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Serum Amyloid A and Tumor Invasiveness in Egyptian Patients with Breast Carcinoma

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Abstract

Many breast cancer (BC) cases are detected with advanced stages and thus worse prognosis. Thus, evaluating sensible biomarkers for BC early detection and prognosis is still a great challenge. Here, we aimed to evaluate the association between serum amyloid A (SAA) and both detection and prediction of poor BC progression. Compared to CEA and CA 15.3 as established tumor markers, our findings found that elevated SAA significantly (P<0.0001) related to BC development (36.74 (21.1-50.2) ng/mL) compared to patients with benign diseases (14.3 (12.0-18.12) ng/mL) and healthy (5.1 (3.2-6.9) ng/mL) controls. It had a great ability (AUC=0.960) to diagnosing BC patients. SAA levels (ng/mL) was significantly (P<0.05) affected the disease progression including tumor late stages (47.3 (37.0-90.5)), lymph node invasion (40.8 (27.5-69.23)), distant metastasis (75.0 (47.8-108.0)), high grades (78.5 (24.8-100.2)) and large size (41 (28.9-71.2)). Moreover, SAA high levels were associated to negative estrogen receptor and progesterone receptor status and negative HER2. SAA was significantly correlated with CA 15.3 (r=0.317; P=0.0020) and CEA (r=0.644; P=0.0001) in BC patients. In conclusion, SAA protein appears to have a pivotal role in BC development and progression. Owing to the association between its increased concentrations and BC severity, it could be a biomarker candidate for BC surveillance and perhaps a therapeutic BC target.

Key words: Breast cancer; Serum Amyloid A; Tumor invasiveness; Biomarker; Diagnosis

Introduction

Nowadays, breast cancer (BC) is a prevalent cancer, particularly affecting females as one of the most frequent forms of malignancy [1]. Among women, it represents the leading cause of tumor-related death, with >680000 deaths [2]. In contrast to difference in rates of BC incidence between developing and developed countries, BC stills the most frequent female cancer type in Egypt with an age-specific incidence rate of 48.8/100000. In 2050, about 46000 incident patients are forecasted [3].

Despite improvements in BC diagnosis, management and therapy, genetic signatures or molecular mechanisms are still crucial challenges owing to other tumorigenesis factors [4]. BC development involves a progression via intermediate until the invasive stages of malignancy and finally into metastatic BC [5]. Initially, a marked portion of cases found with localized disorder, for which curative therapy are effective. In contrast, another major cases portion is detected with metastatic BC with a worse prognosis [6]. Given the variability in clinical progression, it is particularly important to evaluate and identify biomarkers with the ability to predict the tumor behavior in BC [7].

For clinical management in cancer patients, tumor markers determination is a useful tool, assisting in diagnosis, staging, therapeutic response evaluation, metastasis and recurrence detection, and novel therapy modalities development [8]. Serum amyloid A (SAA), a positive acute-phase protein, is generated primarily by the hepatocyte in response to inflammation, infection, trauma, and even neoplastic stimuli [9]. It has been found that tumor and tumor-related cells can also produce SAA in the tumor microenvironment (TME) [10]. Increased SAA serum levels found in the TME are related to poor prognosis. Furthermore, several reports have demonstrated that

SAA contributes to tumor progression by the promotion of angiogenesis, inflammation, proliferation and metastasis in renal cell, ovarian, prostate, lung and breast cancers [11].

The aim of this study is to evaluate SAA potential role as a reliable biomarker in differentiating BC from premalignant benign breast diseases and in prediction of poor disease progression. We evaluated the association of SAA with tumor severity including advanced stages, large tumor size, lymph node invasion, high histological grades, distant metastasis and negative estrogen (ER) and progesterone (PR) receptors and negative human epidermal growth factor receptor 2 (HER-2) and elevated levels of established tumor markers cancer antigen 15-3 (CA 15-3) and carcinoembryonic antigen (CEA).

Material and methods

Patients

Serum samples from 150 Egyptian women (90 with BC, 30 with benign breast diseases and 30 healthy) were collected before any specific interventions. BC patients were age-matched with benign and healthy females. All cases were recruited from Mansoura Oncology center, Mansoura University hospital, Egypt. BC detection was radiologically, clinically, and pathologically confirmed. Clinical and pathological data were collected from all participants. Tumor-Node-Metastasis (TNM) international classification system [12] was used to classify BC patients according to different cancer features. There were no one of healthy individuals or cases with benign disorder had a history of any tumors.

Samples collection and laboratory tests

Serum samples were collected through centrifugation (4500 rpm, 15 minutes) from blood samples of all participants. In an automated closed biochemistry analyzer (Hitachi, Japan), fresh sera was used for measuring kidney (urea and creatinine) and liver (alanine and aspartate aminotransferase (ALT and AST), albumin and bilirubin)) related parameter using specific commercial kits. EDTA-K3 treated blood was used for complete blood count in an automated analyzer (Sysmex, Japan). According to the industrial prescript, both CEA (MyBioSource, San Diego, USA) and SAA (Bioneovan, Beijing, China) were detected by commercial ELISA assay kits.

Statistical analysis

Different parameters were expressed as mean±SD, absolute numbers or median (interquartile range), appropriately. *ANOVA* and *t-test* or the *Kruskal-Wallis* tests were used to evaluate differences in cases of parametric and nonparametric variables, respectively. $P<0.05$ is defined as significant. Pearson correlation was used to assess correlation between serum SAA and CEA levels. Diagnostic power of SAA was evaluated using area under the receiver operating characteristic (ROC) curve. Data statistical analyses were done using SPSS version 20 and GraphPad version 6.0.

Results

Patients' characteristics

Healthy and benign controls were age-matched to BC ($P>0.05$). Patients' clinical and hematological data are shown in Table 1. Due to the exclusion of any chronic diseases, there was no significant difference ($P>0.05$) between BC cases and controls in hematological, liver and kidney related parameters. BC patients were related to significant ($P=0.024$) elevated CA 15.3 levels (Table 1). Table 2 showed BC

classification according to the TNM staging system. It also included data about tumor size and differentiation degree and the expression of estrogen and progesterone receptors and HER2 protein.

Table 1. Clinical characteristics of breast cancer patients and controls

Variables	Breast cancer	Benign	Healthy	*P* value
Number	90	30	30	—
Mean age ±SD, years	50.5±11.9	48.0±4.3	47.8±4.4	0.494
Hemoglobin (g/dL)	11.1±1.8	11.5±2.15	11.6±1.8	0.211
RBCs ($\times 10^{12}$/L)	4.2±0.66	4.4±0.51	4.5±0.52	0.541
WBCs ($\times 10^{9}$/L)	7.6±2.8	8.1±1.9	7.7±1.8	0.132
Platelet count ($\times 10^{9}$/L)	263.4±69.1	271±65.7	280±63.7	0.233
ALT (U/L)	26.5±9.2	23.2±4.1	21.6±6.1	0.513
AST(U/L)	36.14±10.1	30.12±8.12	29.11±7.5	0.109
Bilirubin (mg/dL)	0.7±0.19	0.64±0.12	0.63±0.11	0.610
Albumin (g/dL)	3.81±0.41	3.92±0.39	4.0±0.32	0.422
Creatinine (mg/dL)	0.88±0.29	0.78±0.14	0.75±0.16	0.399
Urea (mg/dL)	26.12±5.31	24.8±5.9	23.6±4.8	0.610
CEA (U/L)	3 (1-6.5)	2 (1-4.3)	2 (1-5)	0.510
CA 15.3 (U/L)	18 (12-44.3)	20 (13-22.3)	12.5 (11-16.5)	0.024

Normally and non-normally distributed data were expressed as mean± SD and median (interquartile range), respectively. RBC: red blood cell; WBC: white blood cell; ALT: alanine aminotransferase; AST: aspartate aminotransferase; CEA: carcinoembryonic antigen. CA15.3: cancer antigen 15.3. Significant differences were determined using *ANOVA* and *Kruskal-Wallis* test, appropriately. $P<0.05$ was significant.

Table 2. Classification of breast cancer patients

Clinicopathological features	No. (%)
Primary tumor stage	
Early stage (T1–T2)	40 (44.4%)
Late stage (T3–T4)	50 (55.6%)
Lymph node invasion	
Negative (N0)	24 (26.7%)
Positive (N1)	66 (73.3%)
Metastasis	
Negative (M0)	69 (76.7%)
Positive (M1)	21 (23.3%)
Histological grade	
Low grade (G1–G2)	45 (50%)
High grade (G3)	45 (50%)
Tumor size	
Small (≤ 2 cm)	23 (25.6%)
Large (>2 cm)	67 (74.4%)
Estrogen receptor	
Negative	41 (45.6%)
Positive	49 (54.4%)
Progesterone receptor	
Negative	40 (44.4%)
Positive	50 (55.6%)
HER2	
Negative	55 (61.1%)
Positive	35 (38.9%)

Elevated SAA was associated with tumor development and severity

Compared to CEA (Figure 1A) and CA 15.3 (Figure 1B), increased serum SAA was related to BC development (Figure 1C), as BC patients (36.74 (21.1-50.2) ng/mL) were associated (P<0.0001) with greater SAA levels compared to patients with

benign diseases (14.3 (12.0-18.12) ng/mL) and healthy (5.1 (3.2-6.9) ng/mL) controls. SAA had great ability (AUC=0.960) to differentiate BC patients from all non-cancer individuals (Figure 1D).

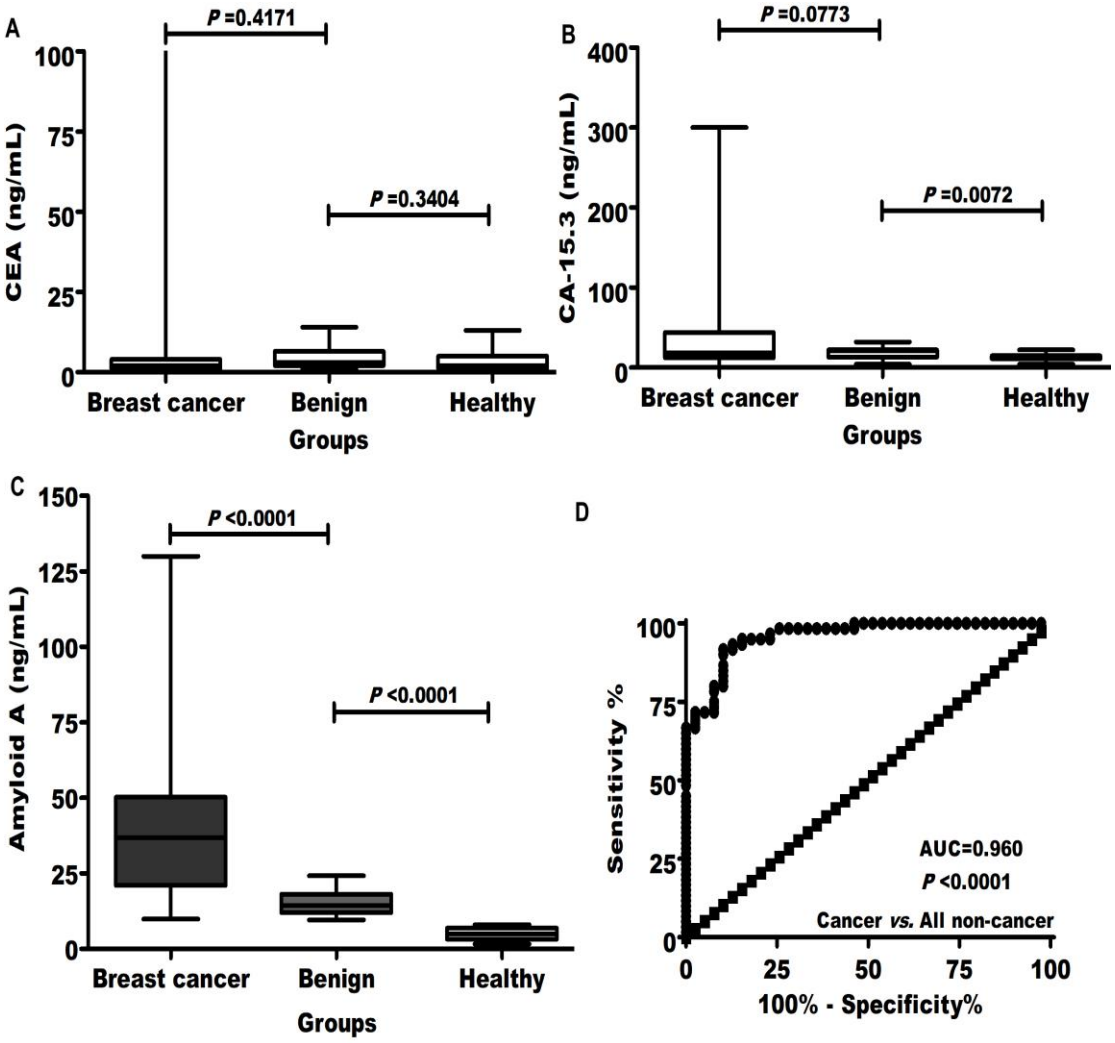

Figure 1. Serum Amyloid A in breast cancer. Compared to (A) CEA and (B) CA 15.3, elevated levels of (C) SAA were significantly related to BC cases compared to benign diseases and healthy controls. (D) ROC curve analysis revealed the great diagnostic power of SAA in differentiating BC from all non-cancer individuals (benign and healthy combined).

In BC patients, SAA levels (ng/mL) was significantly (P<0.05) affected the disease progression including tumor late stages (47.3 (37.0-90.5)), lymph node invasion (40.8 (27.5-69.23)), distant metastasis (75.0 (47.8-108.0)), high grades (78.5 (24.8-100.2)) and large size (41 (28.9-71.2)) (Table 3). Moreover, SAA high levels were associated to negative estrogen receptor and progesterone receptor status and negative HER2 (Figure 2). SAA was significantly correlated with CA 15.3 (r=0.317; P=0.0020; Figure 3A) and CEA (r=0.644; P=0.0001; Figure 3B) in BC patients.

Table 3. Impact of SAA levels on BC progression. Data were expressed median (inter quartile range).

Categories	**Amyloid A (ng/mL)**	***P* value**
Primary tumor stage		
Early stage (T1-T2)	23.9 (19.8-40.7)	0.0001
Late stage (T3-T4)	47.3 (37.0-90.5)	
Lymph node invasion		
Negative (N0)	20.2 (18.4-28.1)	0.0001
Present (N1)	40.8 (27.5-69.23)	
Metastasis		
Negative (M0)	27.3 (20.0-41.0)	0.0001
Present (M1)	75.0 (47.8-108.0)	
Tumor histological grade		
Low grade (G1-G2)	30.2 (20.0-40.7)	0.0008
High grade (G3)	78.5 (24.8-100.2)	
Tumor size		
Small (≤ 2 cm)	21.3 (19.3-28.24)	0.0002
Large (>2 cm)	41 (28.9-71.2)	

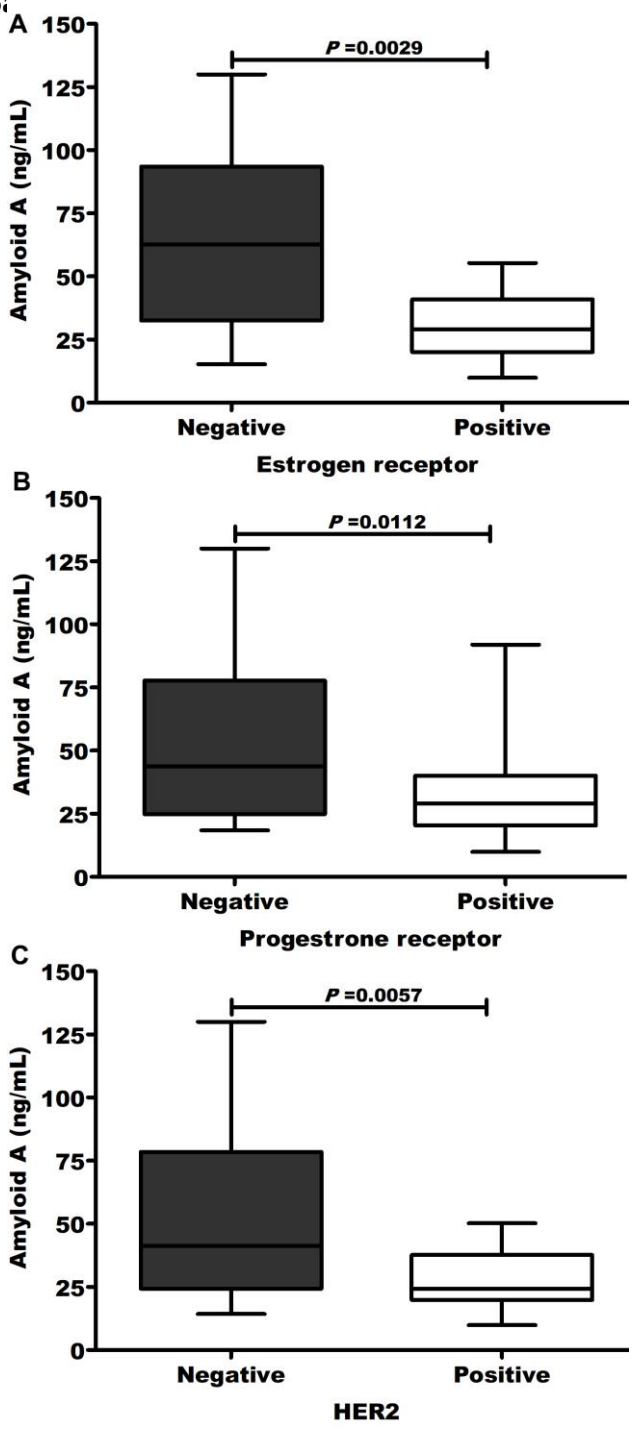

Figure 2. Serum amyloid A and BC progression according to (A) estrogen and (B) progesterone receptors status and (C) HER2 expression. Significant difference was determined using *Kruskal-Wallis* test. $P<0.05$ was significant.

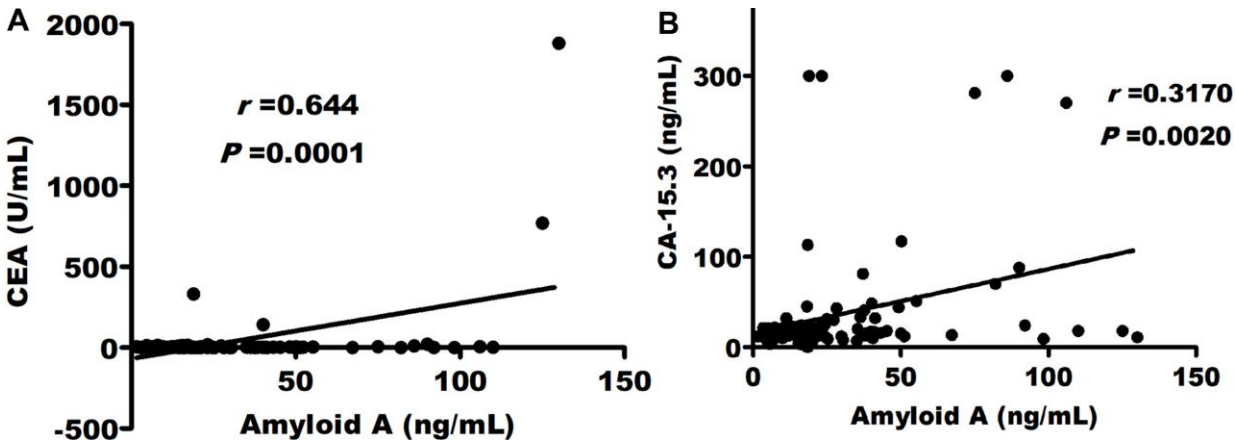

Figure 3. Correlation between serum amyloid A and (A) CEA and (B) CA 15.3 serum levels in breast cancer patients.

Discussion

Worldwide, BC stills a considerable health challenge, necessitating the evaluation and searching of reliable biomarkers for accurate prognosis, early diagnosis, and targeted treatment [13]. Moreover, BC prognostic biomarkers are very important for early tumor detection and aiming in anticipating illness trajectory and enhancing the therapy options [14]. So, it is necessary to evaluate novel biomarkers, particularly blood markers, to detect the disease at an early stage and also to evaluate their relation with cancer aggressiveness to prevent poor progression of the disease [15]. As a reliable blood biomarker, this study revealed serum SAA levels potential role in BC early detection compare to benign breast diseases and its role in prediction of poor BC progression.

In contrast to other established BC tumor markers such as CEA and CA 15.3, our results showed that elevated SAA (ng/mL) was significantly (P<0.0001) related to BC (36.74 (21.1-50.2)) development compared to patients with benign diseases (14.3 (12.0-18.12)) and healthy (5.1 (3.2-6.9)) controls. It had great power (AUC=0.960) to

differentiate BC patients. Moreover, elevate SAA levels significantly (P<0.05) related to tumor aggressiveness including tumor late stages (47.3 (37.0-90.5)), lymph node invasion (40.8 (27.5-69.23)), distant metastasis (75.0 (47.8-108.0)), high grades (78.5 (24.8-100.2)) and large size (41 (28.9-71.2)). SAA high levels were associated to BCs with no estrogen or progesterone receptors or HER2 protein. Also, SAA was significantly correlated with CA 15.3 (r=0.317; P=0.0020) and CEA (r=0.644; P=0.0001) in BC patients.

SAA can function as an endogenous damage associated molecular patterns by binding to pattern recognition receptors like toll-like receptors on both cancer associated fibroblasts (CAFs) and BC cells [16]. SAA can so promote pro-inflammatory cytokines production including IL-1β, chemokines CCL1, CCL3 and CCL4 production and tumor necrosis factor-α (TNFα), thereby producing favourable inflammatory environment to support BC growth [16-18]. Previous reports suggested that SAA could considerably affect carcinogenesis by inducing the expression of matrix metalloproteinases (MMPs) [19, 20] and activating the transcriptional factor nuclear factor kappa-B (NFκB) [21-23]. These genes activation could suppress cancer cells apoptosis [24].

Yang et al. found that SAA protein in many BC patients was expressed in tumor-associated macrophage (TAM) and tumor cells [25]. Also similar to our results, SAA protein immunoreactivities and positivity in tumor cells and TAM were both related to lymph node metastasis, high histological-grade, lymphovascular invasion, large tumor size, negative progesterone- and estrogen-receptor statuses, and HER2 overexpression. Moreover in a multivariable regression model, SAA was associated with worse recurrence-free survival [25]. Recently in both *in vitro* and *in vivo* BC models, du Plessis e al. found that SAA overexpression activated modulators of

autophagy (such as PI3K/Akt and MAPK signaling pathways) [11]. Its overexpression in BC cells caused an increase in cell viability and increased proliferation marker expression. Also, SAA *in vivo* knockout caused in an altered inflammatory profile and promoted resistance to necrosis and apoptosis through autophagy regulation. SAA promote BC tumorigenesis via autophagy regulation in BC cells [11]. In the same line, Niu et al. reported that SAA induces suppressive neutrophils via the Toll-like receptor 2–mediated signaling pathway to stimulate BC progression [26].

Conclusions

Our results suggested that SAA may has a pivotal role in BC development and progression. It had a promising power in the early differentiating of BC from benign breast diseases. Its elevated levels were associated with BC severity and, therefore, it is a biomarker candidate for BC surveillance and perhaps a therapeutic BC target. Some study limitations are noteworthy. The included participants were collected from only one single center and also the small number of healthy and benign controls may affect the statistical analysis of the study. Thus, larger studies with multi-centers are required to validate our findings.

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Disclosure statement

None

Funding

None

Data availability

Data is available via request the corresponding author

Ethics approval

The study protocol was approved by the ethics and scientific committees of Faculty of Medicine, Port Said University and was in accordance with the ethical guidelines of the “Helsinki Declaration”. Informed consent was get from each participant to involve in this work.

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