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Research Article

Keywords: Asprosin, breast cancer, diagnostic indicator

Posted Date: September 21st, 2021

DOI: https://doi.org/10.21203/rs.3.rs-897211/v1

A novel adipose hormone asprosin as a potential breast cancer marker

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Abstract

Purpose: Asprosin is a recently discovered hormone released by white adipose tissue (WAT) that is typically significantly elevated in obese adults. Consequently, the adverse effects of increasing WAT in obesity during breast cancer (BC) development and progression have attracted interest of researchers and clinical practitioners. Thus, the aim of the present study was to determine whether the asprosin levels are associated with the probability of women having BC.

Methods: The study sample comprised of 45 female patients diagnosed with invasive BC and 42 healthy women that served as controls. Asprosin serum level was quantified in all subjects by ELISA, whereas serum levels of CEA and CA 15–3 were measured using an immunology analyzer. The potential association between asprosin and BC was examined through logistic regression analyses, while samples provided by BC patients were further subjected to ROC analysis to assess the diagnostic accuracy of asprosin.

Results: Asprosin levels were significantly higher in BC patients compared to healthy controls (2.38 ± 0.54 vs. 1.39 ± 0.53 ng/mL, $p < 0.001$). Multivariable analysis showed that the increased asprosin levels were associated with a significantly higher risk of breast cancer after adjustments for family history of breast and/or gynecological cancer, dyslipidemia, and BMI (odds ratio = 157.92; 95% confidence interval = 17.22−1447.96). When 1.78 ng/mL was adopted as the cut-off value, AUC, sensitivity, and specificity of asprosin for BC were 0.943, 91.1%, and 88.1%, respectively.

Conclusions: Our findings demonstrate that asprosin is elevated in BC and can thus be an appropriate candidate for breast cancer diagnosis.

Key Words: Asprosin, breast cancer, diagnostic indicator

Introduction

With about 12% worldwide prevalence[3, 7], breast cancer (BC) is the most common type of cancer among women, and the second most frequent cause of death globally [6]. Yet, despite these alarming statistics, BC is often detected in advanced stages due to the dense structure of the breast tissue, especially among obese women [28]. Consequently, the efficacy of cancer treatments is lower in obese patients. Although BC etiology is not fully understood, available evidence points to an association with obesity, as excessive levels of adipose tissue hormones and adipokines can affect cancer cells [14, 20, 24].

Adipokines impair peripheral insulin sensitivity, oxidative capacity, and lipid intake by acting centrally to regulate appetite and energy consumption. As adipose tissue is a dynamic organ, its amount and condition can change the adipokines [22]. In obese individuals, fat cell hypertrophy and/or hyperplasia is caused by pathological expansion of white adipose tissue, which results in hypoxia and oxidative stress, as well as metabolic, inflammatory, immune, and epigenetic changes, all of which promote neoplastic transformation and growth [2]. Asprosin, encoded by the fibrillin 1 (FBN1) gene, is secreted from white adipose tissue and metabolized in the liver, where it promotes rapid glucose release into the bloodstream [26]. As asprosin is an orexinergic peptide, it has been shown to elevate blood glucose levels in mice [9, 26]. Empirical evidence further indicates that its levels are increased in obese women, as well as those affected by polycystic ovarian syndrome (PCOS), type 2 diabetes, and metabolic syndrome, whereby this adverse effect is linked to insulin resistance [19]. However, our study group found that asprosin levels were similar in obese female rats compared to healthy female rats [25].

Although the relationship between asprosin and cancer is insufficiently studied, extant findings indicate that asprosin immunoreactivity is increased in malignant mesothelioma [13], suggesting that it may play a role as a glucogenic peptide in ovarian cancer [11]. However, it remains to be established whether asprosin levels are changed in patients diagnosed with BC.

Therefore, the aim of the present study was to examine the link between asprosin levels and the risk of developing BC.

Materials and Methods

Participants

Prior to commencing the study, approval from the Firat University Ethics Committee (Protocol No: 2018-19) was obtained. Its target population were women diagnosed with primary invasive ductal breast cancer, while excluding those suffering from metabolic diseases, major cardiovascular diseases (such as unstable angina, stroke, or myocardial infarction), liver disease, diabetes mellitus, severe psychiatric conditions, chronic kidney disease, diseases of the central nervous system, and polycystic ovary syndrome, as well as women undergoing immunosuppressive agent therapy or exhausting exercise regimen, and those with history of other malignancies. As 45 female patients recruited from the Pain and Oncology Clinic met these criteria, they formed the BC sample, while 42 healthy female volunteers served as controls. Their demographic profile is shown in Table 1.

Determination of Human Serum Asprosin and Cancer Marker Levels

Serum asprosin levels were measured using Sandwich enzyme-linked immunosorbent assays (ELISA), based on the manufacturer' protocol (Abbexa Ltd., Cambridge, UK, Catalogue no: abx257694). The serum levels of cancer markers CEA, CA 15–3 and Her-2/neu were determined using Advia Centaur Immunology Analyzer (Advia 2400, Automatic Siemens Healthcare Diagnostics Inc., Tarrytown, USA). For this purpose, peripheral venous blood samples were collected from all patients and healthy volunteers and were centrifuged at 4000 rpm for 5 minutes at 4 ^{o}C. The resulting serum samples were aliquoted and kept at [3]–80 ^{o}C until needed for analyses.

Statistical Analyses

The sample size for the present study was determined by the power analysis, indicating that 15 subjects were sufficient to achieve a Type 1 error (α) of 0.05, Type 2 error (β) of 0.10,

and Power = 0.90. Conformity with the normal distribution was evaluated through Shapiro−Wilk test and all normally distributed data was presented as mean ± standard deviation (M ± SD), whereas categorical variables were summarized as absolute (n) and relative frequencies (%) and nonparametric data was presented as median (25–75th percentile). Associations between categorical variables were evaluated using contingency tables and chi-squared tests without continuity correction or Fisher's exact test, if applicable. For normally distributed data, two independent groups were evaluated via Student's t-test while applying Mann–Whitney U test in other cases. Three or more categories were compared by means of one-way analysis of variance (ANOVA) followed by a post-hoc Bonferroni correction. Correlations between asprosin levels and continuous variables were evaluated using the Pearson's correlation coefficient (normally distributed data) or Spearman correlation coefficient (skewed data).

The strength of the association between asprosin levels and BC probability was evaluated using logistic regression analysis, while considering BMI (<22.5, 22.5–25, 25–30, and ≥30 kg/m^2), dyslipidemia, and family history of breast or gynecological cancer as potential confounders. We also calculated adjusted estimates of odds ratios (OR) and 95% confidence intervals (95% CI) for BC risk estimates.

Receiver operating characteristic (ROC) was used to identify the optimal asprosin cut-off level and determine its sensitivity and specificity for BC. In addition, the area under the ROC curve (AUC) was used to assess the diagnostic performance of asprosin in BC. Finally, Hosmer–Lemeshow fit test was conducted to assess the agreement between observed and model-predicted proportions of BC. All statistical calculations were performed using the SPSS version 21.0 commercial software (SPSS Inc, Chicago, II, USA).

Results

Demographic Characteristics

As shown in Table 1, the BC and control group were similar in terms of age, height, weight, BMI, and menopausal status. Moreover, no statistically significant differences were

noted in their total cholesterol, HDL, LDL, and TG (dyslipidemia) levels. On the other hand, the BC group was statistically significantly more likely to have a family history of breast or gynecological cancer than healthy women ($p < 0.001$) and their serum asprosin levels (2.38 ± 0.54 ng/mL) were statistically significantly higher than those measured for controls (1.39 ± 0.53 ng/mL) (Fig.1). The analyses further indicated that a 1-unit increase in asprosin levels increased the BC risk by 100-fold. To validate these findings, all analyses were repeated after adjusting for potential confounders (such as BMI, dyslipidemia, and family history of breast/gynecological malignancy), which increased the BC risk to 158-fold (Table 2). Next, ROC analysis was conducted to establish the sensitivity and specificity of asprosin in the detection of BC.

The obtained results indicated that serum asprosin levels successfully discriminated BC patients from healthy controls (AUC = 0.943, 95% CI: 0.871–0.981), and exhibited acceptable discriminative ability (sensitivity = 0.911; specificity = 0.881) at the optimal cut-off value of 1.78 ng/ml ($p < 0.0001$) as shown in Figure 2. The presence of ER was associated with asprosin levels ($p<0.05$, Fig.3). However, further investigations may reveal that asprosin levels may be related to PR, c-erb-B2 status, tumor size and grade (Table 3), as well as cancer markers (Ki67 and p53).

Discussion

The findings yielded by the present study indicate that asprosin levels in BC patients are significantly higher than in healthy women. Therefore, elevated asprosin values can be an indicator of an increased BC risk. Specifically, the ROC analysis results demonstrate that, when the cut-off point for asprosin is set at 1.78 ng/mL, its sensitivity for BC detection is 91.1% and its specificity is 88.1%. Thus, asprosin could be a useful marker for early detection of breast cancer.

As glucose uptake is elevated in tumor cells, asprosin (as a novel glucogenic adipokine) may play a role in tumor development and progression. Available evidence indicates that asprosin immunoreactivity is considerably raised in malignant mesothelioma, and can thus serve as its possible diagnostic marker [13]. Recently, Kerslake et al.[11] reported that asprosin influences fertility and steroidogenesis in healthy ovaries and may act as a glucogenic peptide in ovarian cancer, whereas Li[18] found that FBN1 (asprosin encoded gene) may be a potential colon cancer marker. According to [4] , reduced asprosin levels may also be the cause of cancer-related anorexia and cachexia syndrome in patients with gastric cancer, colorectal cancer, and other cancers.

However, as the link between asprosin and cancer remains insufficiently understood, the results obtained in this study could lead to the development of tests that can be performed in clinical practice to assess the BC risk in women. It is also hoped that its role in other cancers will be investigated in future studies. Going forward, it would also be beneficial to study asprosin's molecular effects and the mechanisms that influence its development in BC. According to Romere [26], asprosin dysfunction is caused by immunological or genetic factors, and results in a significant decrease in glucose and insulin concentrations. As was shown in this study, asprosin can reach pathological levels in BC patients.

It is well established that prolonged estrogen exposure increases the risk of breast cancer [1], as aromatase (a critical enzyme in estrogen biosynthesis) is secreted by breast adipose tissue, which is the primary source of estrogen in postmenopausal women, as the ovaries are unable to produce estrogen [23]. As a result, early menopause and postmenopausal adipose tissue accumulation significantly increase the risk of developing breast cancer [21, 24, 27]. Recently, however, Li et al.[19] found no correlation between asprosin and progesterone levels in women with PCOS, an endocrine disorder known to affect androgens. On the other hand, their analyses revealed that asprosin and estrogen levels were positively correlated with PCOS in women with normal weight, but not overweight subjects. Findings obtained in the current study indicate presence of an association between asprosin and ER in BC patients.

Therefore, we speculate that, in BC, estrogen sends a signal to cells such as adipocytes by regulating asprosin secretion.

At present, breast cancer is diagnosed using cancer biomarkers (which suffer from low specificity and sensitivity) and mammography (which is incapable of detecting interval tumors and suspicious lesions in women with dense breasts). Thus, to overcome these limitations, it is essential to identify new biomarkers with high sensitivity and specificity for breast cancer detection. Recent studies show that changes in adipokine levels may be a significant risk factor for the development of BC [8, 29]. Empirical evidence further indicates that adiponectin levels are low in breast cancer patients [29], whereas leptin, interleukin-6, interleukin-8, TNF-, resistin, and visfatin values are elevated [5, 8, 10, 12, 15, 16].

In the present study, elevated serum asprosin values were shown to serve as an independent diagnostic factor for BC (Figure 2), but not as an indicator that can be reliably used for staging/grading the BC. However, as the present study was conducted on a small sample recruited from one clinical center, these results need to be validated through further investigations involving larger and more diverse patient populations. A further limitation of this study stems from the fact that the potential effects of premenopausal menstrual cycle and postmenopausal hormone replacement therapy on asprosin levels were not evaluated directly. In the available literature, an association between fasting plasma asprosin levels in healthy women and menstrual cycle, oral contraceptive use, and physical activity levels has been established [17]. However, as the last menstrual period of breast cancer patients and healthy premenopausal women was comparable (20.8 vs 22.1 days) in this study, these findings need to be explored further.

Conclusions

Even though the role of asprosin in a variety of diseases and metabolic conditions has been studied, this work marks the first attempt to establish the clinical significance of asprosin levels in the BC diagnosis and risk assessment. The findings reported here thus provide critical

preliminary evidence indicating that serum asprosin levels may be an appropriate new candidate for breast cancer detection and early diagnosis.

Author contributions

All authors contributed to the study conception and design. SO, NY, MY, MRO and MO contributed to the analysis of data and preparation of manuscript. SO, NY, MY contributed to data collection. MO and SO : Manuscript preparation and critical revision. All authors are accountable for all aspects of the work.

Disclosure statement

No potential conflict of interest was reported by the authors.

Data availability statement

The data used to support the findings of this study are available from the corresponding author upon request.

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Table and Figures Legends

Table 1. Participants' characteristics at baseline

Table 2. Association between asprosin serum levels and the probability of women having breast cancer (logistic regression analysis)

Table 3. Association between asprosin levels and breast cancer charecteristics

Fig 1. Boxplots of serum asprosin levels in breast cancer and healthy women. Mean values ± standard deviation are also denoted. Serum asprosin levels were significantly higher in breast cancer patients compared to controls (2.38 ± 0.54 vs. 1.39 ± 0.53 ng/mL, respectively, $p < 0.001$)

Fig 2. Receiver operating characteristic (ROC) curve analysis in breast cancer detection. ROC curve analysis assessing the feasibility of serum asprosin as a diagnostic indicator of breast cancer. Serum asprosin can discriminate between breast cancer patients and healthy individuals at a cut-off point of 1.78 μg/ml, with 0.943 area under the curve (AUC), 91.1 % sensitivity and 88.1 % specificity.

Fig 3. Boxplots stratified by (A) tumor stage and (B) tumor grade for asprosin levels in breast cancer women

Figures

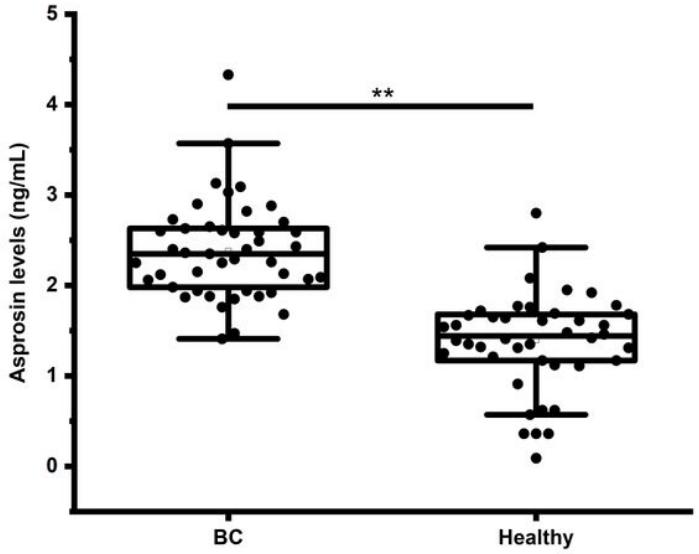

Figure 1

Boxplots of serum asprosin levels in breast cancer and healthy women. Mean values ± standard deviation are also denoted. Serum asprosin levels were significantly higher in breast cancer patients compared to controls (2.38 ± 0.54 vs. 1.39 ± 0.53 ng/mL, respectively, $p < 0.001$)

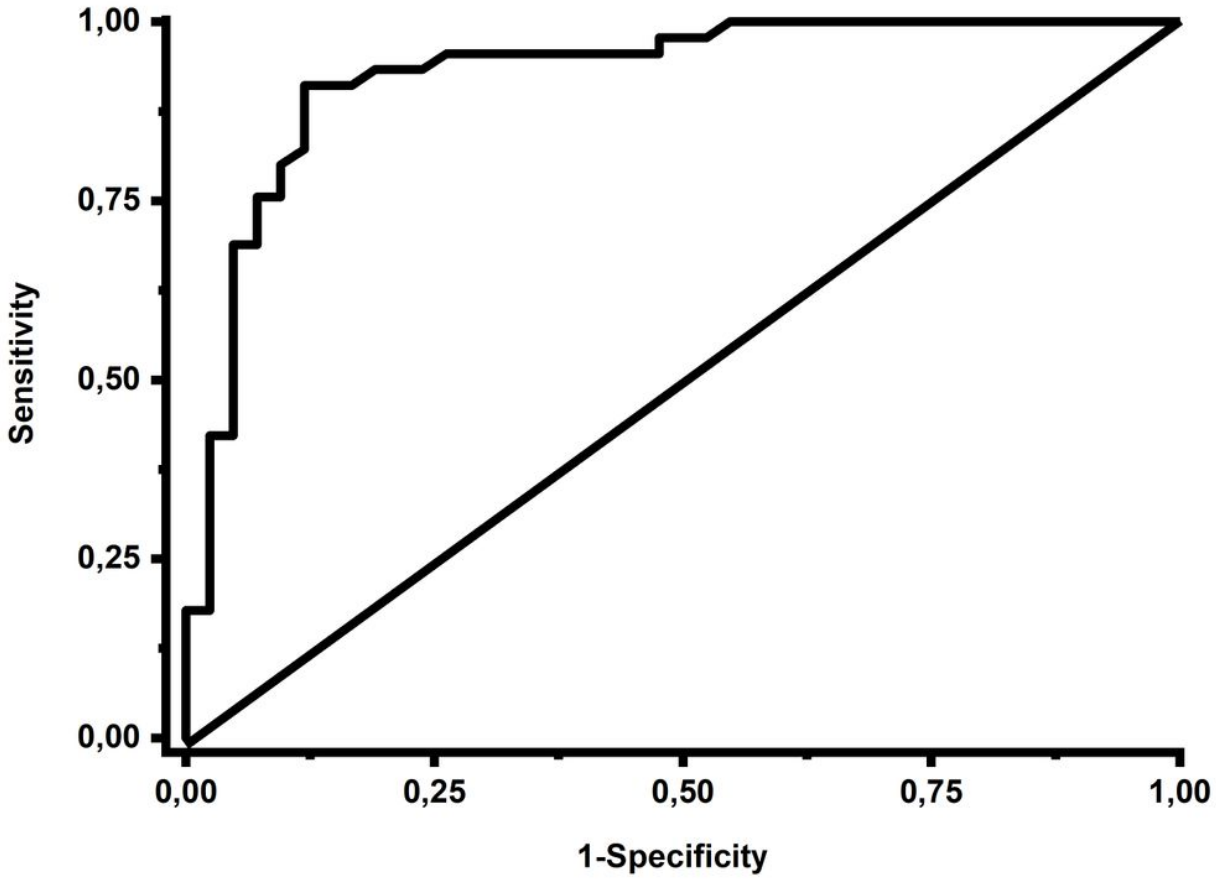

Figure 2

Receiver operating characteristic (ROC) curve analysis in breast cancer detection. ROC curve analysis assessing the feasibility of serum asprosin as a diagnostic indicator of breast cancer. Serum asprosin can discriminate between breast cancer patients and healthy individuals at a cut-off point of 1.78 µg/ml, with 0.943 area under the curve (AUC), 91.1 % sensitivity and 88.1 % specificity.

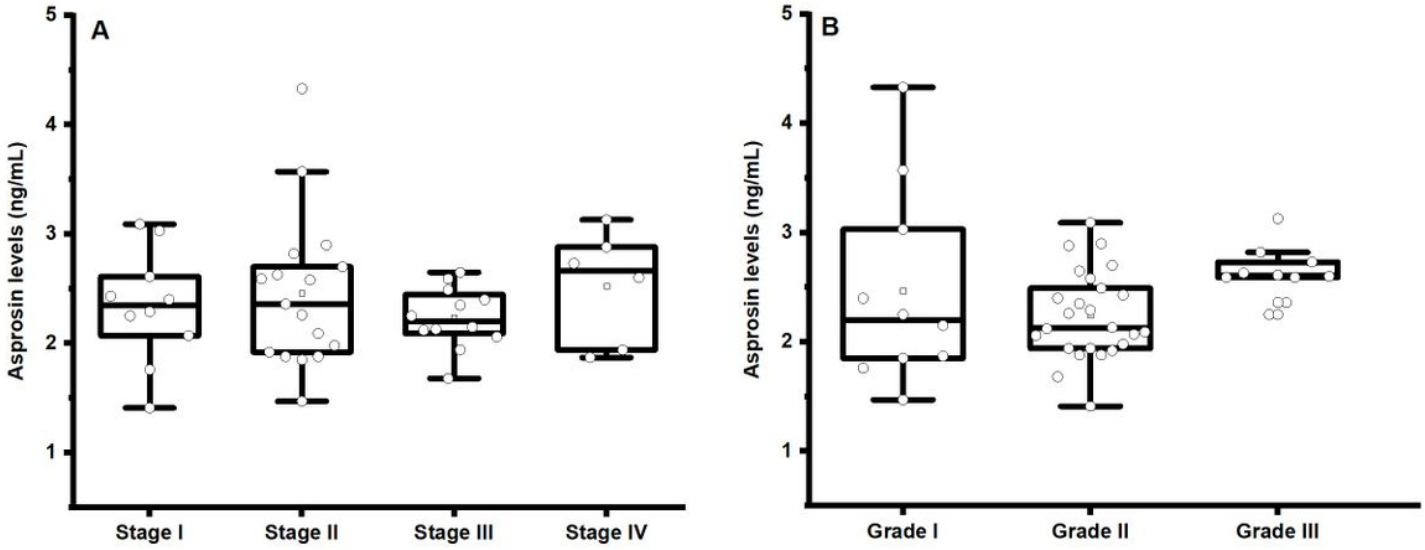

Figure 3

Boxplots stratified by (A) tumor stage and (B) tumor grade for asprosin levels in breast cancer women