Effective Detection of Breast Cancer by the Quantitative Measurement of Thioredoxin 1 from Blood

Youn Ju Lee[1,2], Bo Bae Choi[3], Je Ryong Kim[1,2] , Young Kim[4], Kyuong 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: Breast cancer is one of the leading cancers for women worldwide. Mammography is the only recommended method for breast cancer screening, although it is inaccurate in young women or women with dense breasts in Korea and Asian countries. Therefore, it has been a long time un-met need to have a simple and effective tool that can detect breast cancer by routine blood test to resolve such problem and complement mammography. **Methods**: We have developed an ELISA kit that quantitate thioredoxin 1 (Trx1) from blood to detect breast cancer. In order to estimate the validity and utility of Trx1 ELISA, we have tested the bloods from 116 normal healthy women, 137 confirmed breast cancer patients with stage from 0 to 4, and 30 confirmed patients of each lung, ovarian, gastric, colorectal, and cervical cancer. The test results were analyzed by ROC, one-way ANOVA test, and unpaired t-test. **Result**: The mean value of Trx1 from normal women was 7.506±0.4607 and that from breast cancer patients was 37.75±0.8633. The Trx1 level was effective to detect breast cancers over normal cases with sensitivity of 96.4% and specificity of 99.1% (AUC 0.990, p<0.001). Each mean value of Trx1 level from lung (16.7±1.786), ovarian (15.50±1.972), gastric (15.66±1.551), colorectal (16.39±1.678), and cervical (22.51±2.210) cancer was below the cut-off value (22.8U/ml) for breast cancer detection. Trx1 levels could rescue misread cases of confirmed patients by mammography. **Conclusion**: These results indicated that the blood level of Trx1 estimated by the ELISA kit was effective and accurate to detect breast cancer, and complement mammography.

BACKGROUND

Breast cancer is the most common type of cancer in women. It has been known that a new case of breast cancer is being diagnosed in every 29 second in worldwide, 2 minutes in USA, and probably 20 minutes in Korea. According to statistics, one out of eight women is likely to get breast cancer while they live for 70 years, and 85% of breast cancer patients have nothing to do with family history [1]. Breast cancer is one of cancers with very high survival rate when the cancer is discovered in early stage [1]. Mammography is the only approved modality for early detection of breast cancer by WHO [2]. Even though a pile of reports proves its beneficial effect raising enormously the survival rate from breast cancer, mammography has certain limits to demonstrate its full ability for women with small and dense breasts [3]. Therefore, a simple in vitro diagnostic method using blood has been a long-time unmet need from clinical field to complement or assist 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 or hypoxia, it is widely accepted that Trx1 express high level in malignant cells [4]. We have suggested Trx1 as a biomarker in blood for breast cancer detection by studies of Trx1 gene and protein expression differences in many malignant tissues and bloods from various cancer patients [5, 6]. It has been shown that gene expression level of Trx1 was the highest in breast cancer tissue among many different cancers in contrast to the lowest in normal breast tissue. Its protein level in bloods from various cancer patients was the highest in breast cancer. The quantitation of Trx1 level in blood was superior to detect breast cancer compared to those of CA15-3 and CEA. Since the validity of Trx1 as a biomarker for breast cancer was likely to be assessed, we studied the clinical utility of Trx1 level in blood to identify breast cancer. The Trx1 levels in sera from designated participants were estimated by specifically manufactured ELISA kit, DxMe® BC, to see whether it could detect breast cancer, and the results were compared to mammograms of corresponding participants.

Method

Sample Collection and Clinical Information Blood samples and clinical information of all participants were collected from Chungnam National University Hospital (CNUH) with full adherence to proper informed consent, as well as its strict institutional review board (IRB). Collecting bloods, and preparing and storing of sera followed the standard instructions of the MFDS (Ministry of Food and Drug Safety). Clinical information of each participant was provided in anonymized form. Classification and characteristics of 137 breast cancer patients who had matched mammograms were listed in Table 1.

Sample Sizes In this study, 137 breast cancer patients' bloods, 116 normal healthy women's bloods and their mammograms were analyzed and compared, respectively. In order to evaluate ability of Trx1 level to differentiate breast cancer from other cancers, each 30 bloods from lung, ovarian, gastric, colorectal, and cervical cancer were analyzed.

Quantitation of Blood Trx1 by DxMe® BC The concentration of Trx1 in each serum was estimated by sandwich ELISA using DxMe® BC. DxMe® BC was ELISA kit containing 96-well microplate coated with Trx1 specific antibody. ELISA was carried out as indicated in the instruction for user of the kit. Briefly, all the components were left at room temperature for 2 hr and 100 μl of serum was added to each well. After incubation for 1 hr, all wells were washed and incubated with anti-Trx1 antibody conjugated with HRP for 2 hr. The colorization reagent containing TMB was added for 10 min after washing wells thoroughly, and the reaction was stopped by sulfuric acid. The color density of each well was measured at 450 nm and converted into concentration by extrapolating it onto standard curve. The quantitation of CA15-3 and CEA in the same blood samples by corresponding ELISA kits were also estimated as external references.

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 one-way ANOVA test, and unpaired t-test in order to confirm their statistical validity. The concentration of Trx1 from each sample was compared to the cut-off value (22.8 U/ml) to decide whether the result indicated positive or negative. In addition, it was also compared to BI-RADS category of mammogram of corresponding patient.

RESULTS

Table 1. Classification of Clinical information of Breast cancer patients

	No.	%		No.	%
Total No.	137	100.00	**Receptor expression**		
Mean age (years)	49.97±8.49	30-71	**ER**		
Stage			-	29	21.17
0	3	2.19	+	108	78.83
1	51	37.23	**PR**		
2	61	44.53	-	42	30.66
3	19	13.87	+	95	69.34
4	3	2.19	**HER2**		
T stage			0	34	24.82
≤ 1	61	44.53	1	72	52.55
≥ 2	76	55.47	2	4	2.92
N stage			3	27	19.71
0	87	63.50	**Ki67 test**		
1	32	23.36	≤ 25%	90	65.69
≥ 2	18	13.14	> 25%	47	34.31
Histological grade			**Carcinoma type**		
1	33	24.09	DCIS	3	2.19
2	61	44.53	IDC	119	89.66
3	43	31.39	ILC	8	5.84
			Other carcinoma	7	5.11

A

Sample size	AUC±SE	95% CI	Sensitivity(%)	Specificity(%)	Criterion	P-value
Nor(116), BC(137)	0.990±0.006	0.968 to 0.998	96.40	99.10	22.8	P<0.001

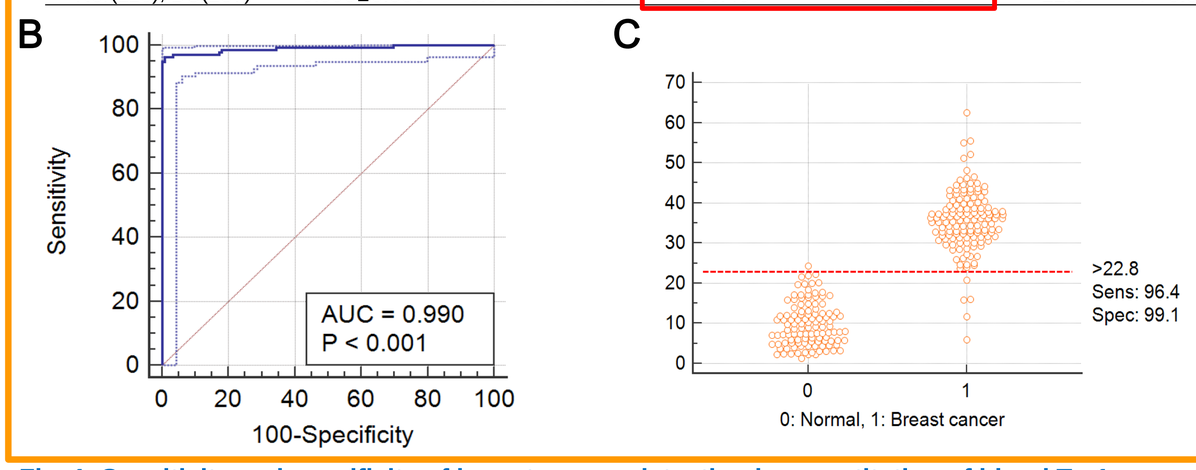

Fig. 1. Sensitivity and specificity of breast cancer detection by quantitation of blood Trx1

Trx1 levels in 116 bloods from normal and 137 from breast cancer were measured and analyzed by ROC curve and dot plot, respectively. (**A**) Summary of results from the blood samples. (**B**) The calculated sensitivity and specificity by ROC analysis were 96.40% and 99.10%, respectively, and AUC was 0.990 (P< 0.001). (**C**) Distribution of Trx1 levels in normal bloods (0) and breast cancer patients' bloods (1) analyzed by interactive dot plot. Red dot line indicates cut-off value of 22.8 U/ml.

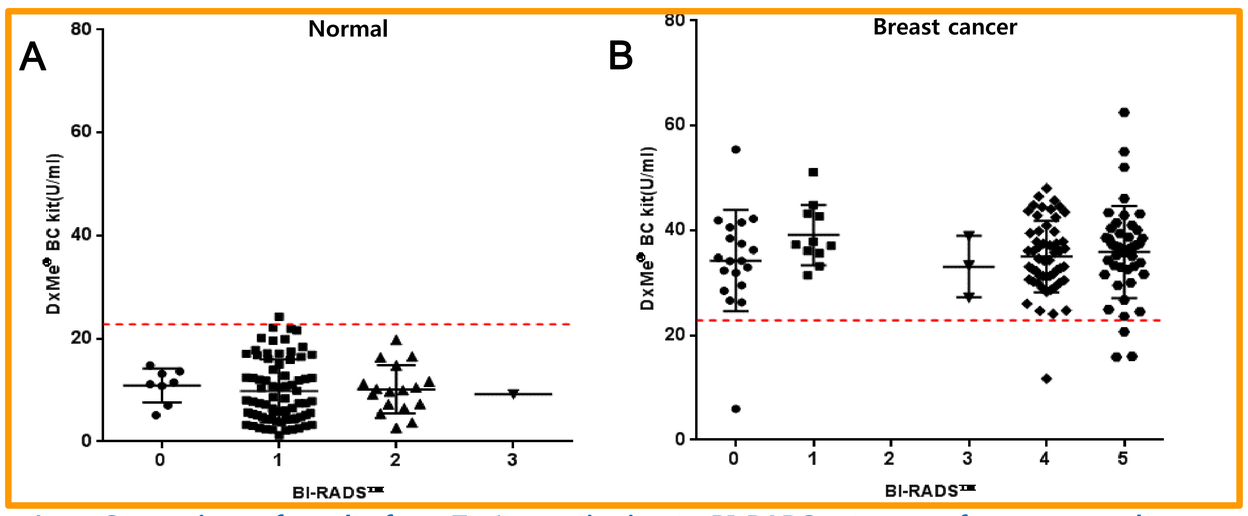

Fig. 2. Comparison of results from Trx1 quantitation to BI-RADS category of mammography

The results of blood Trx1 quantitation were compared to those of BI-RADS categories determined by mammography of matched patients. (**A**) Comparison of normal women's Trx1 levels to BI-RADS categories of corresponding women. Although most of normal healthy women should be judged as negative, the false positive rate of mammography was 26% whereas that of Trx1 quantitation was 1%. Red dot line indicates cut-off value (22.8 U/ml). (**B**) Comparison of confirmed breast cancer patients' Trx1 levels to BI-RADS categories of corresponding women. Since these women were positive, Bi-RADS categories of them was expected to fall to categories above 4. However, many of breast cancer patients with other categories were found. The false negative rate of mammography was 24.82% and that of Trx1 quantitation was 3.76%. Red dot lines indicate the same cut-off value (22.8 U/ml).

REFERENCES

1. American Cancer Society. Breast cancer Facts & Figures 2017-2018. Atlanta: American Cancer Society, Inc. 2017/
2. Elmore et al, Screening for Breast cancer, *JAMA*, 2005; doi10.1001/jama.293.10.1245.
3. Drukteinis et al, Beyoud Mammography: New Frontiers in Breast cancer Screening, *Am J Med*, 2013; doi:10.1016/j.amjmed.2012.11.025.
4. Palde et al, A universal entropy-driven mechanism for thioredoxin-target recognition, PNAS, 2015; vol. 112,. No. 26, 7960-7965
5. Cha et al, Overexpression of peroxiredoxin 1 and thioredoxin 1 in human breast carcinoma, Journal of Experimental & Clinical Cancer Research, 2009; doi:10.1186/1756-9966-28-93
6. Park et al, Thioredoxin 1 as a serum marker for breast cancer and its use in combination with CEA or CA15-3 for improving the sensitivity of breast cancer diagnoses, 2014; doi:10.1186/1756-0500-7-7

A

Register No.	Disease	BI-RADS	Trx1(U/ml)	CA15-3(U/ml)	CEA(ng/ml)
01266777	Breast cancer	5	43.17(22.8)	6.81(26.4)	1.54(5)
01296995	Breast cancer	1	43.26(22.8)	18.61(26.4)	3.35(5)
Claissification	**Disease**	**Composition**	**Calcification**	**Histological grade**	**Stage**
IDC	Breast cancer	Dense	X	1	1A

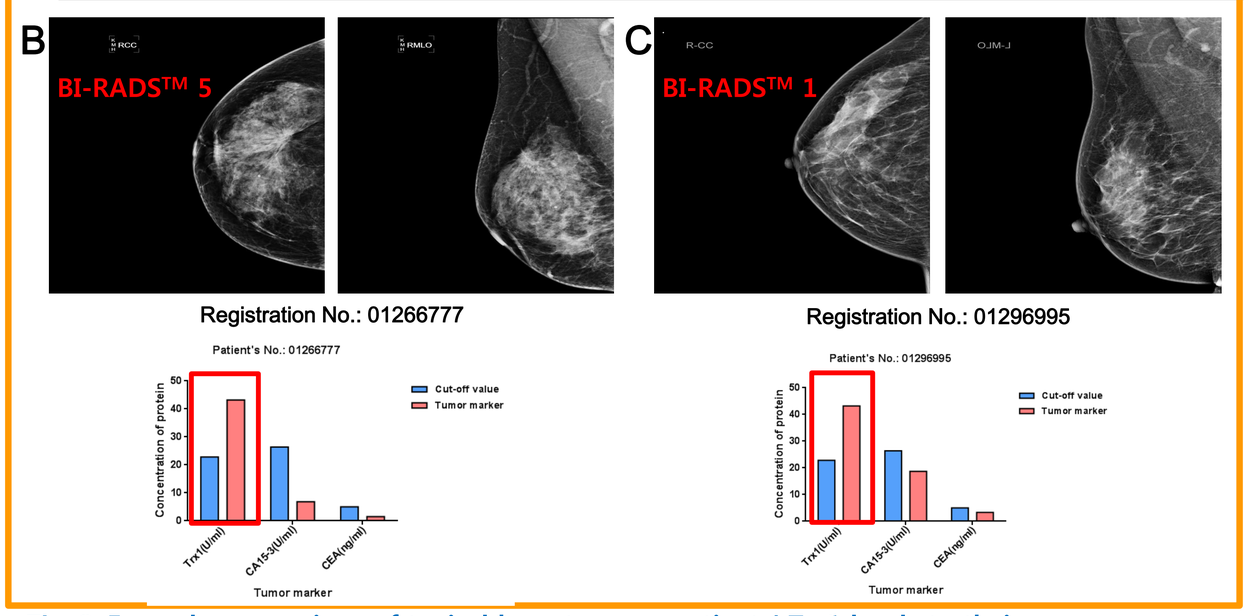

Fig. 3. Example comparison of typical breast cancer patients' Trx1 levels to their mammograms

The blood Trx1 levels of two typical breast cancer patients from participants were compared to their corresponding mammograms. For external references, the blood levels of CA15-3 and CEA were estimated separately and respectively from the same matched bloods. (**A**) Summary of comparison study. The cut-off value of each test was shown in parenthesis. (**B**) Mammographic image of one confirmed breast cancer patient. The BI-RADS category 5 that indicated highly suspicious malignancy agreed with that of Trx1 quantitation (43.17 U/ml) result that implied positive. (**C**) Mammographic image of another confirmed breast cancer patient judged as BI-RADS category 1 that meant negative. Although BI-RADS misread the image of this patient, Trx1 quantitation result (43.26 U/ml) correctly differentiated this case as positive. However, in both cases, CA15-3 and CEA tests as external references judged these confirmed breast cancer patients as negatives since the test values were lower than corresponding cut-off values.

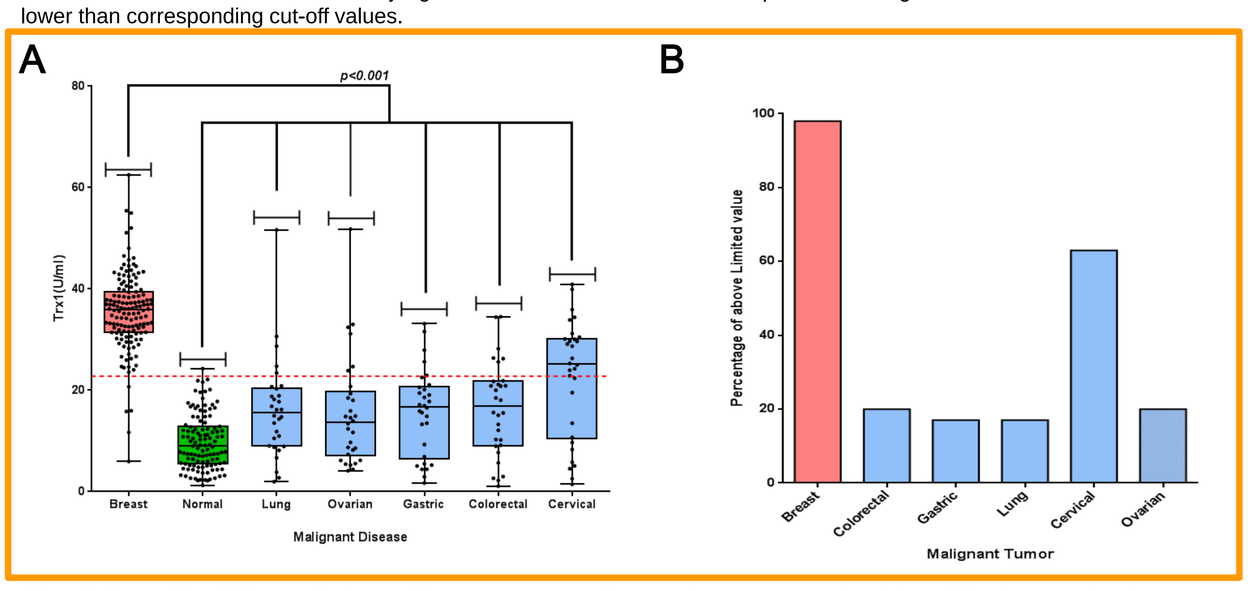

Fig. 4. Specific differentiation of breast cancer from other cancers by blood Trx1 quantitation

Ability of blood Trx1 quantitation to differentiate breast cancer from lung, ovarian, gastric, colorectal, and cervical cancers was assessed. The levels of blood Trx1 from breast cancer patients (137) and normal healthy women (116) were compared to those from other cancers (30 bloods from each cancer). (**A**) Dot box analysis of Trx1 levels among cancers and normal. Each mean value of Trx1 level from lung (16.7±1.786 U/ml), ovarian (15.50±1.972 U/ml), gastric (15.66±1.551 U/ml), colorectal (16.39±1.687 U/ml), and cervical (22.51±2.210 U/ml) was lower than the cut-off value of breast cancer (22.8 U/ml). Red dot lines indicate the cut-off value for breast cancer. (**B**) The percentage of Trx1 level above cut-off value (22.8 U/ml) for breast cancer in each tested cancer group. It has been known that the incidence rate of cervical cancer is about 19% of that of breast cancer in Korea.

CONCLUSIONS

1. **Blood Trx1 quantitation by DxMe® BC showed very high sensitivity (96.40%) and specificity (99.10%) with probability (AUC) of 0.990.**
2. **Blood Trx1 quantitation correctly differentiate positive cases that had been misread as other categories by BI-RADS of mammography.**
3. **Blood Trx1 quantitation was highly specific to breast cancer compared to other cancers.**
4. **Therefore, Trx1 quantitation by DxMe® BC could be an effective and specific modality to detect breast cancer from blood and also could complement current limits of mammography for small and dense breasts.**

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