ORIGINAL ARTICLE

Body composition and serum levels of matrix metalloproteinase-9, adiponectin and AMP-activated protein kinase in breast cancer survivors

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Background: Available data suggest that obesity is related to changes in the several adipocyte-derived proteins levels, which are involved in cancer recurrence. The purpose of this work was to investigate the correlation between obesity with metalloproteinase-9 (MMP-9), adiponectin and adiponectin and AMP-activated protein kinase (AMPK) levels by comparing serum levels of MMP-9, AMPK in normal weight and obese breast cancer survivors. **Materials and Methods:** In this cross-sectional study, 30 normal weight breast cancer survivors (body mass index [BMI] 18.5-25 kg/m^2) and 30 obese breast cancer survivors (BMI ≥30 kg/m^2) were investigated. Anthropometric parameters and serum levels of MMP-9, adiponectin, and AMPK were compared between the two groups. **Results:** No differences were detected in the serum levels of MMP-9, adiponectin, and AMPK in obese patients and normal weight patients ($P > 0.05$). There were no correlations between MMP-9, adiponectin, and AMPK levels with anthropometric measurements in two groups ($P > 0.05$). **Conclusion:** We found that there was a lack of correlation between obesity measures and serum levels of MMP-9, adiponectin, and AMPK. In breast cancer survivors, it seems that circulating levels of adiponectin, AMPK, and MMP-9 do not change in obesity state.

Key words: Adiponectin, AMP-activated protein kinase, breast cancer, matrix metalloproteinase-9, obesity

How to cite this article: Babaei Z, Parsian H, Korani B, Mostafazadeh A, Moslemi D. Body composition and serum levels of matrix metalloproteinase-9, adiponectin and AMP-activated protein kinase in breast cancer survivors. J Res Med Sci 2022;27:48.

INTRODUCTION

Available data suggest that obesity is related to changes in the several adipocyte-derived proteins levels such as leptin, matrix metalloproteinases (MMPs), and adiponectin, which are involved in the cancer development and recurrence.[1-4] MMPs belong to a large proteolytic enzymes family that can degrade extracellular matrix (ECM) components of the basement membrane.[1,2] The ability of MMPs to change the structural integrity is crucial to control tissue remodeling under physiological conditions. Nevertheless, the abnormal expression of MMPs is related to many pathological conditions, including tumor growth.[1] MMP-9, belongs to the MMPs family, has an important role in remodeling of the ECM that facilitates cancer metastasis.[1,2] High level of circulating MMP-9 activity has been related to a worst overall survival rate in patients with breast cancer.[1] Some studies revealed that MMP-9 serves as an effective prognostic marker in patients with breast cancer.[1,2]

Adiponectin is known as an adipocytokine that can modulate various obesity-associated disorders, including cancer.[3] Several studies have revealed a negative relationship between serum levels of adiponectin and the risk of breast cancer.[3] The antitumor function of

Access this article online

Quick Response Code:

Website: www.jmsjournal.net

DOI: 10.4103/jrms.JRMS_453_20

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Submitted: 14-May-2020; **Revised:** 10-Aug-2020; **Accepted:** 06-Oct-2020; **Published:** 30-Jun-2022

adiponectin has also been clarified.[3,5] In breast cancer cells, report has shown that adiponectin can hinder the malignant biological behavior of tumor cell and promote apoptosis.[5] Increased AMP-activated protein kinase (AMPK) activity remains an important mechanisms of actions of adiponectin in cancer prevention.[5] AMPK, a serine/threonine kinase, is regarded as the major regulator of cellular energy that exerts an important function in the glucose and lipid metabolism.[6] However, reports on the correlation between obesity and serum levels of adipocyte-derived proteins have yielded conflicting results.[7-9] Because the identification of high-risk breast cancer patients is important for health management,[10] the aim of our study was to investigate the correlation between obesity with MMP-9, adiponectin, and AMPK levels by comparing serum levels of MMP-9, adiponectin, and AMPK in normal weight and obese breast cancer survivors.

MATERIALS AND METHODS

Study population

The Ethical Committee of Babol University of Medical Sciences (MUBABOL.REC.1394.34) allowed this cross-sectional study, and informed consents were provided by each patient before the study. The study population consisted of 30 normal weight breast cancer survivors [body mass index (BMI) 18.5–25 kg/m^2] and 30 obese breast cancer survivors (BMI ≥30 kg/m^2). These patients were selected from an initial sample of 208 breast cancer survivors who referred for routine follow-up to Shahid Rajaee Oncology Hospital (Babolsar, Iran), between January 2013 and July 2013. The details of the study population for anthropometric parameters, demographics, and clinical data have been described previously.[4] Breast cancer survivors were enrolled in this investigation if breast cancer (Stages II-III) was identified within the past 5 years and primary treatment was completed (except hormone therapy) at least 6 months before to enrolment. Patients with a history of endocrine abnormality, diabetes mellitus, known cardiac disease, uncontrolled thyroid disease, and uncontrolled hypertension were excluded in our investigation.

Anthropometric measurements and biochemical tests

Body weight of patients was weighed with a digital weight scale (Seca, GmbH, Germany) with a precision of 0.1 kg. Standing height was evaluated up to 0.1 cm precision. BMI of the patients was computed as the weight (kg) divided by height (m^2). Waist circumference (WC) was obtained at 2.5 cm above the umbilicus and hip circumference (HC) was performed in the region of maximum width of the buttocks. Waist-to-hip ratio (WHR) was calculated by dividing the WC by the HC. For analysis of MMP-9, adiponectin, and AMPK, 10 ml of venous blood were collected from each participant after an overnight fast (12 h) and the serum samples gained after centrifugation was kept at −80°C before use. Concentrations of MMP-9, adiponectin, and AMPK were determined in serum with commercially available ELISA kits (Crystal Day Biotech Co., Shanghai, China) according to the manufacturer's protocol. The sensitivities of the kits were 0.11 mg/l for adiponectin; 15.12 ng/L for MMP-9, and 0.025 ng/ml for AMPK. The intra- and inter-assay variation coefficients were 8% or less for adiponectin; 10% or less for MMP-9 as well as 10% and 12% for AMPK.

Statistical analysis

SPSS version 18.0 software (SPSS Inc., Chicago, IL, USA) was employed for statistical evaluation. The results were reported as mean ± standard deviation the normal distribution of data was estimated by the Kolmogorov–Smirnov test. The Student's *t*-test and Mann–Whitney *U*-test were employed to compare normally and nonnormally distributed variables, respectively. The relationship between adiponectin, MMP-9, and AMPK with anthropometric measurements were analyzed with Pearson test. P <0.05 was classified as statistical significant.

RESULTS

A total of 60 breast cancer survivors with a mean age of 49.23 ± 10.3 years, in two group 30 normal weight patients (BMI 18.5–25 kg/m^2) and 30 obese patients (BMI ≥30 kg/m^2) were included in this investigation. Mean follow-up time in two groups was 3.2 ± 2 years. No significant differences were detected in the mean age of obese patients (50.8 ± 7.5 years) and normal weight patients (47.6 ± 12.4 years) [Table 1; $P > 0.05$]. Sixty percent of obese patients were diagnosed with Stage II, while 40% had Stage III [Table 1]. Stage of breast cancer and menopausal status did not differ between obese patients and normal weight patients [Table 1; $P > 0.05$].

Weight, WC, HC, and WHR were significantly higher among obese patients than normal weight patients [Table 1; $P < 0.05$]. Mean of BMI in obese patients was 35.4 ± 4.2 kg/m^2 and mean of BMI in normal weight patients was 22.9 ± 2 kg/m^2, which was higher in obese than in normal weight patients [Table 1; $P < 0.05$]. We observed no important change in the concentrations of MMP-9, adiponectin, and AMPK in obese patients than in normal weight patients [Figure 1; $P > 0.05$].

To explore the correlations between anthropometric characteristics and serum levels of MMP-9, adiponectin, and AMPK, Pearson correlation test was employed. As shown in Table 2, no significant correlations were detected between MMP-9, adiponectin, and AMPK with anthropometric measurements in two groups [Table 2; $P > 0.05$]. Adiponectin was positively correlated with the AMPK and MMP-9 [Table 2; $P < 0.05$].

DISCUSSION

The present study on breast cancer survivors showed that serum levels of adiponectin, AMPK, and MMP-9 were not significant differences between obese and normal weight breast cancer survivors [Figure 1; $P > 0.05$]. We found no significant correlations between adiponectin, AMPK, and MMP-9 with anthropometric parameters [Table 2; $P > 0.05$].

Weight gain after breast cancer diagnosis has been described as a risk factor for cancer recurrence.[11] Meanwhile, available data suggest that obesity is related to changes in the several adipocyte-derived proteins levels such as leptin, MMPs, and adiponectin, which are involved in cancer development and recurrence.[12,13] Our previous study on breast cancer survivors showed that obesity was positively related to levels of leptin and acute phase proteins in obese patients.[4] However, reports on the correlations between anthropometric measurements and serum adiponectin levels have yielded conflicting results.[7,14,15] We have shown here that serum adiponectin was not significant differences between normal weight and obese patients [Figure 1; $P > 0.05$]. Moreover, we found no correlations between adiponectin with anthropometric measurements [Table 2; $P > 0.05$]. Our findings regarding adiponectin are in line with previous results showing that there is no significant relationship between increase adiposity and levels of adiponectin.[7] Interestingly, Yasui *et al*. indicated that circulating adiponectin levels were not related to BMI.[14] Another study conducted by Aguilar-Salinas *et al*. on obese subjects also showed that serum adiponectin concentrations were not significantly different in obese subjects than in normal body weight individuals.[15] According to our results, this suggests a diminished relationship between serum levels of adiponectin with adiposity in breast cancer survivors. In contrast, other studies revealed that serum adiponectin concentration was significantly decreased in obesity.[8,16-19] In addition, Jonas *et al*. and Nayak *et al*. both reported that adiponectin levels were inversely associated with BMI.[20,21] We have no reason for the differences between our study findings with other previously reported results.[8,16-19] It is possible that several other parameters, in addition to quantity of fat, may affect circulating adiponectin level.[22]

Recent data have shown that AMPK is emerging as a target for cancer treatment.[23] In addition, AMPK activity is one of the potential mechanisms of adiponectin's action.[5] As

Table 1: Clinicopathological and anthropometric characteristics

Characteristics	Normal weight patients (*n*=30)	Obese patients (*n*=30)	*P*
Age (years)	47.6±12.4	50.8±7.5	0.231
Follow-up time (years)	3.3±2.0	3.0±2.1	0.527
Clinical stage, *n* (%)			
Stage II	20 (66.7)	18 (60)	0.595
Stage III	10 (33.3)	12 (40)	
Menopausal status, *n* (%)			
Premenopause	21 (70)	19 (63.3)	0.587
Postmenopause	9 (30)	11 (36.7)	
Weight (kg)	56.8±6.8	84.9±11.2	<0.001**
Height (m)	1.57±0.06	1.55±0.06	0.110
BMI (kg/m^2)	22.9±2.0	35.4±4.2	<0.001**
Waist (cm)	86.0±7.6	110.7±9.6	<0.001**
Hip (cm)	95.8±7.2	117.8±9.6	<0.001**
WHR	0.9±0.07	0.94±0.06	<0.05*

*P<0.05 and **P<0.01 were performed by Student's *t*-test, aMenopausal status at diagnosis, Results were reported as mean±SD or *n* (%). BMI=Body mass index; WHR=Waist-to-hip ratio; SD=Standard deviation

Table 2: Correlations between anthropometric measurements and biochemical variables

	BMI	Weight	Waist	Hip	WHR	MMP-9	Adiponectin
MMP-9	−0.089	−0.170	0.071	0.186	0.178	1	0.725**
Adiponectin	−0.140	−0.211	−0.101	−0.176	0.101	0.725**	1
AMPK	−0.059	−0.130	0.028	−0.116	0.151	0.619**	0.730**

**P<0.01 were performed by Pearson test. WHR=Waist-to-hip ratio; BMI=Body mass index; AMPK=AMP-activated protein kinase; MMP-9=Matrix metalloproteinase-9

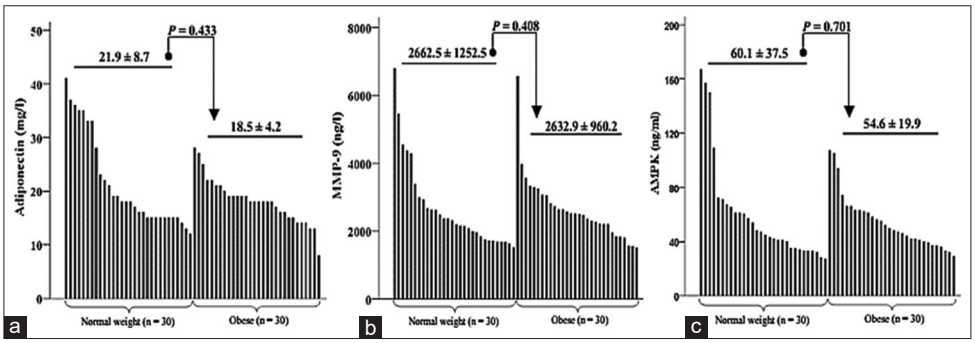

Figure 1: Comparison of adiponectin (a), metalloproteinase-9 (b), and AMP-activated protein kinase (c) serum levels for each individual among obese patients than normal weight patients. Significance evaluation was analyzed by Mann–Whitney test

far as the authors know, serum levels of AMPK have not been studied during obesity state. Accordingly, in this investigation, we compared serum levels of AMPK between obese and normal weight breast cancer survivors. We found no significant correlations between serum levels of AMPK and anthropometric parameters [Table 2; $P > 0.05$]. However, a positive relationship was detected between serum levels of AMPK and adiponectin [Table 2; $P < 0.01$].

Circulating MMP-9 levels has appeared as an effective biomarker for predicting axillary node metastasis.[1] Meanwhile, several authors have demonstrated that circulating MMPs levels could be abnormal in obesity.[9,24,25] Previous studies revealed that MMP-9 and their tissue inhibitors (TIMP-1) plasma levels were increased and MMP-2 plasma levels were decreased in premenopausal obese women.[25] Derosa *et al*. also detected that the levels of MMP-9 and MMP-2 were increased in obese patients.[24] In another study, scientists demonstrated that MMP-9 and TIMP-1 plasma levels were elevated in obese children and adolescents.[26] However, in accordance with previous observations,[24,26] the question is whether serum concentration of MMP-9 may be good biomarker in breast cancer survivors in obesity status. Our findings showed no differences in serum levels of MMP-9 in obese patients compared to normal weight breast cancer survivors [Figure 1; $P > 0.05$]. On the other hand, no relationship was detected between serum concentration of MMP-9 and anthropometric measurements [Table 2; $P > 0.05$]. The results of our investigation are in agreement with previous findings indicating that no differences in total levels of MMP-9 in obese subjects than in nonobese controls.[27,28] The lack of consistency in findings about correlation between serum concentration of MMP-9 and obesity might probably be due to the different effects of obesity-related conditions, including inflammation and metabolic syndrome.[28] Interestingly, Gummesson *et al*. indicated that circulating MMP-9 level was not related to WC and BMI, however, there were significant correlations with blood pressure, fasting glucose, acute-phase protein, and insulin.[28] Besides, there were different metabolic situations may influence circulating levels of MMP-9.[28] Taken together, the lack of association between anthropometric measurements and serum levels of MMP-9 shows that this marker may not be sensitive factor in obese breast cancer survivors.

CONCLUSION

Taken together, our study demonstrated that there was a lack of correlation between obesity measures and circulating serum levels of MMP-9, adiponectin, and AMPK. In breast cancer survivors, it seems serum levels of adiponectin, AMPK, and MMP-9 not be an effective marker in obesity state.

Financial support and sponsorship

The present study was supported by a grant from the Babol University of Medical Sciences (No. 2414).

Conflicts of interest

There are no conflicts of interest.

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