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Evaluation of Serum Mammaglobin as an Alternative Biomarker in the Diagnosis of Breast Tumors

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Abstract

Introduction Breast cancer is the most common cancer in women in India and accounts for 14% of all cancers in women. Rise in mortality is due to lack of awareness and proper screening. Mammography and presently available serum biomarkers have low sensitivity and specificity. In our quest to identify a better biomarker, we studied mammaglobin (MAM) in patients with breast cancer and benign breast tumors.

Aim To evaluate serum mammaglobin in breast cancer patients and compare it with benign breast tumor patients and healthy controls. To compare it with existing biomarkers serum carcinoembryonic antigen (CEA) and cancer antigen 15–3 (CA 15–3). Materials and methods: This is a cross-sectional, case–control study of 77 subjects, of which 27 were breast cancer patients, 20 benign breast tumor patients, and 30 healthy controls. Serum CEA and CA15–3 were estimated by electrochemiluminescence immunoassay (ECLIA) and mammaglobin (MAM) by enzyme-linked immunosorbent assay (ELISA).

Results Mammaglobin and CEA levels were elevated in breast cancer patients, followed by benign breast tumors when compared with controls ($P < 0.000001$). Mammaglobin showed 81.5% sensitivity, 100% specificity, 100% positive predictive value (PPV), and 88.9% negative predictive value (NPV). CEA showed 88.9% sensitivity, 82.5% specificity, 77.4% PPV, and 91.7% NPV. The area under the curve was the highest for MAM (0.892), followed by CEA (0.889) and CA 15–3 (0.555). CA15–3 showed poor diagnostic efficacy. Combined receiver operating characteristic (ROC) curve of the biomarkers MAM and CEA had an AUC of 0.913.

Conclusion Mammaglobin proved to be an efficacious biomarker in diagnosing breast cancer.

Keywords
- breast cancer
- breast tumor
- CA 15–3
- CEA
- MAM
- mammaglobin

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Introduction

Breast cancer is the malignant proliferation of epithelial cells that line the ducts or lobules of the breast. Female breast cancer has now surpassed lung cancer as the leading cause of global cancer incidence in 2020, with an estimated 2.3 million new cases, representing 11.7% of all cancer cases. It is the fifth leading cause of cancer mortality worldwide, with 685,000 deaths. Among women, breast cancer accounts for 1 in 4 cancer cases and for 1 in 6 cancer deaths, ranking first for incidence in the vast majority of countries (159 of 185 countries) and for mortality in 110 countries.[1] The incidence of breast cancer deaths is on the rise and it now represents the leading cause of cancer deaths among women in urban India.[2] Various biomarkers have been studied in breast cancer but none of them show sufficient sensitivity and specificity to be clinically valuable.[3] CEA and CA15–3 are widely used but CEA is elevated in other cancers as well such as colorectal cancer and pancreatic cancer.[4] Hence, there is a crucial need of finding a newer biomarker.

Human mammaglobinA (MAM) has recently been identified as a diagnostic breast cancer marker and is almost exclusively expressed by breast tissue.[5] It is a dimeric protein belonging to the secretoglobin family and found to be associated with lipophilin B and is secreted by breast epithelial cells. It has a transmembrane domain and a signal peptide, which when cleaved is released into the circulation.[5] The exact function of MAM is not known but various studies prove its role in cancer development, immune system regulation, and in the transport of aromatic molecules, such as steroid hormones. Several studies have found that MAM is not elevated in any other cancer.[6] As MAM is relatively a newer marker in serum and most of the studies conducted so far have studied MAM in tissues, we intended to study the role of serum MAM as a diagnostic marker in benign and malignant breast tumors and compare its diagnostic efficacy with available serum markers CA15–3 and CEA.

Materials and Methods

This was a cross-sectional, case–control study comprising 27 histopathologically confirmed breast cancer cases, 20 cases of benign breast tumors, and 30 healthy controls. Based on the α error at 0.05 and β of 0.2, with receiver operating characteristic (ROC) of 0.898, sample sizes in negative/positive groups as 2.15 with p-value < 0.05 from previous study.[5] The sample size calculated was 5 cases and 11 controls that justify our sample size. The study was conducted in the Department of Biochemistry in collaboration with Departments of Surgical Oncology and Pathology of Nizam's Institute of Medical Sciences (NIMS), Hyderabad, from September 2018 to April 2019 after the study was approved by hospital's institutional ethics committee (EC/NIMS/1964/2017). Informed consent was taken from all participants. Women of age group between 18 and 75 years with breast lump visiting the out-patient department of surgical oncology were included either in the benign group or malignant group based on histopathological findings. Controls comprised healthy women volunteers of similar age group. Women with a history of smoking, cancer, patients on radiotherapy, chemotherapy, or those who underwent surgery, women below 18 years, pregnant and lactating women were excluded from the study. Venous blood samples were collected. Serum CA 15–3 (Roche Cobas e 411) and serum CEA (Siemens ADVIA Centaur XP Immunoassay System) were analyzed. Serum was then aliquoted and stored at −40°C to measure serum mammaglobin-A later using the Sandwich ELISA kit (Elabscience; Human SCGB2A2).

Statistical analysis: Statistical analyses were performed using statistical softwaresMedCalc version 20.008 and SPSS version 25. Normality was tested using Kolmogorov–Smirnov test. Parametric data are expressed in terms of mean and standard deviation (SD), and non-parametric data using median and interquartile range (IQR). Pearson's correlation coefficient was calculated for parametric data, and Spearman's coefficient was calculated for non-parametric data. Kruskal–Wallis test was done to compare MAM and CEA among malignant, benign, and control groups. Analysis of variance (ANOVA) was done for comparing the means of CA15–3. The diagnostic performance of each marker in differentiating malignant and benign breast tumor was tested using the ROC curves. For all analyses, $p < 0.05$ was considered statistically significant.

Results

The mean age of malignant patients was 51.2 years (31–72 years), which was significantly higher than that of the benign group 34.4 years (19–64 years) $p < 0.001$. Malignant cases were either of duct cell carcinoma, invasive duct cell carcinoma, ductal carcinoma in situ (DCIS). The most common stage at which breast cancer patients presented was stage IIIB (45%) (►**Fig. 1**).

Serum mammaglobin levels were significantly different among the three groups, $p < 0.000001$ (►**Table 1**). Posthoc analysis showed that the median serum MAM (ng/mL) levels were higher in the malignant group (26.25 [23.03–27.98]) when compared with benign (11.08 [10.87–11.4], $p = 0.004$) and control groups (9.2 [8.78–9.7], $p < 0.000001$) as seen in ►**Fig. 2** and also significant difference was found between benign and control groups ($p = 0.0014$). There was no statistically significant difference in CA15–3 levels (►**Fig. 3**)

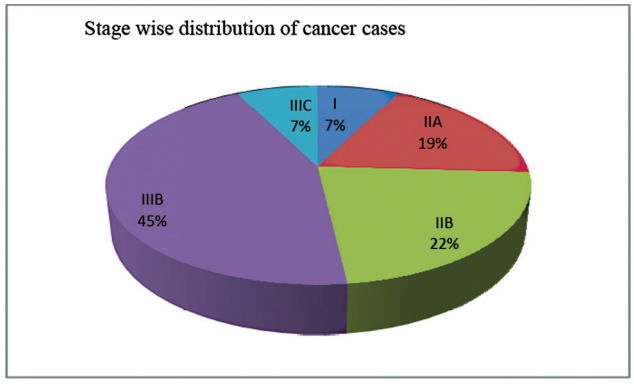

Fig. 1 Stagewise distribution of breast cancer cases.

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Table 1 Serum biomarkers in healthy controls, benign and malignant breast tumor groups

Biomarker	Control group ($N = 30$)	Benign tumor ($N = 20$)	Malignant tumor ($N = 27$)	*p*-Value
Mammaglobin (ng/mL) Median (IQR)	9.2 (8.78–9.7)	11.08 (10.87–11.40)	26.25 (23.03–27.98)	< **0.000001***
CA 15–3 (U/mL) Mean ± SD	15.06 ± 8.95	17.72 ± 9.00	17.39 ± 7.28	0.536
CEA (ng/mL) Median (IQR)	0.41 (0.4–0.425)	1.155 (0.915–1.705)	1.85 (1.53–3.575)	< **0.000001***

Abbreviations: CA 15–3, carbohydrate antigen-15–3; CEA, carcinoembryonic antigen.
*$p < 0.05$ is significant.

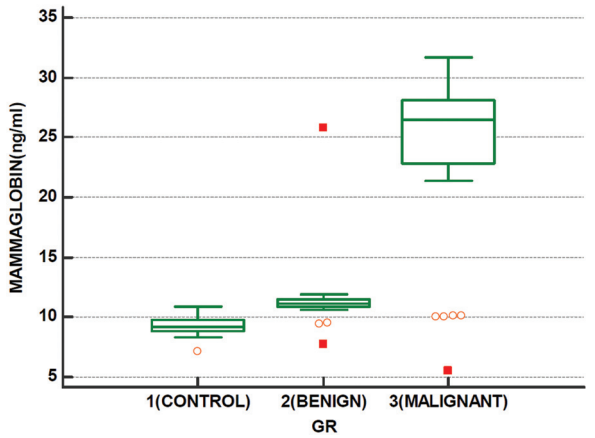

Fig. 2 Box whisker plot depicting the distribution of mammaglobin levels in control and breast tumor groups.

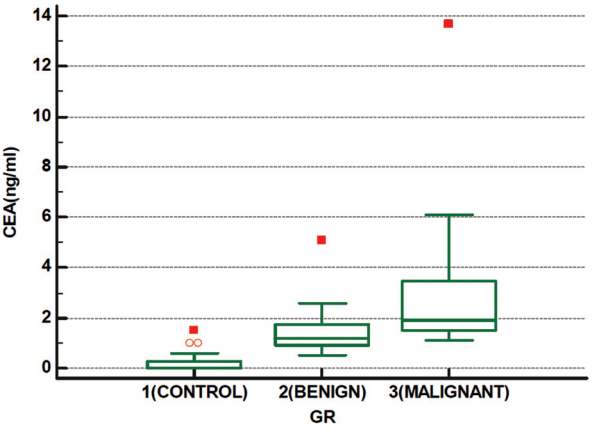

Fig. 4 Box whisker's plot depicting the distribution of CEA among the three groups. CEA, carcinoembryonic antigen, CA 15-3, carbohydrate antigen-15-3.

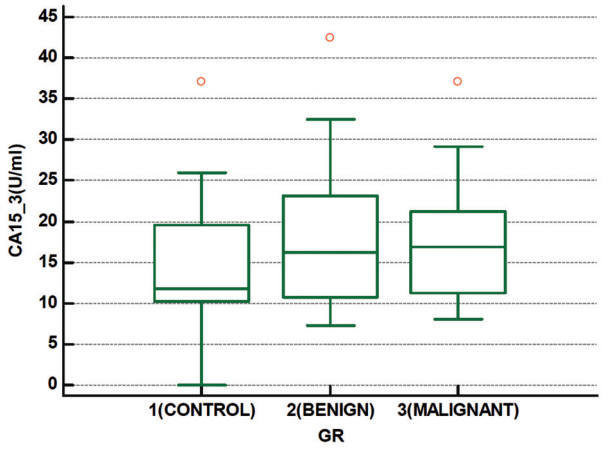

Fig. 3 Box whisker's plot depicting the distribution of CA 15-3 among three groups.

among the three groups ($p = 0.536$). Serum CEA levels were significantly different among the three groups, $p < 0.000001$. The median CEA (ng/mL) levels (►**Fig. 4**) were higher in the malignant group (1.85 [1.53–3.575]) when compared with the benign (1.155 [0.915–1.705], $p = 0.011$) and control (0.41 [0.4–0.425] $p < 0.000001$) groups and also significant difference was found between benign and control groups ($p < 0.00018$). Serum mammaglobin has not shown any significant correlation with either CEA ($r = 0.034$, $p = 0.7$) or CA15–3 ($r = 0.08$, $p = 0.49$).

The ROC analysis showed serum MAM had 81.5% sensitivity, 100% specificity, 100% positive predictive value (PPV), and 88.9% negative predictive value (NPV) at a cut-off of 11.89 ng/mL (►**Table 2**). CA15–3 showed poor diagnostic efficacy with an area of curve (AUC) of 0.555. CEA showed 88.9% sensitivity, 82.5% specificity, 77.4%, PPV, and 91.7% NPV at a cut-off of 1.37 ng/mL. The AUC (►**Fig. 5**) was the highest for MAM (0.892), followed by CEA (0.889) and CA15–3 (0.555). The combined ROC curve of MAM and CEA showed sensitivity of 100%, specificity of 82.5%, and AUC of 0.913.

Discussion

Because serum mammaglobin A is specifically produced by the breast tissue and elevated levels are observed in breast cancer and not seen in any other cancers, this study was done to assess the diagnostic accuracy of serum MAM in the diagnosis of benign and malignant breast tumors and compare it with existing biomarkers.

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Table 2 Diagnostic efficacy of biomarkers

Serial no.	Parameter	Cut-off	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	AUC
1.	Mammaglobin	11.89 ng/mL	81.5	100	100	88.9	0.892
2.	CA 15–3	10.97 U/mL	81.5	35	45.8	73.7	0.555
3.	CEA	1.37 ng/mL	88.9	82.5	77.4	91.7	0.889

Abbreviations: CA-15–3, carbohydrate antigen-15–3; CEA, carcinoembryonic antigen.

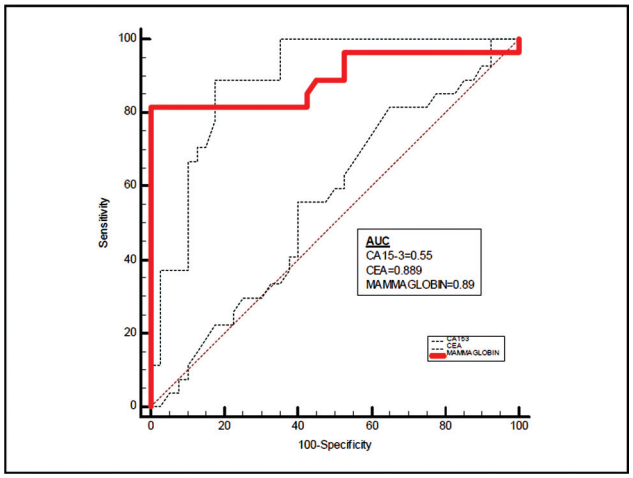

Fig. 5 ROC curves for biomarkers mammaglobin, CA 15-3, and CEA.

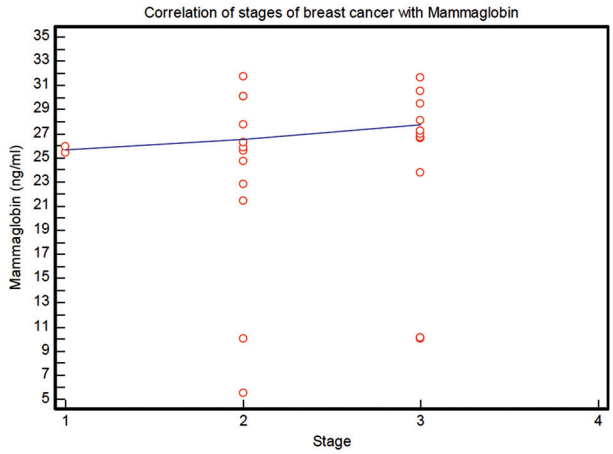

Fig. 6 Correlation of mammaglobin with stages of breast cancer.

The mean age in cancer patients was higher than in those with benign disease possibly due to increase in the incidence of cancers with age. Due to lack of awareness or social inhibition, most of the patients delayed their first visit to the out-patient department after the onset of their symptoms and hence 45% of the cancer patients belonged to stage IIIB at the time of presentation.

Serum levels of markers in patients with breast tumor

Statistically significant differences were found in serum MAM levels (ng/mL) among malignant, benign, and control groups (26.25, 11.08, and 9.20, respectively). In accordance with several studies,[3,7,8] including that by Bernstein et al and Zehentner et al, our study found significant elevation in the levels of serum MAM in malignant cases when compared with benign and healthy controls.

CA15–3 levels did not differ among the three groups. According to many studies, CA15–3 levels are low in the early stages of breast cancer but elevated levels of CA15–3 are observed in metastatic and recurrent conditions. We included only newly diagnosed cases of breast cancer without recurrence and none of them belonged to stage IV and therefore less chances of metastasis; thus, CA15–3 levels might not be elevated in malignant cases when compared with controls or benign groups.

The median CEA value of malignant cases (1.85 ng/mL) was significantly higher than that of benign cases (1.155 ng/mL) and also higher than controls (0.41 ng/mL). Mammaglobin did not correlate significantly with the stage of cancer because of limited number of cases (►**Fig. 6**). Galvis-Jimenez too reported no correlation between MAM levels and clinical stage of cancer.[9]

Correlation of Mammaglobin with CA 15–3 and CEA

We also correlated serum MAM levels with existing biomarkers CA15–3 and CEA and did not find any significant correlation with either of these markers. This might have been because CA15–3 is more of a marker of recurrence than of primary breast cancer. In concurrence with the findings of El Attar et al, who studied 40 Egyptian females with primary breast cancer, we too found no correlation between MAM levels and CEA nor with CA15–3.[10] This was explained by Antonella et al, who reported that higher levels of CA 15–3 and CEA correlated with a larger tumor burden and a more advanced disease.[11] Other tissues such as the liver and bone are capable of producing CA 15–3 CEA, whereas MAM is breast specific. This might be the reason for poor correlation.

Diagnostic Efficacy of Three Markers

Mammaglobin showed excellent ability to detect true disease. At a cut-off of 11.89 ng/mL, mammaglobin had a sensitivity of 81.5% and specificity of 100%. Our study correlated well with the findings of Bernstein et al.[7] The PPV of MAM is 100% in contrast to CEA and CA 15–3. Also mammaglobin had the highest specificity among the three markers (100%). At a cut-off of 1.37 ng/mL, CEA had a sensitivity of 88.9% and specificity of 82.5%. When compared with CEA, CA15–3 showed a lesser sensitivity of 81.5% and poorer specificity of 35%. CA15–3 was useful only to identify breast cancer recurrences and less often in primary cancer as confirmed by several studies including Fejzic.[12]

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ROC Curves and Area under Curve

The AUC for mammaglobin was the highest among the three markers. The AUC for CA-15–3 was the lowest. These results were consistent with the findings of Bernstein et al[7] and Galvis et al.[9] When MAM and CEA were combined, there was a slight increase in the AUC to 0.913 from their individual AUC of 0.892 (MAM) and 0.889 (CEA). The greatest advantage of MAM over CEA is that unlike CEA, MAM is breast specific, whereas CEA is found to be elevated in other cancers such as pancreatic, colon, and lung.[3,7] Mammaglobin in the malignant group is highly elevated than in the control group. In contrast, the elevation in the benign group is not that high. Though CEA showed significant difference among the three groups, its cut-off obtained (1.37 ng/mL) was very low, and with the commonly used reference range cut-off of 2.5 ng/mL, we found that the sensitivity dropped to 37% though the specificity obtained was 97%. This makes CEA not a so useful marker. These findings highlight the valuable role of serum MAM as a diagnostic tool in breast cancer.

Conclusion

Mammaglobin proves to be a very sensitive and specific marker of breast tumors, especially cancer. The usefulness of MAM in diagnosis stems from its specificity in breast tissue and its elevation in breast cancer alone, which is not so in the case of CEA. Mammaglobin can prove as a golden alternative to mammography as a screening test for breast malignancies. CA15–3 has lesser diagnostic accuracy in detecting primary breast cancer when compared with MAM and CEA and must be reserved for follow-up of recurrences. Combining CEA and MAM offers additional diagnostic efficacy in detecting cases of breast cancer. From our observations, we can conclude that serum MAM has the potential to be used as a diagnostic marker in breast cancer.

Limitations

A larger sample size comprising women of wider age group and tumor stages may validate the study further.

Funding
None.

Conflict of Interest
None declared.

References

1 Sung H, Ferlay J, Siegel RL, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2021;71(03): 209–249
2 Malvia S, Bagadi SA, Dubey US, Saxena S. Epidemiology of breast cancer in Indian women. Asia Pac J Clin Oncol 2017;13(04): 289–295
3 Al Joudi FS. Human mammaglobin in breast cancer: a brief review of its clinical utility. Indian J Med Res 2014;139(05):675–685
4 Meng Q, Shi S, Liang C, et al. Diagnostic and prognostic value of carcinoembryonic antigen in pancreatic cancer: a systematic review and meta-analysis. OncoTargets Ther 2017;10:4591–4598
5 Zuo L, Li L, Wang Q, Fleming TP, You S. Mammaglobin as a potential molecular target for breast cancer drug delivery. Cancer Cell Int 2009;9:8
6 Al-Joudi FS, Kaid FA, Ishak I, Mohamed N, Osman K, Alias IZ. Expression of human mammaglobin and clinicopathologic correlations in breast cancer: the findings in Malaysia. Indian J Pathol Microbiol 2011;54(02):284–289
7 Bernstein JL, Godbold JH, Raptis G, et al. Identification of mammaglobin as a novel serum marker for breast cancer. Clin Cancer Res 2005;11(18):6528–6535
8 Zehentner BK, Persing DH, Deme A, et al. Mammaglobin as a novel breast cancer biomarker: multigene reverse transcription-PCR assay and sandwich ELISA. Clin Chem 2004;50(11): 2069–2076
9 Galvis-Jiménez JM, Curtidor H, Patarroyo MA, Monterrey P, Ramírez-Clavijo SR. Mammaglobin peptide as a novel biomarker for breast cancer detection. Cancer Biol Ther 2013;14(04):327–332
10 Abd El-Hafez ZA, Khaled SMI, Rizk M, El-Gammal YT. Assessment of human mammaglobin (hMAG) as a new marker for early diagnosis of breast cancer. Med J Cairo Univ 2018;86:1263–1270
11 Guadagni F, Ferroni P, Carlini S, et al. A re-evaluation of carcinoembryonic antigen (CEA) as a serum marker for breast cancer: a prospective longitudinal study. Clin Cancer Res 2001;7(08): 2357–2362
12 Fejzić H, Mujagić S, Azabagić S, Burina M. Tumor marker CA 15-3 in breast cancer patients. Acta Med Acad 2015;44(01):39–46

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