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Original Article

Association of Serum Leptin with Prognostic Factors in Breast Cancer

Amirreza Hajati[1], Farshad Talebian[2], Asrin Babahajian[1], Nasrin Daneshkhah[3], Bayazid Ghaderi[1]*

[1]Liver and Digestive Research Center, Research Institute for Health Development, Kurdistan University of Medical Sciences, Sanandaj, Iran
[2]Student Research Committee, Kurdistan University of Medical Sciences, Sanandaj, Iran
[3]Faculty of Nursing and Midwifery, Kurdistan University of Medical Sciences, Sanandaj, Iran

Abstract

Background: Nowadays, cytokines such as Leptin and adiponectin are introduced as prognostic factors which, according to some studies, are also associated with body mass index. This study aimed to determine serum leptin level and its relationship with prognostic factors in breast cancer patients.

Methods: This case–control study was conducted in the oncology department of Tohid Hospital, Sanandaj, Iran, between 2019 and 2020. Hundred new cases of breast cancer patients with histological evidence were enrolled in this study. Additionally, 100 age- and BMI-matched healthy individuals were recruited as the control group. The serum leptin level was measured using the ELISA method.

Results: Serum leptin levels were significantly higher in breast cancer patients compared to the control group (21.68 ± 9.16 vs 11.89 ± 4.45; $p < 0.001$). There was no significant relationship between plasma leptin levels with ER, PR, and HER2 expressions ($p > 0.05$). Also, no significant associations were noted between leptin levels and grading and disease staging ($p > 0.05$).

Conclusion: The study found that leptin is higher in breast cancer patients than in healthy individuals, however, it did not prove that leptin is a predictive or prognostic factor.

Keywords: leptin, breast cancer, staging, grading

Corresponding Author:
Bayazid Ghaderi; email:
bayazidg@yahoo.com

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1. Introduction

Breast cancer (BC) is the most common malignancy in women worldwide and the most common type of cancer in Iran. It accounts for 30% of all cancers and 15% of all cancer-related deaths in women. BC is a multifactorial disease, and various factors are involved in its development [1]. Late-onset menopause, advanced maternal age, Nulliparity, long-term use of birth control pills, hormone therapy with estrogen and progesterone for a long term, obesity and estrogen use alone, miscarriage, infertility, history of pregnancy-induced hypertension, a family history of BC, and vitamin D deficiency are associated with an increased risk of BC [2, 3]. Some other factors, such as breastfeeding, oophorectomy, and multiple pregnancies, are associated with a reduced risk of BC [1].

To treat this disease like other cancers, it is necessary to evaluate the survival rate and find prognostic factors. Disease-free survival is defined from the onset of the disease to the time of the metastatic event or death of the patient [4]. The results of previous studies have shown that surgery and chemotherapy in younger women and hormone therapy using tamoxifen and letrozole in postmenopausal women with early BC significantly reduce the risk of cancer recurrence (43%) and improve disease-free survival. Other practical factors include the degree of tumor malignancy, tumor size, number of lymph nodes involved, age, estrogen (ER) and progesterone receptors (PRs), metastasis, type of tumor, and lymphovascular invasion [5]. Today, studies have identified cytokines such as leptin and adiponectin as prognostic factors that, according to some studies, are also associated with BMI [6].

Leptin is a 167-amino acid protein mainly produced by white adipose tissue that enters the bloodstream; it can appear both in free and bounded forms in serum [7]. Leptin is a neurohormone whose primary function is to regulate energy balance and food intake in the hypothalamus [8]. Plasma leptin levels reflect body fat mass [9]. In addition to adipose tissue, leptin can be present in other tissues such as the placenta, gastric and colon mucosa, liver cells, and epithelial cells of the breast [10, 11]. Some studies have shown an association between leptin, obesity, insulin resistance, and the risk of BC [7, 12]. However, some studies have not been able to find this association [13]. The controversy is even more remarkable, while some studies have even reported a negative relationship between leptin levels and BC [14].

Studies have shown that weight gain is associated with increased mortality from cancers in various body parts [15]. Multiple studies have also suggested a positive association between obesity and an increased risk of endometrial, kidney, colon, and gallbladder cancers in women and BC in postmenopausal women [16]. It has been proven that obesity can increase the risk of BC in postmenopausal women by 30–50%. In addition, high BMI is correlated with an increased risk of inflammatory BC both before and after menopause [17]. Because adipose tissue produces leptin, leptin levels are higher in obese people than in normal-weight people. In addition, there is a relatively higher level of resistance to the hormone in obese people, which indicates an increase in their serum levels [18]. Leptin stimulates the proliferation of benign and malignant breast epithelial cells in the laboratory [19]. Leptin is higher in women than men, which can be explained by differences in sex hormones as estrogen increases leptin expression while testosterone decreases it [20]. And given the prevalence of BC and the high number of prognostic factors, it is necessary to recognize the relationship

between these factors and serum leptin levels. This study aims to determine serum leptin level and its relationship with prognostic factors for BC.

2. Materials and Methods

2.1. Study design, setting, and patient population

In this retrospective case–control study, patients with BC presenting to Tohid Hospital, Sanandaj, Iran, between 2019 and 2020 were studied. The inclusion criteria were: age >18 years, informed consent, and diagnosis of BC. Census sampling method was used to select the study population. Patients were excluded from the study if they were pregnant, had liver disorders, or had other types of cancer. Accordingly, 100 people with BC and the same number of age- and BMI-matched healthy women were recruited as the case and control groups, respectively.

2.2. Data collection

Data were collected using a checklist containing information regarding demographic data of all the subjects (age, sex); disease-related indicators in the group of patients such as BMI, Ki67 cell proliferation factor, type of cancer histology, (histopathology), disease stage, disease grade, ER, PR, lymphovascular involvement, and HER2/neu. As prognostic and therapeutic indicators, the aforementioned items were collected as the mandatory items required for the assessment of patients.

2.3. Paraclinical investigation

Fasting blood samples (5 ml) were collected from the patients and the control group to measure the leptin level. Blood samples were centrifuged at 3000 rpm for 5 min to separate serum. Then, it was stored at a temperature below –24°C until the time of the tests. Leptin levels were measured via the radioimmunoassay method, using a MultiSciences kit manufactured in Iran (sensitivity 15.63–1000 pg/ml).

2.4. Statistical analysis

Data were analyzed using the SPSS software, version 22. The Kolmogorov–Smirnov test confirmed the normality of the data. Parametric statistical tests, including Pearson

Table 1: Comparison of the mean leptin level between the two groups of the BC patients and the control group.

Group	*N*	Leptin (ng/mL) Mean ± (SD)	*t*	*P*-value	Mean differences	Confidence interval 95%	
						Min	Max
BC patients	100	11.89 ± (4.45)	7.99	0.000	–9.79	–13.22	–6.36
Control	100	21.68 ± (9.16)					

Table 2: Association of leptin level with the status of hormone receptors in the BC patients.

		N	Leptin (ng/mL) Mean ± (SD)	*t*	*P*-value	Mean differences	Confidence interval 95%	
							Min	Max
ER	Negative	23	23.21 ± (9.42)	0.91	0.36	1.98	–2.34	6.30
	Positive	77	21.23 ± (9.09)					
PR	Negative	28	21.46 ± (9.90)	0.15	0.88	–0.30	–4.37	3.76
	Positive	72	21.77 ± (8.93)					
Her2	Negative	66	21.87 ± (8.69)	0.30	0.76	0.56	–3.29	4.42
	Positive	34	21.31 ± (10.14)					
Histo2ogy	Negative	88	21.40 ± (9.29)	0.84	0.40	–2.36	–7.96	3.24
	Positive	12	23.76 ± (8.19)					

test, independent *t*-test, and analysis of variance, were performed. *P*-value < 0.05 was considered as a significant level.

3. Results

The mean age of the BC patients and the control group was 47.60 ± 9.69 (range, 26–72) and 49.12 ± 8.10 (range, 28–70) years, respectively, with no significant difference ($P = 0.62$). The mean BMI of the patients was 29.42 ± 4.83 kg/m^2.

As shown in Table 1, serum leptin concentration was significantly higher in the BC patients than in healthy individuals (21.68 ± 9.16 ng/Ml vs 11.89 ± 4.45 ng/mL, $p < 0.001$).

The results of this study showed that there was no significant association between serum leptin levels and the status of hormone receptors in the study patients ($p > 0.05$) (Table 2).

The mean leptin levels in BC patients with different stages and grades of the disease are presented in Tables 3 and 4. Based on the reported results, no significant association was observed between serum leptin levels and disease staging ($P = 0.51$) and grading ($P = 0.50$).

Table 3: Mean values of leptin concentration in different stages of BC.

Stage	*N*	Leptin (ng/mL) Mean ± (SD)	*P*-value
1	33	20.31 ± (9.92)	0.514
2	24	23.72 ± (7.54)	
3	20	22.55 ± (8.36)	
4	23	20.78 ± (10.28)	

Table 4: Mean values of leptin concentration in different grades of BC.

Grade	*N*	Leptin (ng/mL) Mean ± (SD)	*P*-value
1	32	23.22 ± (9.25)	0.50
2	40	20.69 ± (9.38)	
3	28	21.34 ± (8.81)	

4. Discussion

The mean age of the patients in the current study was 47.60 ± 9.69 (range, 26–72) years. This is consistent with other studies, in which the mean age of patients ranged from a minimum of 47 to a maximum of 53 years [21–23]. However, there are a few studies in which the average age of patients is higher, ranging from 67 to 69 years [24, 25]. Moreover, the mean BMI in our study was 29.42 ± 4.83 kg/m^2, ranging from a minimum of 20 to a maximum of 44 kg/m^2, which is consistent with most studies [26, 27]. This indicates that, in general, the mean BMI in patients with BC is higher than in healthy people.

As another finding of the present study, the mean leptin level was 21.68 ng/mL in the patient group and 11.89 ng/mL in the healthy group, there was a significant difference between them (P <0.001). Many studies have investigated the association of leptin with BC, and majority of them have confirmed a significant relationship between them. However, in some studies, this relationship has not been significant. For instance, the study of Chen *et al.* [28] showed that serum leptin levels were significantly increased in patients, as compared with the control group (P = 0.025), and those with high leptin levels had larger tumors (P = 0.036). Researchers concluded that elevated leptin levels were associated with a higher risk of BC and even more advanced cancers. In a study by Wang *et al.*, a significant relationship between leptin levels and BC was reported. It was concluded that serum leptin levels and the Free Leptin Index (FLI) could be considered potential indicators for assessing the prognosis of patients with BC [29]. In a meta-analysis, 43 eligible studies were reviewed. In general, serum levels of leptin in BC patients were significantly higher than in the control group (SMD = 0.61, P < 0.001). Even when the study of subgroups was limited to the ethnicity and status of

the menstrual cycle, the serum leptin concentrations remained higher in BC patients. In addition, serum leptin levels were significantly higher in BC patients with a BMI > 25 (SMD = 1.48, P = 0.034). Moreover, in patients with lymph node metastasis, serum leptin concentration was significantly higher (SMD = 0.53, P <0.015). This meta-analysis revealed that serum leptin level might play an essential role in the pathogenesis and invasive progression of BC. Furthermore, the analysis of the ethnicity subgroup showed that the mean leptin levels were significantly higher in patients with BC among the Asian population (P < 0.001) [30]. On the other hand, according to Grossman *et al.* [31], the high adiponectin ratio to leptin indicates a favorable risk profile for BC than the low adiponectin ratio to leptin. Rose *et al.* concluded that leptin might be a strong candidate for predicting the destructive role of fat in BC. In a study by Niu *et al.*, the results showed that higher leptin was associated with increased prevalence and growth of BC [32].

Pan *et al.* conducted a systematic review of 35 eligible articles and concluded that there is a significant correlation between serum leptin levels and the risk of BC (SMD = 0.46, 95% CI = 0.31–0.60). In the mentioned study, the analysis of the BMI subgroup showed a correlation between BC and serum leptin levels in overweight patients (P < 0.01), the results of this meta-analysis showed that leptin could be a potential biomarker for the risk of BC in women, especially overweight women [33]. Other studies on the relationship between leptin and BC have confirmed a significant correlation between them [34, 35]. However, minimal studies do not approve or support this relationship [13].

Laboratory studies investigating the mechanism of action of leptin in connection with BC show that leptin circulation level is the interface that informs the brain about the state of energy storage. These levels indicate the present amount of adipose tissue and are directly related to an increase in BMI. In addition, serum leptin levels are higher in women than in men even after weight correction. This can be explained by subcutaneous synthesis and regulation of estrogen and androgen [20]. One of the environmental functions of leptin is to monitor and regulate the role of energy in the interaction between energy metabolism and the immune system in a part of the body that is responsible for obesity-related inflammation [36]. It has been shown that leptin signaling, as in breast carcinogenesis, regulates the expression of cyclin D1, p53, survivin, IL1, E-Cadherin, VEGF and its AT2, and various tissue factors. In addition, it regulates molecules involved in proliferation, migration, invasion, adhesion, angiogenesis, and inflammation [37, 38]. It has been suggested that leptin at the carcinogenic level may act as a direct activator of short-term ROS production in human epithelial breast cells [39]; however, other studies have previously shown that chronic leptin therapy reduces ROS levels and oxidative stress in MCF-7 cells [40]. Leptin has also been introduced as a mediator

of tumor-stroma interaction [41], where it appears to interact between BC cells and M2 tumor-associated macrophages through stimulating IL-18 production. IL-8, in turn, enhances the proliferation and metastasis of tumors [42]. In addition, a recent study showed that obesity increased leptin expression at the mRNA level by reducing the level of p16INK4A as a tumor suppressor protein in breast adipocytes and promoting precancerous processes [43].

Based on other results of our study, comparing leptin levels in terms of the presence and absence of ER, PR, Her2, Histo2opy, grade, and stage in the patient group, there was no statistically significant difference between the studied items ($P > 0.05$). This finding is consistent with all reviewed studies and indicated that there was no significant difference between BC patients with positive and negative ER in terms of leptin level ($P > 0.05$) [27, 44]. Furthermore, in studies by Kim *et al.* [45] and Wang *et al.* [35], there was no significant relationship between leptin and HER-2, which indicates that these markers may not be associated with an increase or decrease in leptin level in patients. The results of other studies also support our findings on BC grade and stage.

5. Conclusion

The study proves that leptin is higher in BC patients than in healthy individuals; however, it did not prove that leptin is a predictive or prognostic factor. A larger study is needed.

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Ethical Considerations

The study protocol was approved by the ethics committee of Kurdistan University of Medical Sciences, Sanandaj, Iran (No. IR.MUK.REC.1398.266), and informed consent was obtained from all participants prior to the study.

Competing Interests

The authors report no potential conflict of interest.

Availability of Data and Material

All relevant data and methodological details pertaining to this study are available to any interested researchers upon reasonable request to corresponding author.

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