Quantitation of Serum Thioredoxin 1 Could Mitigate Difficulty of Mammography to Detect Breast Cancer in Dense Breasts

Youn Ju Lee[1,2], Hyemi Ko[1,2], Bo Bae Choi[3], Je Ryoung Kim[1,2] , Young Kim[4], Kyoung Hoon Suh[4], Jin Sun Lee[1,2*]

[1]*Department of Surgery, Chungnam National University Hospital, Daejeon, Korea*

[2]*Department of Surgery and Research Institute for Medicinal Sciences, Chungnam National University School of Medicine, Daejeon, Korea*

[3]*Department of Radiology, Chungnam National University Hospital, Daejeon, Korea,*

[4]*E&S Healthcare Co., Ltd., Daejeon, Korea*

Background: Even though almost half of women have dense breasts (DB), the density of breasts has been an obstacle to detect breast cancer (BC) correctly using mammography, the gold standard of screening. It is strongly recommended to consider breast density when analyzing mammograms. We have reported the possibility of using the serum level of thioredoxin 1 (Trx1) as a novel means to assess the risk of BC regardless of various breast conditions. In the present study, we evaluated how the serum level of Trx1 can be used mitigate the difficulty of using mammography to detect BC in dense breasts. **Methods**: We have generated monoclonal antibodies against Trx1 and developed an ELISA kit that quantitates Trx1 in serum. The level of Trx1 was determined in each serum from BC patients (n=106) who had been confirmed as having BC by various examinations, including mammography and surgery. Each serum's Trx1 level was analyzed with the corresponding patient's mammography results, such as pattern, asymmetry, shape, margin, and density. The analyzed results were compared with those from other biomarker tests, including CA15-3 and CEA, to evaluate the clinical validity of Trx1 in this study. Each test was duplicated two times, and test results were analyzed by ROC analysis, one-way ANOVA tests, and unpaired t-tests. **Results**: Most BC patients (82.6%, n=86) were classified as mammography pattern 3 or 4 and had BI-RADS scores higher than 4 (74.0%, n=77). More than half of patients' breast density was in the high density group (54.8%, n=57), whereas 5.8% were iso-density (n=6) and 39.4% were not determined (n=41). The blood Trx1 levels of patients were higher than the cut-off value of 14 U/ml, indicating BC, regardless of their breast characteristics. Although other breast cancer biomarker tests, such as CA15-3 and CEA, could not identify BC, the Trx1 level could correctly detect BC even in the cases when mammography could not show the mass clearly. The sensitivity/specificity of mammography and the Trx1 test were 75.2%/74.0% and 96.2%/99.0%, respectively. When mammography and Trx1 test were combined, the sensitivity/specificity were 99.3%/100.0%. **Conclusion**: Even though it is obvious that further study with a larger sample size is necessary, the results indicated that serum Trx1 level could identify BC in dense breasts easily. These results indicated that the serum Trx1 level could be used to efficiently mitigate the difficulty of using mammography to detect BC in dense breasts.

Introduction

Breast cancer(BC) is the most common type of cancer in women. One out of eight women is likely to get BC before age 70, and 85% of BC patients have no family history [1]. Breast cancer has one of the highest survival rates when it is discovered in an early stage [1]. Mammography is the only modality for early detection of BC approved by the WHO [2]. Even though many reports prove that mammography significantly raises the survival rate of BC, the procedure has limitations when used with women who have small or dense breasts (DB) [3]. High breast density raises BC risk and can mask malignant tumors, decreasing the sensitivity of mammography.

Thioredoxin 1 (Trx1) is a 12 kDa redox-active protein ubiquitously expressed from bacteria to mammals with a universally conserved dithiol active site [4]. Since tumor cells are often under extremely high oxidative status or hypoxia, it is widely accepted that Trx1 express high level in malignant cells [4]. We reported Trx1 as a biomarker in blood for BC detection by studies of Trx1 gene and protein expression differences in many malignant tissues and bloods from various cancer patients [5, 6]. The protein level of Trx1 in bloods from various cancer patients was the highest in BC. The protein level of Trx1 in bloods from various cancer patients was the highest in BC. It showed superior ability to detect BC compared to those of CA15-3 and CEA. As the blood level of Trx1 could be a novel means to detect BC and mammography seemed to be assisted by other diagnostic modality to detect breast cancer from DB, we have examined the ability of blood Trx1 to detect BC from DB along with mammography.

Method

Sample Collection and Clinical Information Blood samples and mammographic information of all breast cancer patients were collected from Chungnam National University Hospital (CNUH) under ethical committee approval. Blood collection and serum preparation followed the corresponding standard instructions of the Ministry of Food and Drug Safety. Mammographic information about each patient was provided anonymously. Clinical classification and information of 106 breast cancer patients who had matched mammograms were listed in Table 1.

Sample Sizes In this study, blood samples from 104 breast cancer patients (104 out of 106) and their mammograms were analyzed and compared. Among 106 patients, 2 patients did not participate in the follow up process and thus were excluded from final analysis.

Quantitation of Blood Trx1 by Custom-made ELISA The concentration of Trx1 in each serum was estimated by custom-made sandwich ELISA kits. We have generated a pair of highly specific monoclonal antibodies to Trx1 and made an ELISA kit with them. The ELISA kit specifically differentiated BC patients from normal healthy women with a reproducible sensitivity and specificity both higher than 93% (Ref). ELISA was carried out as indicated elsewhere (Ref).

Data Analysis Each test was duplicated, and the average value was analyzed by ROC curve to calculate sensitivity and specificity. When it was necessary, data were analyzed by a one-way ANOVA test and an unpaired t-test in order to confirm their statistical validity. The concentration of Trx1 from each sample was compared to the cut-off value (11.4 U/ml) to decide whether the result indicated positive or negative. In addition, it was also compared to the BI-RADS category of mammogram of corresponding patient.

RESULTS

Number of patients	106	100.00%
Followed	104	98.11%
Not followed	2	1.89%

BI-RADS	Number of patient sPatient's number	Percentage
0	13	12.50%
1	11	10.58%
2	0	0.00%
3	3	2.88%
4	43	41.35%
5	34	32.69%
6	0	0.00%

Mammography Pattern	Number of patients	Percentage
1	0	0.00%
2	18	17.31%
3	74	71.15%
4	12	11.54%

Asymmetry	Number of patients	Percentage
Finding mass	63	60.58%
Asymmetry	12	11.54%
Focal asymmetry	7	6.73%
Developing asymmetry	4	3.85%
Global asymmetry	3	2.88%
Not visible	15	14.42%

Mass shape	Number of patients	Percentage
Oval	18	17.31%
Round	8	7.69%
Irregular	37	35.58%
Not determined	41	39.42%

Mass margin	Number of patients	Percentage
Circumscribed	14	13.46%
Spiculated	23	22.12%
Microlobular	10	9.62%
Indistinct	1	0.96%
Obscured	6	5.77%
Angular	9	8.65%
Not determined	41	39.42%

Density	Number of patients	Percentage
High density	57	54.81%
Iso density	6	5.77%
Not determined	41	39.42%

Table 1. Classification and clinical information of breast cancer patients

Among the patients analyzed, 77 (74.04%) out of 104 patients showed Bi-RADS grade 4 or 5. According to mammography pattern and mass density, 86 (82.69%) were in pattern 3 and 4, and 57 (54.81%) had highly dense breasts.

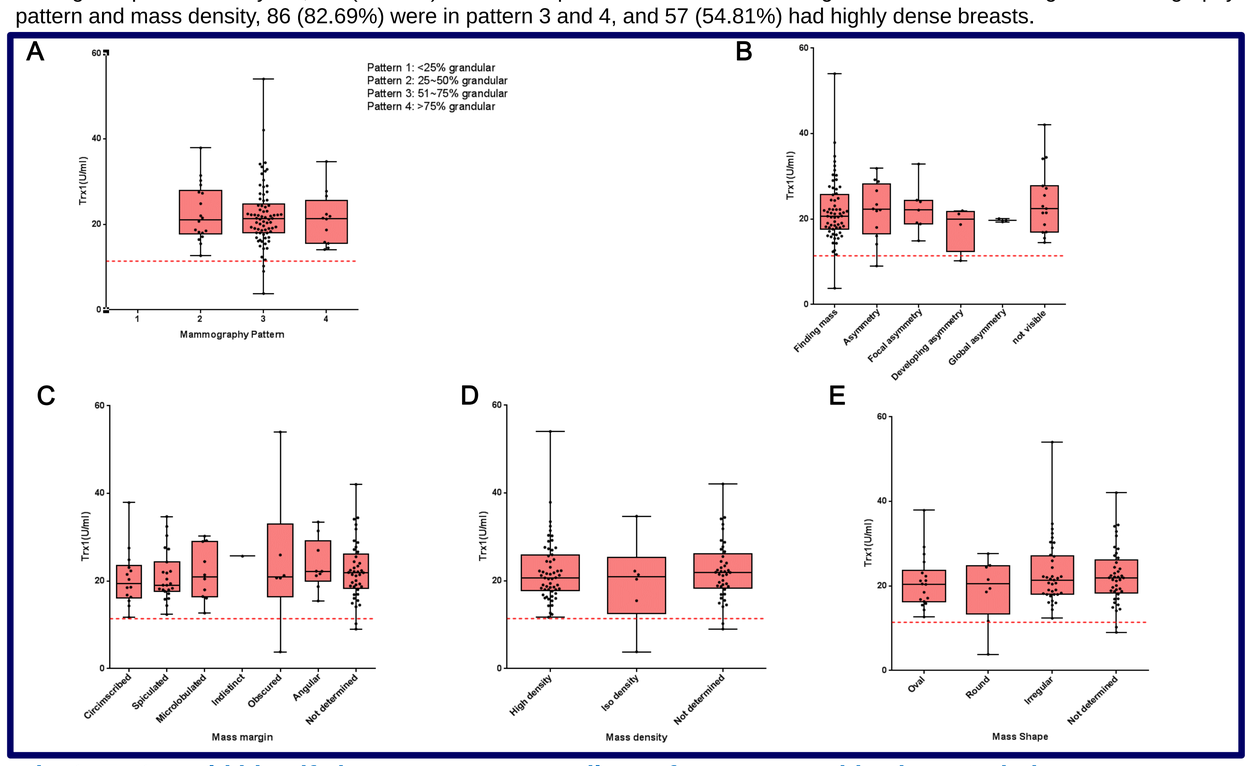


Fig. 1. Trx1 could identify breast cancer regardless of mammographic characteristics

Trx1 levels in 104 sera from BC patients were measured and analyzed according to their mammographic characteristics by box and whisker graph. (**A**) Trx1 levels from BC patients were categorized in accordance with mammography patterns in order to see whether dense breasts had an influence on Trx1 level. The effect on the Trx1 levels by (**B**) Asymmetry, (**C**) Mass margin, (D) Mass density, and (E) Mass shape.

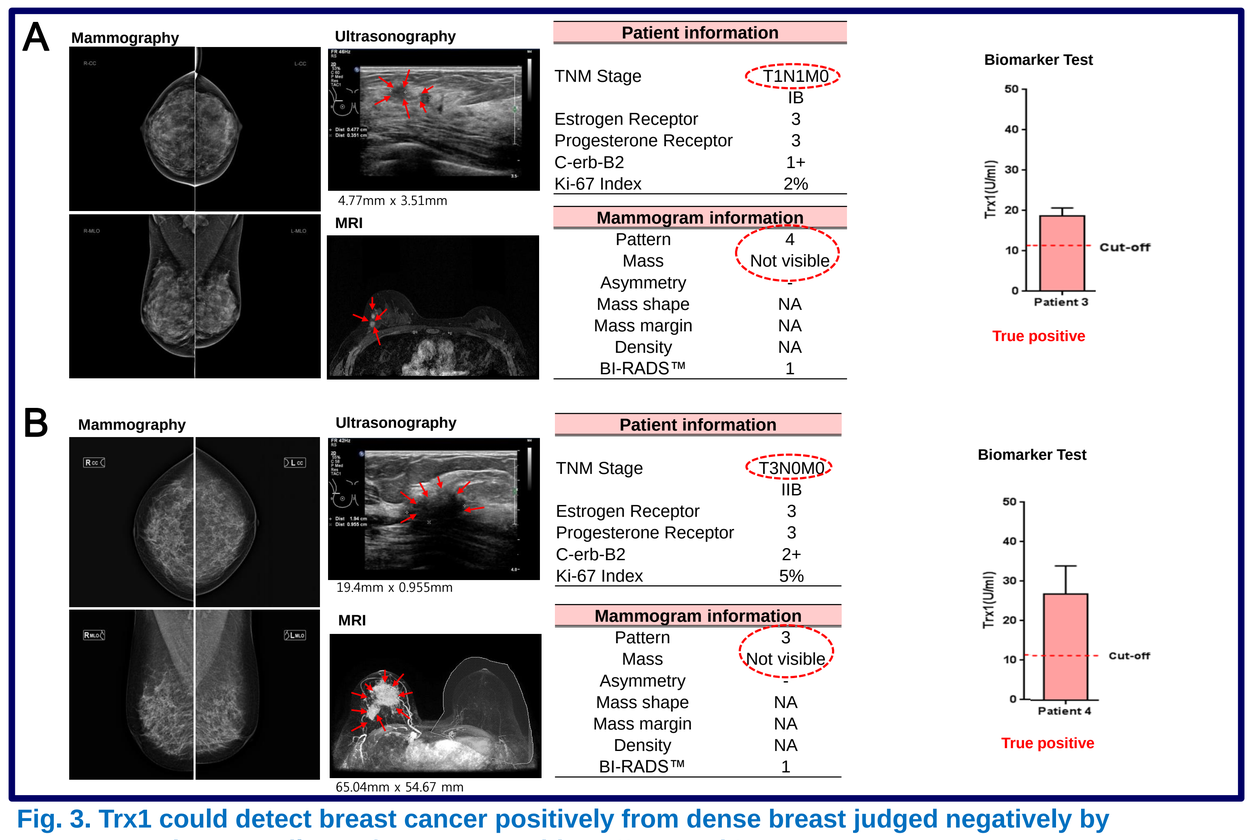


Fig. 3. Trx1 could detect breast cancer positively from dense breast judged negatively by mammography, regardless of mammographic pattern and tumor mass

The ability of the Trx1 test to detect BC regardless of mammographic pattern and tumor mass. **(A)** The BC patient was confirmed as BI-RADS 1 (Negative) and the mammographic pattern was 4. However, in Trx1 test, the patients was correctly determined to be positive. **(B)** Although T stage (Tumor size) was 3, the BC patient was confirmed as BI-RADS 1 initially. However, in Trx1 test, the patients was correctly identified as positive.

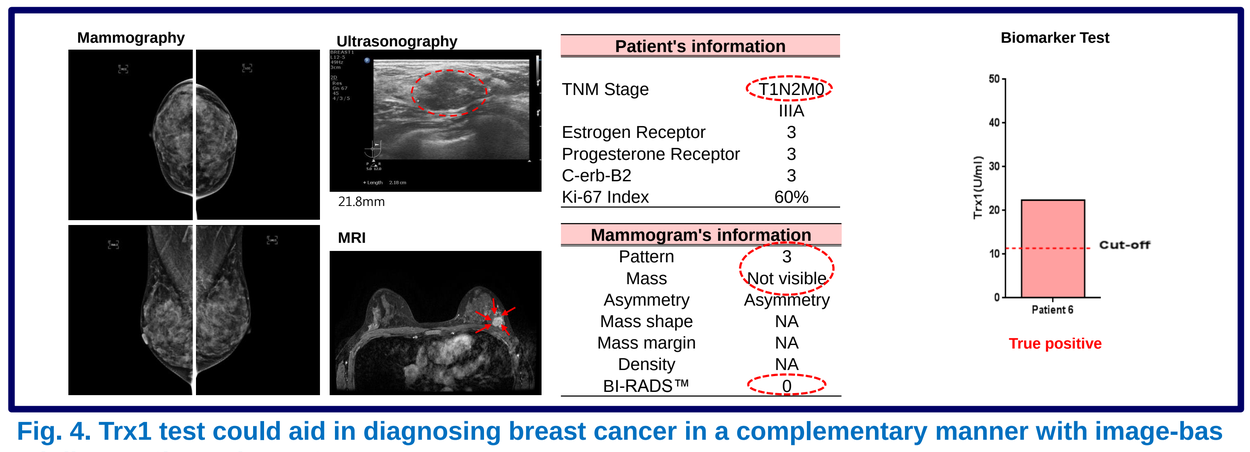


Fig. 4. Trx1 test could aid in diagnosing breast cancer in a complementary manner with image-based diagnostic equipment

The complementary ability of the Trx1 test to detect BC with image-based equipment. The BC patient was confirmed as BI-RADS 0 (Incomplete) and ki-67 value was extremely high. Furthermore, the ultrasonography results were ambiguous. However, in Trx1 test, the patients was correctly determined to be positive.

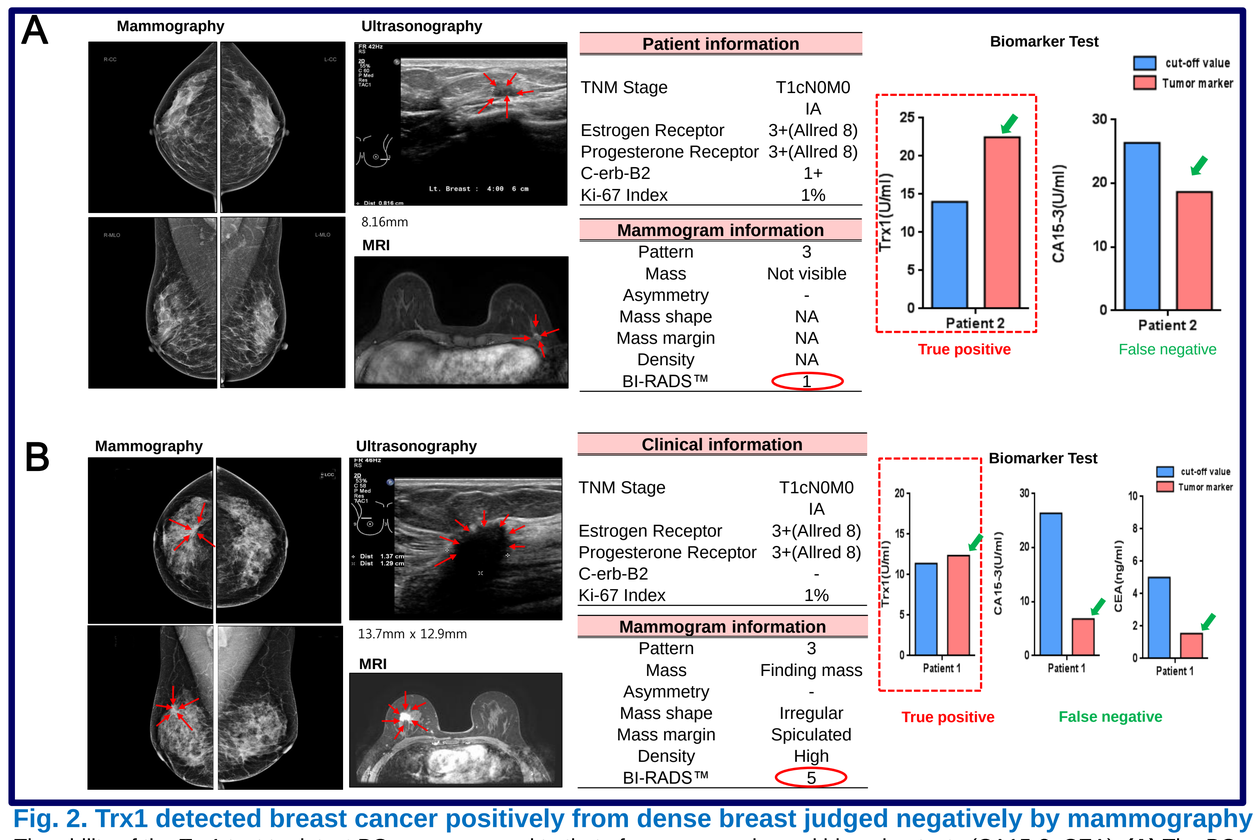
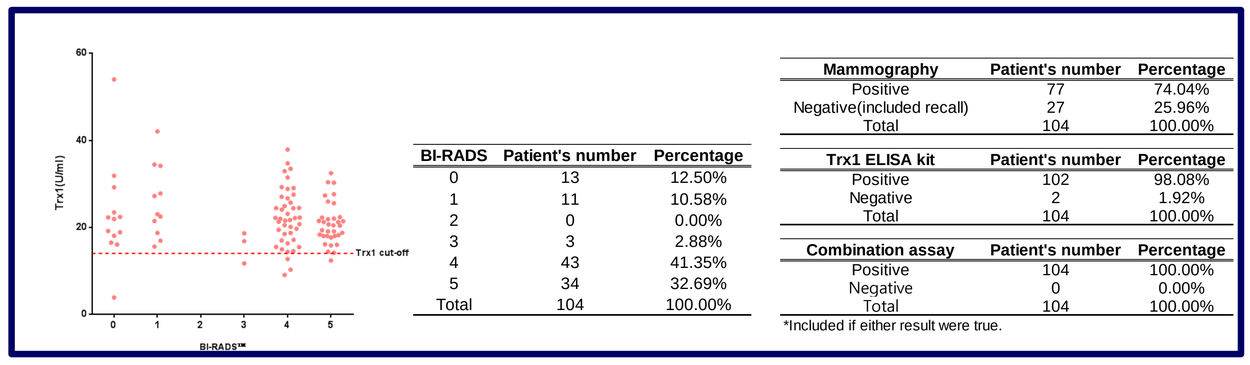


Fig. 2. Trx1 detected breast cancer positively from dense breast judged negatively by mammography

The ability of the Trx1 test to detect BC was compared to that of mammography and biomaker tests (CA15-3, CEA). **(A)** The BC patient was confirmed as BI-RADS 1 (Negative) when carried out the mammography initially, but, in Trx1 test, the patients were correctly identified as positive. **(B)** The BC patient was confirmed as BI-RADS 5 when carried out the mammography initially, but, in Trx1 test, the patients was correctly identified as positive.



BI-RADS	Patient's number	Percentage
0	13	12.50%
1	11	10.58%
2	0	0.00%
3	3	2.88%
4	43	41.35%
5	34	32.69%
Total	104	100.00%

Mammography	Patient's number	Percentage
Positive	77	74.04%
Negative(included recall)	27	25.96%
Total	104	100.00%

Trx1 ELISA kit	Patient's number	Percentage
Positive	102	98.08%
Negative	2	1.92%
Total	104	100.00%

Combination assay	Patient's number	Percentage
Positive	104	100.00%
Negative	0	0.00%
Total	104	100.00%

*Included if either result were true.

Fig. 5. The combination assay with both Trx1 and Mammography could be an improved diagnostic tool for breast cancer.

The complementary diagnostic ability of Trx1 test and mammography: Trx1 levels in 104 sera from BC patients were analyzed according to cut-off value. 102 of 104 (98.08%) patients were detected positively by Trx1 ELISA kit, but only 77 of 104 (74.04%) patients were detected positively by mammography. However, the combination assay could judge 104 of 104 (100.00%) patients as BC.

CONCLUSIONS

- **The blood Trx1 levels were higher than the cut-off value in BC patients regardless of mammographic characteristics.**
- **Even though it is obvious that larger sample size studies are necessary, the study showed that the serum Trx1 level could identify BC in DB correctly.**
- **These results indicated that the serum Trx1 level could efficiently mitigate the difficulty to detect dense breasts by mammography.**

*** Contact information ; Young Kim, Jin-Sun Lee, MD (correspondence).**
Tel : +82-042- 220-7175 ; FAX : +82 - 042 - 257- 8024 ;
E-mail : leejin0520@cnuh.co.kr
youngkim@ens-h.com