Value of Serum MACC-1 as a New Diagnostic Biomarker in Breast Cancer Patients

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Abstract

Breast cancer (BC) is the leading cause of cancer-related death among females. The current study is one of the initial studies to evaluate the diagnostic value of novel serum metastasis-associated in colon cancer-1 (MACC-1) markers in BC patients. Various other serum biomarkers have been used for the diagnosis of BC but this marker proved a good sensitivity and/or specificity. This cross-sectional study included 48 BC patients (Group-I) and 48 normal healthy controls (Group-II). Study subjects demographic, pathologic, and clinical information were recorded. Random blood samples were collected from the antecubital vein after aseptic precaution with 0.5% chlorhexidine gluconate. About 4.0 mL of venous blood was collected into a red screw-capped tube. Serum MACC-1 was assessed by a double-antibody sandwich ELISA method by using the commercially available kit in the Department of Laboratory Medicine, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh. In this study, serum MACC-1 levels were significantly elevated ($P<0.0001$) in BC patients compared to the control group. The mean serum MACC-1 was elevated in BC patients (55.21 ± 14.75 pg/mL) compared with healthy controls (38.19 ± 11.42 pg/mL) ($P<0.0001$). Also, the receiver operating characteristics (ROC) analysis showed an area under the curve (AUC) of 0.98, signifying a good discriminatory ability of serum MACC-1 marker for BC patients. At the cut-off value of optimal cut-off value (38.35 pg/mL), the sensitivity and the specificity of serum MACC-1 were 96.7% and 92.5%, respectively. The median age was 46.3 years in BC patients and 40.2 ± 8.4 years in the control group ($P<0.0001$). Therefore, this study showed that serum MACC-1 can be a potential biomarker for the diagnosis of BC.

Keywords: Serum MACC-1, New Biomarker, Breast Cancer, ROC, AUC

Introduction

Breast cancer (BC) is the leading cause of cancer-related death among females (Bray *et al.*, 2018). It is the most common cancer type among females worldwide affecting 1 in 8 women (Shamsi, 2021). As of 2015, BC is still a leading cancer of women in Bangladesh. It has become a hidden burden which accounts for 69% deaths of women within the country. The rate grows up day by day due to unawareness of the people, lack of confidence about

medical treatment, improper screening, maltreatment, and lack of motivation to go for institutional treatment and management (Shamsi. 2021). Although the outcome of the localized BC patients has improved over time, the survival of patients with metastatic disease (stage IV) remains dismal with a five-year survival rate of only 27% (Smith *et al.*, 2016; Desantis *et al.*, 2019). However, in the developing counties the survival of patients with BC is low, owing to the advanced stage at

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presentations (Fan *et al.*, 2014). The outcome of the BC patients can be greatly improved by early detection of the disease coupled with effective treatment (WHO, 2014). There are various biomarkers to screen, diagnose or predict the outcome for BC. Many identified biomarkers such as cancer antigen 19-9 (CA19-9), carcino-embryonic antigen (CEA), and CA125, CA15-3 and CA27, CA29, P53, Ki67 have little clinical value due to low sensitivity, specificity and reproducibility (Harris *et al.*, 2007). Therefore, novel biomarkers are urgently needed to detect early stage BC (Weige *et al.*, 2016).

Metastasis associated in colon cancer-1 (MACC-1) is a newly identified tumor marker, first identified in colon cancer tissue as a prognostic indicator and inducer of metastasis (Stein *et al.*, 2009). This gene is located on human chromosome 7p21q and regulates hepatocyte growth factor HGF-MNET pathway-a key part in cellular growth, angiogenesis, invasiveness and metastasis (Mueindlin *et al.*, 2014). It is also found to express in other normal and cancerous tissue of gastrointestinal tract, pancreas, ovary, breast, pituitary gland, kidney, lung, bone marrow etc. (Stein *et al.*, 2009). A previous study associated MACC-1 polymorphisms with HER2-positive BC patients suggesting that MACC-1 is a potential BC biomarker (Mueindlin *et al.*, 2014). The expression of MACC-1 in BC and its correlation with outcomes is little known (Ahmed and Aslam, 2020). With the aim, this study was conducted to evaluate the serum MACC-1 level as a new diagnostic marker of BC.

Materials and Methods

This was a cross sectional study. The serum metastasis associated in colon cancer-1 (MACC-1) was assessed as a new diagnostic biomarker for breast cancer (BC) patients and evaluated the sensitivity, specificity, and validity of MACC-1 for BC patients. The study was conducted from July 2022 to June 2023 at the Department of Laboratory Medicine, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh. Potential study participants were any adult (≥18 years) patients who were histopathologically diagnosed cases of BC. Purposive sampling methods were used as per inclusion-exclusion criteria. Inclusion criteria: age ≥18 years. Ninty six patients who had their clinical assessment, radiological investigations and

histopathologically diagnosed cases of BC (stage 0 to III) from the tumor were enrolled. The control subjects were matched with the study group who had no disease at the time of study or in the past. Exclusion criteria: age ≤18 years, did not give written consent, patients who received neoadjuvant chemotherapy before surgery, recurrent BC.

The blood samples of MACC-1 were taken from the study and the control groups before starting any surgery or chemotherapy. Then study subjects were divided into group I (BC patients) and group II (Healthy control). Venous blood (4.0 mL) were collected with aseptic precaution. Blood were collected with no anticoagulant for collecting serum in red tubes for MACC-1 level. Tubes were kept standing for 30 minutes. Then blood was centrifuged at 3000 rpm for 5 minutes and supernatant serum were separated into an Eppendorf tube with the help of a micropipette for storage and analysis. Separated serum were stored at $-20\,°C$ until analysis were done and for further use. Serum MACC-1 were assessed by ELISA method with the commercially available kit in the Department of Laboratory Medicine, BSMMU. A double-antibody sandwich ELISA was conducted to detect serum MACC-1 according to the manufacturer's protocol. Cut-off points of serum MACC-1 level was 38.25 pg/mL.

Results and Discussions

This study is one of the initial studies to evaluate the diagnostic value of novel serum MACC-1 markers in BC patients. The study included 48 breast cancer (BC) patients (Group-I) and 48 normal healthy controls (Group-II). Study subjects' demographic, pathologic, and clinical information is provided in Table 1. Table 1 showed patients mean age 46.7±10.6 years and 40.2±8.4 years in control group ($P<0.0001$). A study found the patient's mean age was 48.3 years in BC patients and 41.7 years in healthy subjects which were nearly consistent with this study (Weige *et al.*, 2016). Ahmed and Aslam (2000) found in their study that all the patients were females with the mean age of 46.7±10.6 years and 40.2±8.4 years in the control group ($P=0.0001$) which was exactly similar to this study.

Table 1. Age distribution of the study subjects (N=96)

Age group (years)	Group-I (n=48)	Group-II (n=48)	*P* value
<20	02 (4.16%)	15 (31.25%)	<0.001[s]
21–30	20 (41.66%)	16 (33.33%)	
31–40	10 (20.83%)	14 (29.16%)	
41–50	08 (16.66%)	02 (4.16%)	
51–60	05 (10.41%)	01 (2.08%)	
>60	03 (06.25%)	00 (0.0%)	
Mean±SD	46.7±10.6	40.2±8.4	
Range	(20−61) years	(22−58) years	

Table 2 depicted the distribution of study subjects according to histopathological grading in BC patients (Group-I) (n=48). Distribution for the Grade I was 9 (18.75%), Grade-II was 33(68.75%) and Grade-III was 6 (12.5%), respectively. The comparison of the serum MACC-1 value on histopathological grading of BC (Group-I) (n=48) was provided in Table 3. Serum concentrations of MACC-1 among the study subjects according to histopathological grading, 51.06±3.24 pg/mL were found in Grade-I, 53.12±5.65 pg/mL in Grade-II and 55.07±7.29 pg/mL in Grade-III respectively (Table 3). Compared to the healthy control, serum MACC-1 were found elevated in BC patients with increasing trend in grade I, II or III. (Table 3). Serum MACC-1 on ROC analysis, the AUC was 0.98 ($P \leq 0.0001$; 95% CI=0.97–1.0). At the cut-off value of 38.35 pg/mL, the sensitivity and the specificity of serum MACC-1 were found 96.7% and 92.5%, respectively (Figure 1). The mean serum MACC-1 was elevated in BC patients (55.21±14.75 pg/mL) compared to healthy controls (38.19±11.42 pg/mL) ($P<0.0001$, Table 4).

This study showed most BC patients belongs to Grade II which was 68.75%. Ahmed and Aslam in 2000 also found maximum number of patients in Grade II tumor (61.7%). Current study also showed the mean serum MACC-1 was elevated in BC patients (55.21±14.75 pg/mL) compared to healthy controls (38.19±11.42 pg/mL) ($P<0.0001$, Table 4). Another study found the mean serum MACC-1

level in BC patients was 3.46±1.3 ng/mL which was significantly higher than control mean serum MACC-1 level (1.90±0.2 ng/mL) ($P<0.0001$) (Ahmed and Aslam, 2020).

Table 2. Distribution of the study subjects according to histopathological grading in BC patients (Group-I) (n=48)

Histopathological grading	Frequency	Percent	*P* value
Grade I	9	18.75%	<0.001[s]
Grade II	33	68.75%	
Grade III	6	12.5%	
Total	48	100 %	

Compared to the healthy control, serum MACC-1 was elevated in BC patients of grade (I, II or III) (Table 3). Higher serum MACC1 levels were also observed with increasing tumor grade ($P=0.007$) in a different study (Ahmed and Aslam, 2020).

Table 3. Comparison of the serum MACC-1 value on histopathological grading of BC (Group-I) (n=48)

Variables	Grade I	Grade II	Grade III	*P* value
Serum MACC-1 (pg/mL)	51.06±3.24	53.12±5.65	55.07±7.29	<0.02[s]

Table 4. Comparison of the serum MACC-1 value between BC and control group (N=96)

Variables	Group I (n=48)	Group II (n=48)	*P* value
Serum MACC-1 (pg/mL)	55.21±14.75	38.19±11.42	<0.0001[s]

Furthermore, serum MACC-1 on ROC analysis, the AUC was 0.98 ($P \leq 0.0001$; 95% CI=0.97–1.0) *i.e.*, a good predictor for BC. At the cut-off value of 38.35 pg/mL, the sensitivity and the specificity of serum MACC-1 were 96.7% and 92.5%, respectively. This study showed that serum MACC-1 can be a potential biomarker for diagnosis and tumor progression in patients with BC. In a similar study, the ROC analysis showed an AUC of 0.98, signifying a good discriminatory ability of serum MACC-1 markers for breast cancer.
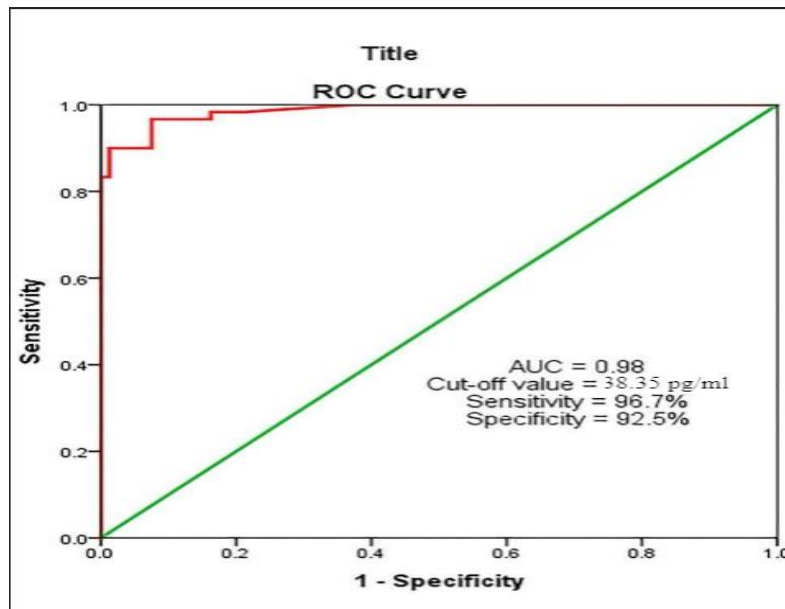
Figure 1. Receiver-Operating Curve analysis for serum MACC-1 between breast cancer patients and healthy control groups (N=96)

At the cut-of value of 2.12 ng/mL and this marker has a high sensitivity and specificity (96.7% and 92.5%, respectively) compared to our study (Ahmed and Aslam, 2020). Weige *et al.*, 2016 showed serum MACC-1 as a diagnostic marker with a lower sensitivity and specificity (71.4% and 89.1%, respectively) in BC patients. Our study demonstrated that serum MACC1 levels were elevated in BC cancer patients compared to control groups, suggesting that MACC1 might act as a useful serum biomarker for distinguishing breast cancer patients and healthy controls.

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Conclusions

This study showed that serum MACC-1 can be a potential biomarker for diagnosis in patients with

breast cancer. Being the least invasive and easily detectable, serum MACC-1 can replace other biomarkers that require tissue samples. Further prospective studies are warranted to validate our findings. Our study demonstrated that serum MACC1 levels were elevated in breast cancer patients compared with control groups, suggesting that MACC1 might act as a useful serum biomarker for distinguishing breast cancer patients from healthy controls.

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Declaration

The author declares that this research findings reported in this article do not have any conflicting interest.

Author's Contribution

MSY is solely responsible for the concept and design, data analysis and interpretation, manuscript drafting, critical revision for essential intellectual content, and final manuscript approval.

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