Diagnostic role of collagen III in the diagnosis of breast cancer in Egyptian women

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ABSTRACT

Mammography, magnetic resonance imaging (MRI) and ultrasound are useful tools that can be used in breast cancer (BC) screening but these methods have various limitations. Thus, finding diagnostic biomarkers may be a useful alternative choice. A total of 142 females with breast diseases (40 females with benign conditions and 102 females with BC) were included in the study. In addition to age- and sex-matched 29 normal females as a negative control. The level of collagen III was detected using the ELISA technique. It was (21.29±3.14 µg/ml) in sera of females with BC higher than in benign (13.91±2.65 µg/ml) and in normal (5.69±0.43 µg/ml) females. Collagen III yielded an AUC of 0.86 to differentiate BC from normal females and an AUC of 0.62 to differentiate BC females from those with benign conditions. Moreover, AUC was risen to 0.72 to differentiate females with BC from those without BC. While the index provided values that were significantly ($P<0.01$) higher in BC (9.1±1.3) than benign (6.3±1.1) and normal (2.5±1.1) females. It yielded an AUC of 0.98 (sensitivity = 90.0 %) to differentiate BC from normal females and an AUC of 0.84 to differentiate BC females from those with benign conditions. Moreover, AUC was risen to 0.87 to differentiate females with BC from those without BC (sensitivity = 84.0 %). In conclusion, serum collagen III concentration represents a candidate biomarker for BC. Moreover, its combination with cancer antigen 15-3 (CA 15.3) and age provided a valuable index for BC diagnosis with high accuracy.

INTRODUCTION

Breast cancer is one of the fatal cancers that threaten the lives of women in the world. It is the most frequent cancer among females and the most frequent cause of cancer-associated death comprising 24.2% of total cancer new cases and 15% of all cancer death (**Bray et al. 2018**). The incidence rate increases day by day, therefore, research ongoing on the development of a superior method for early diagnosis of BC is a must to overcome this serious problem and to reduce mortality (**Joshi, 2019; Henriksen et al. 2019**).

Breast cancer screening method including mammography, clinical breast examination, MRI, and ultrasonography provide potential outcomes in BC diagnosis, but these methods improperly utilized (**Weier et al. 2015**), and also have various limitations including the mammography inaccurate outcomes in case of high breast density females (**Shao et al. 2013; Zhao et al. 2015**), the risk of ionizing radiation accompanied its use when repeated more than one time yearly. In addition, the MRI high cost, the long holding up list in developing countries and the inaccurate result of imaging techniques (**Kuhl, 2019**). So, finding diagnostic biomarkers may be a useful alternative choice (**Tan et al. 2018).**

On the other hand, using serum biomarkers may be favorable and more comfortable for patients, easily repeated and may also provide better outcomes. However, CEA, CA15-3, CA27.29, tissue polypeptide specific antigen and tissue polypeptide antigen have been suggested as diagnostic markers for BC, they have limited sensitivity and specificity for early disease (**Lumachi and Basso, 2004; Menon et al. 2015; Kazarian et al. 2017).**

Interestingly, the extracellular matrix (ECM) is considered one of the most important controllers in breast cancer. ECM proteins especially collagens play a major role in regulating breast health. Additionally, they undergo various changes in composition and organization in tumor progression, invasion, and metastasis (**Angel et al. 2019**). Therefore, the present study aimed to detect collagen III in the serum of women with BC. Then, evaluate its ability to be used as a candidate marker and in combination with other established BC markers (CA 15.3) and age for BC diagnosis.

METHODS

Sample collections and laboratory assays

In the present study, blood samples from 142 females with breast diseases, collected from the Oncology center, Mansoura University Hospitals, Mansoura, Egypt, were classified into 40 females with benign conditions and 102 females with BC. In addition, serum samples from age- and sex-matched 29 normal females were included in the study as a negative control group. None of the women with benign conditions or the normal women had a history of any malignancy. The study was commenced after obtaining approval from the university ethical and scientific committees of the Mansoura University Hospitals, Mansoura, Egypt.

Blood samples from all subjects were collected 3 days after diagnosis and before starting any cancer-specific treatment. Complete blood count (CBC) was performed on the blood portion which previously kept in K3- ethylenediamine tetraacetic acid (EDTA-K3) anti-coagulant by using automated hematology-analyzer. The last portion of blood was left to clot and serum was separated by centrifugation at $1,000 \times g$ for 10 min. Liver and kidney function tests were all measured on fresh serum on an automated biochemistry analyzer. The level of collagen III was detected using the ELISA technique according to Attallah et al protocols (**Attallah et al. 2007**).

Statistical analysis

Using statistical software package "SPSS 15.0, SPSS Inc.", all statistical analyses were done. Descriptive results were expressed as mean ± SD. Differences in

continuous variables were assessed using analysis of variance (ANOVA). All tests were two-tailed and statistical significance assessed at the 0.05 level. Receiver-operating characteristic (ROC) curves were used to assess and compare the diagnostic accuracy of single and combined biomarkers for BC identification. Stepwise linear regression analysis was used to develop a novel index for BC diagnosis. The diagnostic sensitivity and specificity values were calculated from a 2×2 contingency table.

RESULTS

Patient's characteristics

Laboratory tests including liver, kidney functions, and hematology tests were carried out on normal women and women with breast diseases. As shown in (Table 1). It was found that there was no significant difference for ALT, hemoglobin, RBCs count, HCT, platelet count, WBCs and lymphocytes count ($P > 0.05$), but there was significant difference for AST activity ($P < 0.05$), albumin level ($P < 0.001$), level of creatinine ($P < 0.01$) and neutrophil ($P < 0.01$) among the three groups.

Table 1: Patients' characteristics

Variables	Normal n=29	Benign disorder n=40	Breast cancer n=102	*P value*
Age (Years)	46.2±15.6	47.4±11.2	50.2±11.9	> 0.05
ALT (U/L)	28.7±6.9	33.4±9.2	37.2±10.4	> 0.05
AST (U/L)	22.6±7.3	23.7±7.6	25.4±6.4	< 0.05
Albumin (g/dL)	4.3±0.5	4.1±0.4	3.8±0.7	< 0.001
Creatinine (mg/dL)	0.60±0.07	0.61±0.16	0.68±0.15	< 0.01
Hb (g/dL)	12.3±0.9	12.4±1.3	12.6±1.8	> 0.05
RBCs (10^6 cells/mL)	4.5±0.4	4.6±0.4	4.7±0.5	> 0.05
HCT (%)	35.7±2.7	37.1±3.7	38.1±4.6	> 0.05
WBCs (10^6 cells/mL)	7.5±1.7	8.6±3.0	9.0±3.3	> 0.05
Lymphocytes (%)	34.5±7.7	31.8±10.8	29.3±12.7	> 0.05
Neutrophil (%)	53.0±11.0	60.5±12.7	62.8±13.8	< 0.01
Platelets (10^9 cells/L)	245.5±45.8	239.0±66.2	229.8±73.8	> 0.05

Determination of collagen III in women with breast diseases

The level of collagen III was quantified in serum samples of females with breast diseases and normal control using ELISA. It was found that the serum collagen III level was (21.29±3.14 µg/ml) in serum samples of females with BC higher than in samples of females with benign breast conditions (13.91±2.65 µg/ml). While it was (5.69±0.43 µg/ml) in samples of normal females (Figure 1A).

Diagnostic performance of collagen III for BC identification

Using ROC curves, we assessed the diagnostic accuracy of serum collagen III antigen to differentiate between females with BC and those normal females. It yielded an area under ROC curve of 0.86 with sensitivity = 80.4 % and specificity = 93.1 % (Figure 1B). In addition, collagen III yielded an AUC of 0.62 and $P < 0.05$ to differentiate BC females from those with benign conditions with sensitivity = 80.4 % and specificity = 60.0 %. Moreover, AUC was risen to 0.72 ($P < 0.05$) to differentiate females with BC from those without BC with sensitivity = 80.4 % and specificity = 73.9 % (Figures 1C-D).

Development and diagnostic performance of BC diagnostic index

The AUC was calculated for providing the accuracy of candidate markers for BC identification. The AUC of these candidate markers is in the order collagen III= 0.86> CA15.3=0.81 > age=0.69. Then we developed a novel index for BC identification, it can be represented as Index= 0.02×CA15.3+0.09×age+0.07×collagen+3.2 as consequence, the diagnostic index provided values that were significantly ($P<0.01$) higher in BC (9.1±1.3) than benign (6.3±1.1) and normal (2.5±1.1) women (Figure 2A).

Using ROC curves, we assessed the diagnostic accuracy of the index to differentiate between females with BC and those normal females. It yielded an area under ROC curve of 0.98 with sensitivity = 90.0 % and specificity = 96.0 % (Figure 2B). In addition, the index yielded an AUC of 0.84 to differentiate BC females from those with benign conditions with sensitivity = 86.0 % and specificity = 79.0 %, **Figure 2C**. Moreover, AUC was risen to 0.87 to differentiate females with BC from those without BC with sensitivity = 84.0 % and specificity = 80.0 % (Figure 2D).
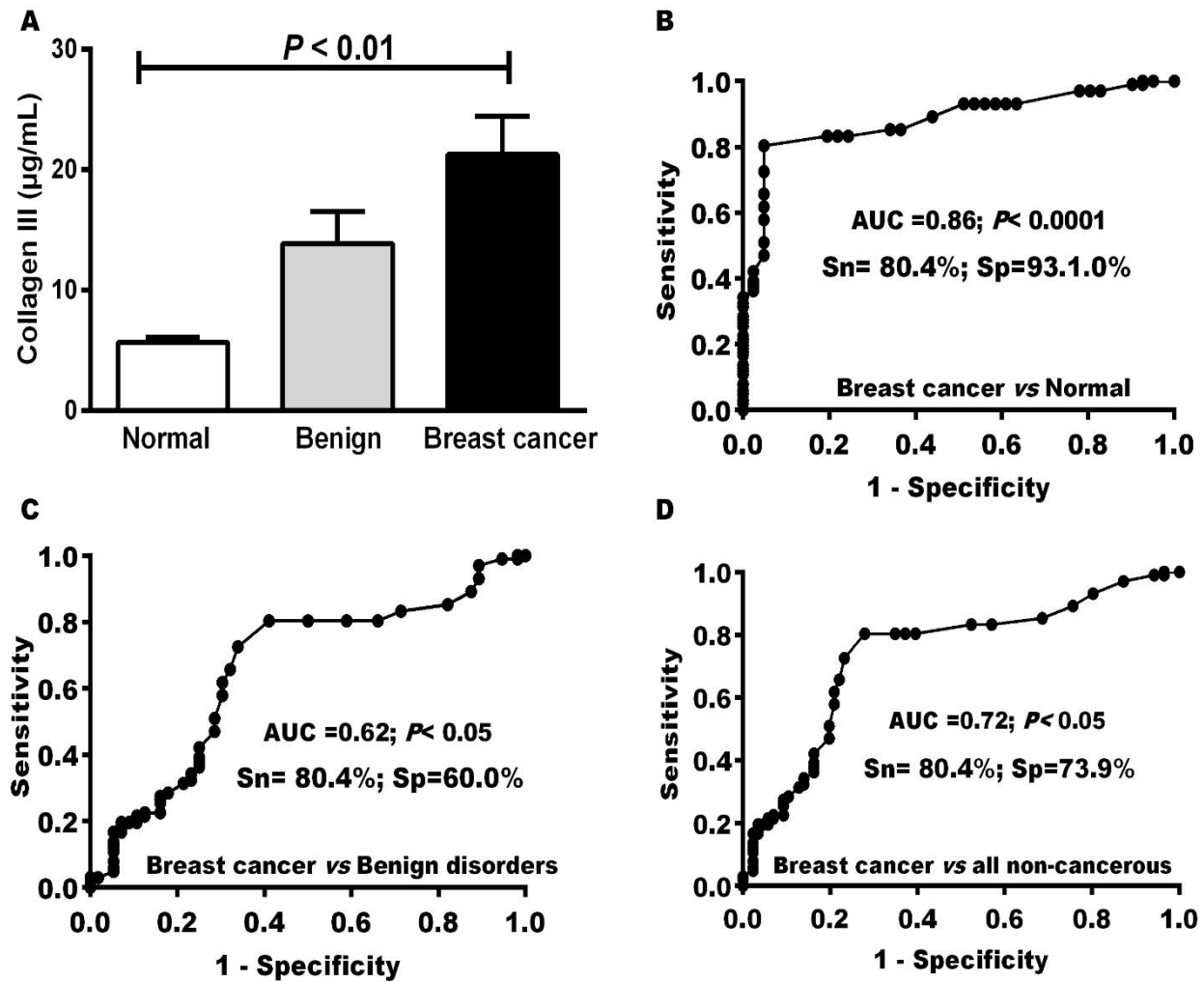
Figure 1: Diagnostic performance and distribution of collagen III. A: collagen III level among three group of subjects. Receiver operating characteristic curve of collagen III for distinguishing between breast cancer women and B: normal control, C: women with benign disorders and D: all non-cancerous women.
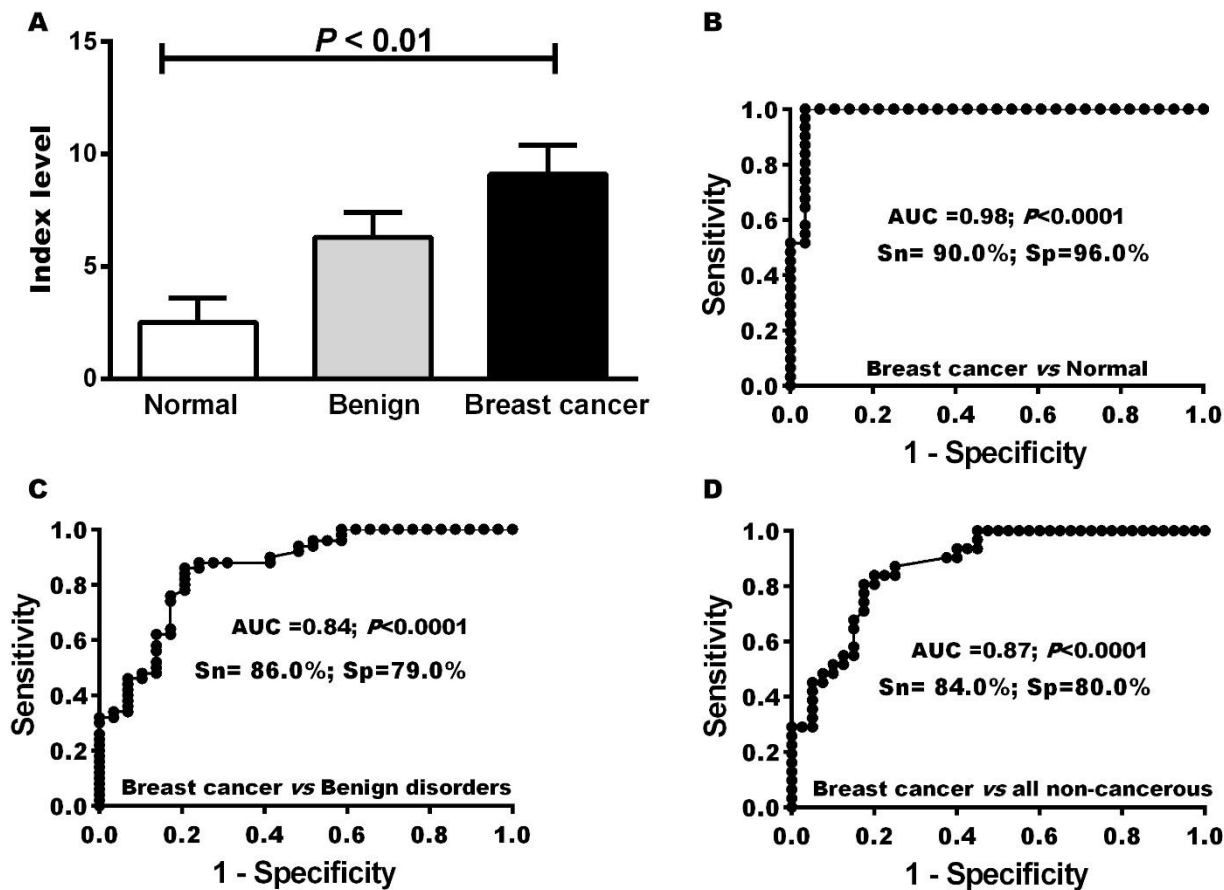
Figure 2: Diagnostic performance and distribution of Index. A: level of Index in the three group of subjects. Receiver operating characteristic curve of Index for distinguishing between breast cancer women and B: normal control, C: women with benign disorders and D: all non-cancerous women.

Index= $0.02 \times CA15.3 + 0.09 \times age + 0.07 \times collagen + 3.2$.

DISCUSSION

Breast cancer is commonly related to extraordinary modifications of ECM structure, function, and composition (**Xiong and Xu, 2016**). It is also associated with the increased breast density and the over deposition of collagens such as collagen I, II, and III which involved in tumor progression and invasion as it involved in altering tumor stroma structure through series of enzymatic reaction that results in increasing its thickness, stiffness, crosslinking, degradation, redeposition and accordingly altered ECM remodeling and facilitate tumor progression (**Fang et al. 2014**). Collagen III is one of the main fibrillar structural proteins of ECM which comprises 10-15% of skin collagen (**Riekki et al. 2004; Mazurek et al. 2018**). Mutation in this collagen involved in several malignancies and it can be used as a biomarker (**Nielsen and Karsdal, 2016**).

In this study, we found that there was a high significant increase ($P < 0.01$) in serum collagen III levels in BC women than in women with a benign condition ($P < 0.01$) and normal control. These results were in agreement with **Bager et al. (2015)** who found that there was a high significant increase in serum level of collagen type I, III and IV in BC patients ($P < 0.04$–0.0001) compared to healthy controls. **Rizwan et al. (2015)** also found that there was up-regulation in the gene expression of collagen III alpha 1($P = 0.00206$). These findings are in accordance with previous reports which found elevated levels of type III collagen in the tissue of BC patients **(Kauppila et al. 1998).**

In breast cancer, several matrix metalloproteases expression are significantly increased that correlated with lysyl oxidase expression which increases the ECM stiffness as it has a collagen crosslinking activity and induces stromal collagen to secrete more matrix metalloproteinase which degrades collagen and promote tumor progression **(Motrescu et al. 2008; Erler et al. 2009)**

In this study, AUC of collagen III to differentiate women with BC from those normal women was 0.86 with high sensitivity and specificity (80.4% and 93.1%; respectively). Also, it yielded high sensitivity and specificity to differentiate BC women from those women without BC (AUC=0.72, sensitivity = 80.4% and specificity = 73.9%) with significant difference ($P < 0.05$). Moreover, collagen III still had a good diagnostic performance to differentiate women with BC from those women with benign conditions (AUC=0.62, yield sensitivity = 80.4 % and specificity = 60.0 %) with significant difference ($P < 0.05$).

While the AUC of the index to differentiate women with BC from those normal women was 0.98 with high sensitivity and specificity (90.0% and 96.0%; respectively). Also, it yielded high sensitivity and specificity to differentiate BC women from those women without BC (AUC=0.87, sensitivity = 84.0%

and specificity = 80.0%) with significant difference ($P < 0.0001$). Moreover, the index still had a good diagnostic performance to differentiate women with BC from those women with benign conditions (AUC=0.84, yield sensitivity = 86.0 % and specificity = 79.0 %) with significant difference ($P < 0.0001$).

Collagen III provided a diagnostic performance superior to other BC markers, such as CEA, CA 15.3, CA125, CA 19-9, CA 27.29, total and free prostate-specific antigen which they yielded sensitivities (18%, 31.9%, 29.8%, 14% **(Zaleski et al. 2018), 62% (De Paterna et al. 1995)**, 57% and 36%; respectively) **(Black et al. 2000)**. Furthermore, it is superior to CEA, CA 15.3, CA125, CA 19-9, total and free prostate-specific antigen in distinguishing women with BC from women with benign conditions which they yielded sensitivities (20%, 23.4%, 19.1%, 6% **(Zaleski et al. 2018),** 57% and 36%; respectively). However, free the prostate specific-antigen is more specific in distinguishing women with BC from women with benign conditions than total prostate specific-antigen and collagen III Where the specificities were 72%, 25%, and 60%; respectively **(Black et al. 2000).**

The ROC curve also showed diagnostic performance of the epidermal growth factor to differentiate patients with BC from those without BC superior to collagen III with AUC= 0.89 and sensitivity 94.0% but collagen III is more specific than the epidermal growth factor which they yielded specificities (73.9% and 70.6%, respectively) **(Kim et al. 2009)**.

Concerning the diagnostic performance of the index, it provided AUC superior to the combination of CEA with CA15-3 and the combination of CEA or CA15-3 with miR-34a to differentiate women with BC from those normal women which they yielded AUC of 0.74, 0.84 and 0.80; respectively and the sensitivities were (38.5, 34.1 and 56.1; respectively). Moreover, it is superior to the combination of CEA with miR-34a and

the combination of CA15-3 with miR-34a   to differentiate women with BC from those women with benign conditions which they yielded sensitivities (31.8 and 53.7; respectively) **(Zaleski et al. 2018).**

Furthermore, the index provided AUC superior to the combination of collagen III, nuclear matrix protein-52 and matrix metalloproteinase-1to differentiate women with BC from those with benign conditions which they yielded AUC of 0.73 and the sensitivity of 78%. However, the AUC of the index is slightly lower than the AUC of this combination (AUC, 0.99) in distinguishing women with BC from normal women **(Attallah et al. 2019).**

Moreover, the index diagnostic performance is lower than the diagnostic performance of the combination of vascular endothelial growth factor, tissue inhibitors of metalloproteinase-2 and CA 15-3 which they yielded a sensitivity of 93% **(Ławicki et al. 2017).**

CONCLUSION

In conclusion, serum collagen III concentration represents a candidate biomarker for BC, which was detected using ELISA, in higher concentration in BC females compared to normal and females with benign breast conditions. It had also a potential role in detecting BC with high sensitivity and specificity. Moreover, the combination of collagen III with other established BC marker (CA 15.3) and age provided a valuable index for BC diagnosis with high accuracy.

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