commercial RIA kits, and their levels were correlated with BMI, clinicopathological parameters and relapse-free survival. The median duration of patients' follow-up was 32 months. **Results:** 73.7% of the cohort was overweight/obese. Compared to breast cancer patients with normal BMI, overweight/obese patients had a significantly higher tumor size and higher histological grade. Overweight/obese patients had higher leptin and lower ghrelin and adiponectin levels. Adiponectin was lower in patients with higher tumor grade and lymph node involvement, while ghrelin decreased with increasing tumor size and histological grade. Only serum ghrelin levels were significantly correlated to better disease-free survival. **Conclusion:** Ghrelin, adiponectin and leptin are significant factors in controlling BMI in breast cancer patients but only ghrelin is a significant predictor of better outcome and recurrence-free survival.

Key words: Adiponectin, body mass index (BMI), breast cancer, ghrelin, leptin, survival

INTRODUCTION

In most cases, obesity is the result of inadequate energy balance, in which the amount of energy gain exceeds the amount of energy expenditure. Body Mass Index (BMI) is usually used as an index to measure adiposity.(1) Epidemiological data have shown that overweight and obesity are risk factors for breast cancer, particularly hormone-responsive tumors.(2,3) Women with breast cancer who are overweight or gain weight after diagnosis have a poorer prognosis and higher risk of death compared with lean wom-

[a]Radiation Sciences, Medical Research institute, Alexandria University
[b]Experimental and Clinical Surgery, Medical Research institute, Alexandria University
[c]Cancer Management and Research Departments, Medical Research institute, Alexandria University

en.[4,5] Several large-scale cohort studies such as the Cancer Prevention Study II and the study of the International Breast Cancer Study Group have also confirmed this association between obesity and increased mortality from breast cancer.[2,6]

Given the increasing rates of obesity in women, it is critical to understand the physiological mechanisms underlying obesity and its effect on tumorigenesis. Besides excess adipose tissue, obesity has been associated with many factors including serum levels of ghrelin, adiponectin and leptin, all of which are likely to affect tumor prognosis.[2,6]

Ghrelin is a 28-amino acid orexigenic hormone produced in the stomach by the oxyntic gland that is identified as an endogenous ligand for GH secretagogues receptor. It affects energy regulation by increasing food intake and lowering the metabolic rate. It has been shown to cause a positive energy balance by decreasing fat utilization through GH-independent mechanisms.[7] Ghrelin is up-regulated during starvation, cachexia, anorexia and hypoglycemia, and down-regulated during feeding and obesity.[8] It exerts its actions via activation of neuropeptide Y neurons in the hypothalamic arcuate nucleus.[9]

Adiponectin, also known as Acrp30, is an adipocyte-derived protein hormone consisting of 244 amino acids which belongs to a family of adipokines. It is established that adiponectin is an insulin-sensitizing hormone with anti-diabetic, anti-inflammatory and anti-atherogenic properties. It promotes adipocyte differentiation, fatty acid catabolism and insulin sensitivity and is negatively correlated with obesity.[10]

Leptin is one of the most studied adipocyte-derived hormones. Circulating levels of leptin are directly proportional to the amount of adipose tissue and nutritional status. Therefore, leptin levels are increased during obesity and are thus decreased by caloric restriction. Furthermore, alterations in leptin metabolism or function can result in obesity, diabetes and imbalance in energy homeostasis. Leptin is involved in several biological

processes including food intake regulation, immune function, and sexual development.[11] In vitro, leptin was found to induce cell proliferation in both normal and cancerous cells.[12]

The study aims to evaluate the association of ghrelin, adiponectin and leptin with BMI in breast cancer patients as well as their effect on disease prognosis.

SUBJECTS AND METHODS

For this prospective cohort study, 80 females recently diagnosed with breast cancer were randomly recruited from the Experimental and Clinical surgery department and Cancer Management and Research Department, Medical Research Institute, Alexandria University, during the period from February 2007 till September 2007. All patients were diagnosed with primary breast cancer, as indicated by fine-needle biopsy or core biopsy. Eligibility criteria included confirmed diagnosis of breast cancer, no metastasis at time of recruitment, no previous treatment, no previous malignancies and good cardiac, liver and kidney functions. All patients were subjected to complete physical examination and complete diagnostic evaluation consisting of chest X-rays and ultrasound of abdomen and pelvis. Whole-body bone scan, computed tomography scan and magnetic resonance imaging were performed if clinically indicated.

All patients were treated primarily with modified radical mastectomy.[13] followed by adjuvant FAC-based chemotherapy (6 cycles; each one consisted of 5-flourouracil 500 mg/m^2, Adriamycin 50 mg/m^2 and cyclophosphamide 500 mg/m^2, given every 21 days). Patients who were positive for estrogen and progesterone receptors received hormonal therapy (Tamoxifen 10mg twice/day for 5 y) after chemotherapy.[14] Patients were clinically followed up for a mean period of 45 (50-39) months for relapse.

A single blood sample was collected from each patient before surgery and from controls between 7 and 8 a.m. after an overnight fast. Serum was collected, fractionated and stored at -80°C until it was used.

BMI was calculated as body weight (Kg) divided by the square height (m^2). Subjects with BMI <18.5 were considered underweight, 18.5-25 were considered normal, >25-30 were considered overweight and >30 were considered obese.

Total ghrelin and adiponectin were assayed by commercial radioimmunoassay (RIA) kits (from Millipore, Missouri, USA) and leptin was assayed by a commercial RIA kit (from Linco Research Inc., Missouri, USA). In RIA, a fixed concentration of labeled tracer antigen is incubated with a constant dilution of antiserum. When an unlabeled antigen is added and incubated in this system, there is competition between the labeled tracer and the unlabeled antigen for the limited and constant number of binding sites on the antibody. At equilibrium, the amount of tracer bound to antibody will decrease as the concentration of unlabeled antigen increases. The antibody-bound fraction is separated from free tracer by the double antibody technique and is counted on a gamma counter. A standard curve is set up with increasing concentrations of standard unlabeled antigen and from this curve the amount of antigen in unknown samples can be calculated.

The lowest level that can be detected by these assays when using a 100 µL sample size are 93 pg/mL of ghrelin, 1 ng/mL of adiponectin and 0.437 ng/mL of leptin.

In addition, 50 normal healthy individuals of matched age, menopausal status and BMI as the patients' group were included in the study as controls to confirm the normal range of the parameters included.

Ethical Considerations:

All samples were collected after the subjects' informed written consent, and confidentiality of data was insured at all stages of the study. The study was approved by the Ethics Committee of the Medical Research Institute.

Statistical Analysis

For variables description, the percent was used for qualitative variables and the mean with the standard deviation for quantitative

normally distributed variables. The correlation between parameters was tested by Pearson correlation (r). The differences of these factors according to other variables were tested with Chi-Square test. Disease-Free Survival (DSF) was assessed by Kaplan-Meier curves, using median value of the control group for each parameter as a cutoff. Univariate and multivariate analyses were performed using Cox proportional hazard regression model to calculate the relative risk and its 95% confidence interval (CI). All computations were done with the SPSS (Statistical Package for the Social Sciences) software version 18.0 (SPSS Inc., Chicago, IL, USA). All statistical tests of significance are two-sided and considered significant at $p<0.05$.

RESULTS

The study included 80 breast cancer patients and 50 normal healthy females of matched age as controls. The control group comprised of normal healthy volunteers with no previous history of breast cancer and who are matched in age and BMI distribution to the cancer group. The characteristics of both patients and controls are summarized in table 1. The two groups were of matched age and BMI distribution as indicated by the absence of a significant difference between the two groups regarding age and BMI distribution ($p=0.774$ and 0.875 respectively). Almost half of the cohort of patients were overweight with the other half equally distributed between normal and obese patients. The majority of patients were of histological grades and clinical stages II and III, with lymph node metastasis and positive hormone receptor expression.

Table 1. Clinicopathological characteristics of breast cancer patients (n=80) and control subjects (n=50), including age, body mass index (BMI), tumor size, histological grade, clinical stage, lymph node metastasis, vascular invasion and hormone receptor status

Clinicopathological Parameter	Breast cancer patients		Control subjects		
	n (80)	%	n (50)	%	*p*
Age (years)					
21-40	26	32.5	16	32.0	
41–60	39	48.8	24	48.0	0.774
> 60	15	18.7	10	20.0	
Body mass index (BMI)					
Underweight	2	2.5	1	2.0	
Normal	19	23.8	12	24.0	0.875
Overweight	38	47.5	24	48.0	
Obese	21	26.2	13	26.0	
Tumor size (cms)					
<3cm	32	40.0			
> 3 cm	48	60.0			
Histological Grade					
I	12	15.0			
II	38	47.5			
III	30	37.5			
Clinical Stage					
I	10	12.5			
II	36	45.0			
III	34	42.5			
Lymph node metastasis					
Negative	20	25.0			
Positive	60	75.0			
Vascular Invasion					
Negative	34	42.5			
Positive	46	57.5			
Hormone Receptor					
Negative	14	17.5			
Positive	66	82.5			

Table 2 represents the association of BMI, ghrelin, adiponectin and leptin with clinicopathological parameters of breast cancer patients group. The mean level of serum ghrelin among breast cancer patients was significantly lower than among controls (983±364 versus 1327±573 pg/ml, *p*=0.036), while the mean leptin level among breast cancer patients was significantly higher than among controls (26.8±7.3 versus 18.4 ± 2.1 ng/ml, *p*=0.023). On the other hand, adiponectin

levels among breast cancer patients were not significantly different from control subjects (9.6±6.1 versus 11.3±5.2 ng/ml, *p*=0.211). Both ghrelin and adiponectin were significantly lower among patients with higher BMI (*p*=0.023 and 0.037 respectively), while leptin was significantly higher among patients with higher BMI (*p*=0.014). Increased BMI was also significantly associated with higher tumor size (*p*=0.027) and higher histological grade (*p*=0.015). Adiponectin levels were significantly lower in patients with higher tumor grade (*p*=0.032) and with lymph node involvement (*p*=0.029), while ghrelin levels were significantly lower in patients with higher tumor size (*p*=0.046) and histological grade (p=0.034).

All three parameters were significantly correlated to BMI; ghrelin and adiponectin showed a negative correlation (*p*=0.001 and 0.001) while leptin showed a positive correlation (*p*=0.001). The three parameters were also correlated with each others. Adiponectin and ghrelin were positively correlated with each others (*p*=0.016) while they were negatively correlated with leptin (*p*=0.010 and 0.013 respectively).

By the end of the follow up period, 42 of the 80 patients (52.5%) had relapsed. For disease-free survival analysis, the Kaplan–Meier test was performed to study the effect of BMI, serum ghrelin, adiponectin and leptin on disease prognosis. BMI did not reflect any impact on breast cancer prognosis as indicated by the absence of a significant difference between underweight, normal, overweight and obese subgroups (*p*=0.351). Patients with low versus high ghrelin levels had an average survival of 36.3 months versus 28.7 months, which was significantly different (*p* = 0.024). There was no significant difference in disease free survival between patients with low versus high adiponectin (*p*=0.498) or leptin (*p*=0.058).

Table 2. Association between clinicopathological parameters and studied markers in breast cancer patients (n=80)

Clinicopathological Parameter	Number	BMI <25 / >25	Ghrelin (pg/ml)	Adiponectin (ng/ml)	Leptin (ng/ml)
Breast cancer	80	21 / 59	983 ± 364	9.6 ± 6.1	26.8 ± 7.3
Controls	50	13 / 37	1327 ± 573	11.3 ± 5.2	18.4 ± 2.1
P		0.875	0.036	0.211	0.023
BMI			0.023	0.037	0.014
Underweight	2		1421 ± 614	10.4 8.1	17.4 ± 4.1
Normal	19		1123 ± 237	9.6 ± 6.1	19.4 ± 4.7
Overweight	38		986 ± 261	9.8 ± 5.7	22.6 ± 5.3
Obese	21		785 ± 244	7.5 ± 4.3	33.2 ±6.7
Tumor size (cms)		0.027	0.046	0.561	0.661
<3cm	32	12 / 20	1143 ± 507	9.6 ± 6.5	25.4 ± 4.6
> 3 cm	48	9 / 39	952 ± 361	8.7 ± 6.7	27.1 ± 6.1
Histological Grade		**0.015**	**0.034**	**0.032**	**0.117**
I	12	9 / 3	1049 ± 381	10.7 ± 8.4	23.4 ± 6.3
II	38	9 / 29	996 ± 364	8.1 ± 5.6	25.3 ± 4.7
III	30	3 / 27	758 ± 517	6.5 ± 5.7	28.0 ± 5.9
Clinical Stage		**0.443**	**0.121**	**0.186**	**0.276**
I	10	3 / 7	1112 ± 329	9.9 ± 6.3	24.1 ± 4.6
II	36	11 / 25	943 ± 489	8.1 ± 5.5	26.1 ± 4.1
III	34	7 / 27	958 ± 503	8.2 ± 6.5	27.5 ± 6.6
Lymph node metastasis		0.586	0.341	0.029	0.438
Negative	20	6 / 14	1017 ± 388	10.3 ± 5.9	25.3 ± 5.2
Positive	60	15 / 45	965 ± 416	8.1 ± 6.7	26.1 ± 7.8
Vascular Invasion		**0.710**	**0.137**	**0.081**	**0.876**
Negative	34	10 / 24	1017 ± 388	9.1 ± 5.6	26.2 ± 6.5
Positive	46	11 / 35	965 ± 416	8.9 ± 7.6	26.8 ± 6.9
Hormone Receptor		**0.623**	**0.724**	**0.938**	**0.729**
Negative	14	4 / 10	985 ± 501	9.7 ± 6.3	26.1 ± 6.6
Positive	66	17 / 49	916 ± 536	8.6 ± 6.1	27.3 ± 6.4

Table 3. Correlation between various obesity markers in breast cancer patients (n=80)

	BMI		**leptin**		**Ghrelin**	
	r	***p***	**r**	***p***	**r**	***p***
Adiponectin	-0.421	0.001	-0.352	0.010	0.239	0.016
Ghrelin	-0.393	0.001	-0.206	0.013		
leptin	0.632	0.001				

Significant Pearson Correlation at $p<0.05$.

Results of multivariate Cox regression analysis are summarized in table 4. None of the tested parameters (high BMI, low ghrelin, low adiponectin and high leptin) significantly increased the relative hazard ratio (RHR) of relapse in breast can cer patients.

Table 4. Cox regression analysis for the breast cancer patient cohort (n=80)

Predictor	Hazard Ratio	95% CI	*p*
Body mass index (BMI)			
Underweight	1.94	0.59 - 6.45	
Normal	1.00		0.371
Overweight	0.91	0.49 - 1.38	
Obese	1.14	0.54 - 1.51	
Ghrelin	1.13	0.85 - 1.29	0.067
Adiponectin	1.02	0.96 - 1.07	0.490
Leptin	1.07	0.88 - 1.15	0.167

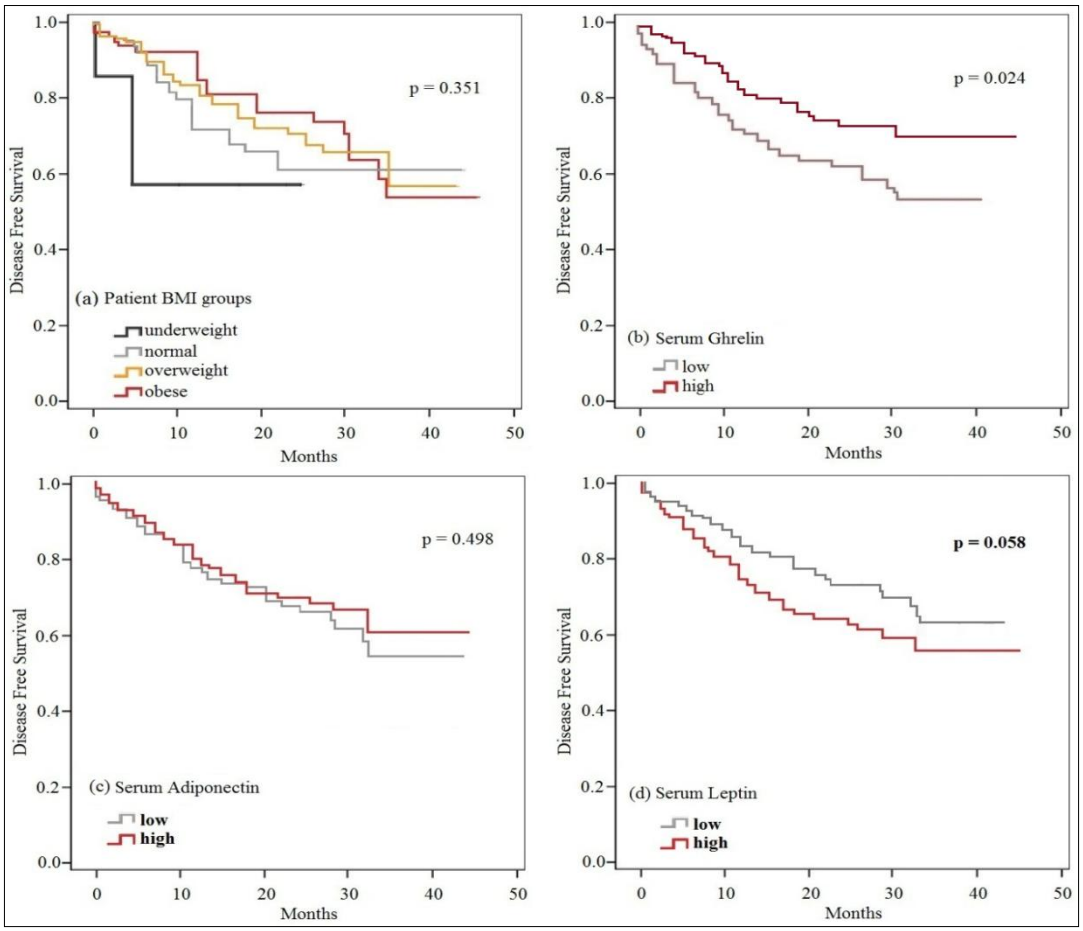

Figure 1. Kaplan-Meier disease-free survival curves of; (a) various BMI subgroups; (b) low versus high levels of ghrelin; (c) low versus high levels of adiponectin and (d) low versus high levels of leptin

DISCUSSION

Obesity is an increasingly important public health concern in Egypt. An estimated 35% of Egyptian adults are considered to be obese, the majority of them are females (18.7% males and 48.1% females).[15] In recent years, interest has grown in the relationship between obesity and cancer. Increasingly, obesity has been identified as a significant risk factor for many cancers and, after tobacco use, may be the single greatest modifiable cancer risk factor.[16,17] For breast cancer, a clear association between obesity and disease risk seems to have been established (particularly in post-menopausal women), and new studies confirming this observation and investigating explanatory hypotheses for the relationship continue to appear regularly.[18,19] In accordance with that, almost three-quarters of the breast cancer cohort included in this study were overweight/obese, which reflects the high prevalence of breast cancer among these subgroups.

With respect to prognosis, however, the evidence is much less clear. Since the often cited review by Goodwin and Boyd in 1990,[20] numerous studies have been conducted in a variety of settings. While many studies concluded that obesity is indeed prognostic, many others indicated a very modest or in some cases absent influence of obesity on outcomes.[5,6,18] In the current study, there was no difference in DFS between various BMI subgroups of breast cancer patients. Although most studies with sufficient study population sizes have found associations between obesity and overall survival,[5,6,21,23] the results vary more with respect to disease-free survival. Some studies reported that patients who were obese and had breast cancer had a poorer disease-free survival,[22-24] while others reported no association between obesity and disease-free survival.[21,25] This is likely a result of the definition of

disease-free survival, the distinction between locoregional and distant metastases, and differences between study populations.

One of the strongest correlations found between BMI and tumor characteristics was tumor size; 66.1% of patients with having tumors of ≥3 cm diameter compared to 42.8% of patients with normal BMI. This is in accordance with previous studies.[21,26] Another strong association was found between BMI and histological grade, where the majority (94%) of patients with BMI>25 were of grades II and III compared to 57% of patients with normal BMI. Numerous hypotheses have been put forward to explain these findings. Obese patients tend to have large breasts, which may make palpation of a tumor difficult and therefore delay diagnosis. Another suggested reason for the association between larger BMI and larger, more advanced tumors is enhancement of carcinogesesis by endogenous factors. Obesity has been associated with increased insulin, estrogen and growth factor signaling, all of which are likely to stimulate tumorigenesis.[27] Also, Daling et al[26] found a higher Ki-67 expression ratio and a higher mitotic count in tumors from patients with higher BMI, indicating a possibly more rapid growth rate. However, no correlation could be found in our data between obesity and tumor grading or lymph node involvement, which contradicts many earlier reports.[21,28]

Although cancer and control groups were of the same BMI distribution, ghrelin levels were significantly lower and leptin levels were significantly higher in breast cancer patients than in controls. That may indicate a role for both ghrelin and leptin in cancer progression. Ghrelin levels in serum were significantly lower in patients with higher tumor size or higher histological grade. It was also a significant factor in DFS with patients with higher gherlin levels having higher DSF.

The role of ghrelin in cancer has witnessed great controversies. Recent evidence supports a role for ghrelin in the regulation of the proliferation of both normal and neoplastic cell lines. The peripheral actions of ghrelin result from modulation of function and from regulation of survival and proliferation of target cells. Several endocrine and nonendocrine neoplastic cells synthesize ghrelin. Evidence that ghrelin and ghrelin receptors are coexpressed in several tumors and related cell lines indicates that ghrelin system is likely to have an important autocrine/ paracrine role in the development of neoplasms. Experiments in vitro demonstrate that ghrelin either stimulates or inhibits the proliferation of several human tumor cell lines. An explanation for the dual action of ghrelin on the viability of neoplastic cells is not known, although it might be related, at least partially, to its concentration. In addition, ghrelin might also regulate neoplasm proliferation by exerting an inhibitory effect on angiogenic factors.[29]

Jeffery et al[30] have reported that ghrelin exerts a proliferative effect on breast cancer cell lines, which contradicts our results. Several other reports described an antiproliferative effect of ghrelin,[31-32] which well agree with our results, where high ghrelin concentration was associated with lower tumor size and lower grade. Our results also proved ghrelin to be a good prognostic marker.

The circulating levels of adiponectin are decreased in obese individuals,[33] an observation that agreed with our findings. Adiponectin was lower in patients with higher histological grade and lymph node metastasis. This can be explained, at least in part, by the proposed functions of adiponectin. In addition to its function as a metabolic hormone, growing evidence suggests that adiponectin plays a regulatory role in cell growth, angiogenesis and tissue remodeling.[33,34] Adiponectin has

been shown to inhibit proliferation of aortic smooth muscle cells, myelomonocytic cells, endothelial cells and hepatic stellate cells.[35] Also, an anti-proliferative effect of adiponectin has been suggested, as adiponectin selectively binds with several mitogenic growth factors that can induce cell proliferation in many types of cells. The interaction of adiponectin with these growth factors can preclude their binding to the membrane receptors and lead to the attenuation of their mitogenic actions, suggesting that the anti-proliferative effect of adiponectin is at least partly due to its selective sequestration of growth factors at a prereceptor level.[10] However, adiponectin was not found to affect DFS and did not increase the relative risk of relapse in breast cancer patients.

Leptin belongs to the same family of adipokines as adipnectin, but has an opposite action on lipid tissue. Leptin is involved in several biological processes including food intake regulation, immune function and sexual development.[11]

The current results found leptin to be increased in breast cancer patients compared to matched controls, which may indicate a role for leptin in carcinogenesis. Since leptin levels are increased during obesity and obesity is a risk factor for postmenopausal breast cancer, several studies have assessed the mechanisms by which leptin may affect breast cancer growth. There are several signaling pathways that are proposed to respond to leptin proliferative effects. In vitro, leptin induces cell proliferation in both normal and cancerous cells as a consequence of a bidirectional cross-talk between leptin and IGF-1 signaling, suggesting that elevated levels of these factors during obesity can contribute to increased breast cancer cell growth.[10] Leptin exerts its growth promoting effects on the cell through activation of the JAK/STAT3, ERK1/2 and/or PI3K/akt signaling pathways and regulating aromatase expression, estrogen synthesis and

vascular endothelial growth factor expression.[36,37]

However, increased leptin levels were not associated with any of the clinicopathological parameters of cancer, did not affect DFS, although it was close to being significant (p=0.058) and it did not increase the hazard of relapse in breast cancer patients.

Obesity has been for long stated as a risk factor for breast cancer but it might not be a poor prognostic factor. However, underweight emerges as a poor prognostic factor as the underweight patients in the current study were among the first to relapse, but the number of patients in that group was so low. That strongly recommends studying the effect of being underweight on breast cancer prognosis in a larger scale study.

In conclusion, ghrelin, adiponectin and leptin are considered significant factors in controlling BMI in breast cancer patients but only ghrelin is a significant predictor of better outcome and recurrence-free survival.

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