Impact of serum leptin and adiponectin levels on breast cancer in postmenopausal Iraqi women: an observational study [version 1; peer review: awaiting peer review]

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Abstract

Background: Breast cancer is the most common malignant tumor and the second most significant cause of death for women in Iraq, behind cardiovascular diseases. Obesity has been linked to a substantial increase in the risk of breast cancer. Adipose tissue functions as an endocrine gland, controlling the body’s metabolism by secreting adipokines, which play a significant role in metabolism and inflammatory reactions.

Methods: Overall, 90 postmenopausal women participated in this research. Of these, 60 patients with breast cancer were recruited at Baghdad’s Oncology Teaching Hospital between October 2021 and February 2022: 30 were obese with a body mass index (BMI) of > 30 kg/m² (group 1), and 30 were not obese (group 2). The third group consisted of 30 participants without breast cancer or obesity (group 3). Each person donated five milliliters of venous blood. The blood levels of adiponectin and leptin are determined using enzyme-linked immunosorbent assay (ELISA) kits.

Results: Control individuals who were not obese (group 3) had greater blood adiponectin levels than patients with cancer who were both obese and non-obese (groups 1 and 2), with no significant difference in serum adiponectin levels seen between groups 1 and 2. The findings also showed that group 1 (patients with breast cancer and obesity) had greater serum leptin levels than both group 2 (patients with breast cancer without obesity) and the control group (group 3), with no significant difference in serum leptin levels between groups 2 and 3.

Conclusions: Adiponectin levels in the blood of women with breast cancer and obesity were low which may be due to high BMI, which reduces adiponectin’s protective effects. Conversely, Leptin levels were more significant in the blood of women with breast cancer and obesity than in the control group, which may be due to its pro-inflammatory effects in obesity, among other variables.
Keywords
Obesity, Breast Cancer, Leptin, Adiponectin, Iraq

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Introduction

According to GLOBOCAN 2020 statistics, breast cancer is one of the most prevalent malignancies diagnosed today and the fifth most prominent cause of cancer-related deaths, with around 2.3 million new cases diagnosed globally. According to the Iraqi Cancer Registry report of 2020, breast cancer is the most prevalent malignant tumor, with an incidence rate of about 19%. Additionally, breast cancer is regarded as the second-leading cause of death from malignancy in the entire Iraqi population, with an incidence rate of roughly 13%, and the first leading cause of death from malignancy in Iraqi women, with a rate of incidence of 25%. World Health Organization classified obesity as a serious world-wide medical issue, affecting a large proportion of the population and correlating with many chronic conditions, such as diabetes mellitus, cardiovascular disease, hypertension, as well as certain types of cancer, which all can cause a significant burden on the medical systems of the countries worldwide, the connection with breast cancer is substantial. Adipose tissue is considered not only a reservoir for the storage of lipids but also an endocrine gland with a significant role in the regulation of the body’s metabolism and the different immune responses, which can, in turn, affect the metabolism of other cells and enzymes which in turn affect the steroid hormone metabolism. White adipose tissue secretes adipokines, which have a wide range of effects on various biological processes, including inflammation, insulin signaling, glucose metabolism, and lipid metabolism. Adiponectin or adipocyte complement-related protein is a monomer of 30 kDa that circulates in the plasma as low- and high-molecular-weight multimers.

Leptin, encoded by the obese (Ob) gene, is a multifunctional polypeptidic protein (16 kDa). Its production and the levels of plasma rise in proportion to fat accumulation. Leptin can influence a diverse range of biological processes. This includes breast tumorigenesis in addition to its neuroendocrine function. This adipokine can affect the biology of breast cancer in endocrine, paracrine, and autocrine, and this has been amply demonstrated. Distant and local adipocytes primarily produce it, but it is also produced by the cells of the epithelial tumor as well as by other stromal cells in the tumor (cancer-associated fibroblasts), numerous intracellular downstream signaling pathways, including the JAK2/STAT3, MAPK, and PI3K/Akt pathways, which are involved in the regulation of cell proliferation, differentiation, survival, migration, and invasion, are activated as a result of leptin binding to the long ObR isoform. White adipocyte hypertrophy and hyperplasia limit their vascularization, lowering oxygen availability. Due to the hypoxic state, there is increased oxidative stress, insulin resistance, ischemia, adipocyte necrosis, and the release of inflammatory and angiogenic proteins. Adipose tissue dysfunction in obesity is associated with an aberrant mediator release profile that results in a chronic inflammatory state. Adiponectin is downregulated due to an increase in leptin and pro-inflammatory cytokines; as a result, anti-inflammatory and anti-tumor actions are reduced.

The objective of this study was to compare the levels of serum adiponectin and leptin in postmenopausal breast cancer patients who had obesity or were not obese, to a non-obese control group, to discover possible correlations between these biomarkers and the development of breast cancer.

Methods

Ethical consideration

The research protocol received formal clearance from the University of Baghdad’s ethics committee on April 10, 2021 (ethics board approval code: 193220). All participants in the study signed an informed consent form.

Study design

This is an observational study including postmenopausal women with breast cancer to ascertain whether there is a correlation between obesity biomarkers (leptin and adiponectin) and breast cancer.

Sample size

The size of the sample was calculated using G*Power version 3.1.9.7 software. The smallest total sample size was 70 patients with 90% power at a 95% confidence interval two-tailed alpha of 0.05, and an effect size of 0.80. The research involved 90 individuals: 60 women with breast cancer and 30 healthy controls.

Eligibility criteria and recruitment

This research involved 90 participants, all of them were postmenopausal Iraqi women. In total, 30 participants were obese (BMI value of ≥30 kg/m²), and diagnosed with breast cancer (group 1), 30 were not obese (BMI of < 30 kg/m²) and diagnosed with breast cancer (group 2), and 30 did not have obesity (BMI of < 30 kg/m²) or breast cancer (group 3).

The participants were randomly recruited from the consulting clinic of Oncology Teaching Hospital \ Medical City, Baghdad, Iraq using the simple random selection; the healthy volunteers were recruited from the general population. Blood samples from the participants were obtained at the same hospital in the period from October 2021 to February 2022. All the participants enrolled in this study were informed about the aim of the study, and consent was taken.
addition, information about age, illness history, medication history, and menopausal status were obtained and recorded from the participants themselves.

Exclusion criteria
Any type of metastasis, steroids, some blood pressure medications, certain Human Immunodeficiency Virus (HIV) treatments (Protease inhibitors (PI) including atazanavir, darunavir, fosamprenavir and indinavir or nucleoside analog reverse transcriptase inhibitors (NRTI) including lamivudine, stavudine, zidovudine), and some psychiatric medications (for example: olanzapine, quetiapine, clozapine and risperidone), male subjects were excluded, history of chronic renal failure, history of hepatic diseases, history of autoimmune diseases, subjects with an acute infection or acute myocardial infarction.

Study procedure
Measurements
BMI is an anthropometric value considered a disease-related factor when it occurs upper or lowers the reference range. It can be measured from the formula:

\[
\text{BMI (kg/m}^2\text{)} = \frac{\text{Weight}}{\text{Height}^2}
\]

In addition, waist circumference was measured in (cm) for each participant using a tape measure.

Laboratory investigation
Each participant provided a sample of five millilitres (ml) of venous blood. It was then collected into a gel tube and centrifuged for 10 to 15 minutes at a speed of 4,400 rounds per minute (rpm) to extract the serum for testing. Serum adiponectin and leptin levels were measured using enzyme-linked immunosorbent assay (ELISA) kits. Table 1 lists the chemical components, tools, and equipment used in this investigation.

Bias
The ideal study population is well-defined, easily reachable, reliable, and highly likely to produce the desired result. Bias in selection could occur when choosing the study sample. This is especially evident in retrospective cohort studies where participants are recruited after exposures and outcomes have already occurred. However, sampling error is less likely in this prospective study because the results are unknown at the time of enrolment. One of the strongest strategies for reducing sample bias in this study is simple random sampling. The population as a whole has an equal probability of getting chosen for being selected.

Statistical analysis
The statistical analysis was done using IBM SPSS Statistics version 25 for Windows. The Shapiro-Wilk test was used to determine whether the data were regularly distributed. The median and interquartile range (IQR) for each participant was computed. To compare the groups, the Kruskal-Wallis test was performed. Spearman’s correlation test was employed for correlation studies, and a P-value of 0.05 or higher was considered significant.

Results
The study recruited 90 participants as shown in Figure 1. The studied groups demonstrated non-significant differences in median (IQR) of age or height, including the three studied groups, as shown in Table 2. In addition, group 3 (control

<table>
<thead>
<tr>
<th>Table 1. list of the study’s kits and equipment.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chemicals</strong></td>
</tr>
<tr>
<td>Human Leptin Elisa Kit</td>
</tr>
<tr>
<td>Human Adiponectin Elisa Kit</td>
</tr>
<tr>
<td><strong>Instrument</strong></td>
</tr>
<tr>
<td>Automatic ELISA reader PKL PPC 230</td>
</tr>
<tr>
<td>Centrifuge</td>
</tr>
<tr>
<td>Gel tube</td>
</tr>
</tbody>
</table>
participants without breast cancer or obesity) showed higher serum adiponectin levels than both group 1 (participants with breast cancer and obesity) and group 2 (participants with breast cancer without obesity), while no significant difference in serum adiponectin levels was showed between group 1 and group 2, as shown in Table 3 and Figure 2.

Group 1 (participants with breast cancer and obesity) showed a higher Serum Leptin level than both group 2 (participants with breast cancer without obesity) and group 3 (control participants without breast cancer or obesity). In contrast, no significant difference in serum leptin levels was shown between group 2 and group 3, as shown in Table 3 and Figure 3.

This study found a moderate negative correlation between BMI and adiponectin, while a robust positive correlation between leptin and BMI was found. The study also found the correlation between serum adiponectin and leptin levels was moderately negative, as shown in Table 4.

Discussion
From this study, the levels of serum adiponectin showed significantly higher values in the control group (group 3) when compared to both groups 1 (participants with breast cancer and obesity) and group 2 (participants with breast cancer

![Study participants flowchart.](image)
without obesity), as shown in Table 3. This may be due to central adiposity and the negative association with body mass index.\textsuperscript{18} In addition, the down-regulation of the receptors, AdipoR1, and AdipoR2, plays a crucial role in the low adiponectin levels in individuals with obesity.\textsuperscript{19} Along with the previously listed factors, obesity is a chronic state of inflammation defined by high inflammatory cytokines levels, including IL-6, IL-8, TNF-\textgreek{a}, and others.\textsuperscript{20,21} These cytokines inhibit adiponectin transcription, resulting in low levels of adiponectin.\textsuperscript{22} This study agreed with many previous studies, where Kredy \textit{et al.}; and Miyoshi \textit{et al.}; found that patients with breast cancer had lower serum adiponectin levels than the healthy control subjects.\textsuperscript{23,24} At the same time, it disagreed with the study by Shafika \textit{et al.}, who found that serum adiponectin levels in breast cancer patients were higher than in the control group.\textsuperscript{25}

In this study, participants with breast cancer and obesity (group 1) showed significantly higher serum leptin levels compared to non-obese breast cancer (group 2) and non-obese control (group 3). This result may be explained by the high quantity of leptin expressed at the gene level in adipose tissue in people with obesity.\textsuperscript{26} Furthermore, there are significant positive relationships between plasma leptin levels and body fat percentage.\textsuperscript{27} And this agreed with the finding of a study

**Table 2. Anthropometric Result among Study Groups.**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Group 1 (participants with breast cancer and obesity) N=30</th>
<th>Group 2 (participants with breast cancer without obesity) N=30</th>
<th>Group 3 (participants without breast cancer or obesity) N=30</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>54.5 (11)</td>
<td>52 (6)</td>
<td>55.5 (5)</td>
<td>0.24</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>85.00 (10)</td>
<td>61.5 (9)</td>
<td>66.00 (12)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.5800 (0.09)</td>
<td>1.6100 (0.06)</td>
<td>1.6250 (0.09)</td>
<td>0.113</td>
</tr>
<tr>
<td>BMI (kg/m\textsuperscript{2})</td>
<td>31.8150(3.64)</td>
<td>24.0850 (5.43)</td>
<td>24.7150 (4.91)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Waist Circumference (Cm)</td>
<td>103.00 (12)</td>
<td>98.00 (8)</td>
<td>100.00 (8)</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

Data presented in the table in terms of the median (IQR) * Indicates a significant difference between the groups.

**Table 3. Serum leptin and adiponectin levels in the studied groups.**

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (participants with breast cancer and obesity) N=30</th>
<th>Group 2 (participants with breast cancer without obesity) N=30</th>
<th>Group 3 (control participants without breast cancer or obesity) N=30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leptin (ng/ml)</td>
<td>29.030 (14.45)a</td>
<td>16.3900 (3.28)b</td>
<td>14.9150 (8.34)b</td>
</tr>
<tr>
<td>Adiponectin (mg/l)</td>
<td>5.5395 (1.08)b</td>
<td>5.9865(1.28)b</td>
<td>7.3705(4.29)a</td>
</tr>
</tbody>
</table>

**Figure 2. Median of serum adiponectin levels.** * B.C: breast cancer.

![Figure 2](image-url)
by Zeinab et al. which illustrated that breast cancer patients had significantly higher serum leptin levels than healthy controls.28 The result of this study also agreed with other studies which also compared patients with breast cancer and a healthy control group with the insignificant difference in BMI between the two groups; where Manar et al. and Adel et al.; found that the levels of serum leptin higher in patients having breast cancer compared to healthy controls.29,30 In this study, both group 2 (non-obese breast cancer) and group 3 (non-obese control) patients showed no significant difference in serum leptin levels, since leptin levels in the body are directly related to BMI, and both of these groups have nearly the same BMI, and this finding agreed with by study done by Marc et al.; who showed the same finding in their study.31 In this study, BMI showed a significantly robust positive correlation (\(\rho = 0.922\)), as leptin levels directly relate to body fat percentage.32,33 On the other hand, BMI showed a significant moderate negative correlation with adiponectin (\(\rho = -0.306\)) since the adiponectin levels have an inverse relation with body fat content.9 While the Spearman correlation test showed a significantly moderate weak correlation (\(\rho = -0.352\)) between leptin and adiponectin, this may be explained by continuous inflammatory status in obesity, which leads to altered transcription of adiponectin due to high levels of leptin in the state of obesity.22

**Limitations**

There are various considerations for the limitations of the current research design. This research used a single assessment of adipokines from a single baseline blood specimen. Nevertheless, a single test of circulating leptin and adiponectin concentrations seemed to be a valid predictor of an individual’s long-term levels of both adipokines, even when samples were obtained 1–4 years apart.16,35 Second, to determine obesity, we employed BMI rather than waist circumference. Third, The relatively homogenous group of postmenopausal women may restrict the generalizability of our findings. Fourth, the study employed several exclusion criteria to reduce the confounding effect. Fifth, a small number of patients are enrolled in this current research, so we strongly recommend expanding the number of participants in future studies.

**Conclusions**

Women with obesity and breast cancer were found to have low serum levels of adiponectin, which reduces the protective actions of adiponectin and may promote the growth of breast cancer. On the other hand, leptin levels were higher in the serum of women with obesity and breast cancer than in women who were not obese, which may be due to leptin’s pro-inflammatory activities in addition to other variables.
Data availability
Underlying data
Zenodo: Serum Leptin and Adiponectin levels. https://doi.org/10.5281/zenodo.7223948.17

This project contains the following underlying data:

- Article data.xlsx (Measurements of Leptin and Adiponectin in Serum)

Data are available under the Creative Commons Attribution 4.0 International license (CC-BY 4.0).

Acknowledgments
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