

Determination of Thymidine Kinase 1 (TK1) Level as a Risk Warning Biomarker to Improve Early Detection of Breast Cancer

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Abstract: Early detection of breast cancer (BC) is a global target to reduce mortality and morbidity also to improve therapeutic and survival outcomes. Currently, mammography is the gold standard in BC diagnosis followed by biopsy when warranted. Thymidine Kinase 1 (TK1) is a proliferative biomarker that succeeded in discovering premalignant transformations of breast cancer before the appearance of any symptoms. This study aimed to provide a non-invasive method to early detect BC by measuring TK1 in sera of women with breast lesions alongside mammography. The study included 271 women divided into five BIRADS categories. Methods and Material: only one blood sample was collected from each woman to detect TK1 concentration, before undergoing mammography and Fine Needle Aspiration Cytology (FNAC) or true cut. Results: TK1 levels were significantly different between BI-RADS categories. It was correlated with clinical stage, histological grade, lymph node metastasis, and vascular invasion. TK1 levels could distinguish between healthy individuals and patients who had breast lesions with a sensitivity and a specificity as follows 91.3 and 87.5%, respectively. Furthermore, this test could discriminate between benign and malignant breast lesions with a sensitivity of 92.5% and a specificity of 91.2%. Conclusion: These findings suggest the determination of TK1 levels as a risk warning biomarker to improve early detection of BC.

Keywords: BI-RADS, Thymidine Kinase 1 (TK1), Breast Cancer, Benign, Proliferative Marker

1. Introduction

Breast cancer (BC) is considered the second major cancer among women worldwide [1]. Late diagnosis of BC reduces survival and quality of life [2]. Thus, early detection of the tumor is a prerequisite to effective treatment and to minimize morbidity and mortality rates of BC, as an early diagnosis in a localized site increases 5-year survival rates to 98% [3, 4].

Currently, mammography is the gold standard in breast cancer diagnosis followed by biopsy when warranted [2]. The Breast Imaging and Reporting Data System (BI-RADS®) is a

number system that categorizes mammography results into seven categories from 0 through 6 [5]. The most difficult assessment category for the radiologists is BI-RADS 3 (probably benign) [6]. As many previous studies displayed significant differences between radiologists' interpretation of breast lesions as BI-RADS 3 [7].

Patients assigned as a BI-RADS 3 should undergo follow up imaging for 2 years concerning a 6-month interval to document steadiness or resolve of a finding [8]. However, if 6-month intervals imaging follows up of BI-RADS 3 results in undetermined radiological lesions, this can initiate anxiety for radiologists, women, and breast surgeons. Also, this follow up

is ineffective when patients do not return for follow-up imaging and that leads to delayed in diagnosis if the progression of lesions occurred over time. Additionally, repeated exposure to ionizing radiation increases the risk of cancer and is costly [8-11].

Therefore, there is a necessity for non-invasive biomarkers that identify precancerous breast lesions. Thymidine kinase 1 is a proliferative biomarker that is associated with a cellular division and intended to be a tumor growth marker [3, 12, 13]. It controls the intracellular thymidine pool via salvage pathway, as it catalyzes the transfer of phosphate group from ATP to 5' hydroxyl group of thymidine to create thymidine monophosphate which is then incorporated in DNA replication [13]. TK1 levels depend on cell cycle considering that its levels are hardly detected in resting cells, but once passing G1 checkpoint its levels increased, reaching the maximum at S phase then disappeared at mitosis [13].

Therefore, this study aimed to provide a non-invasive method to early detect BC by measuring TK1 in sera of women with breast lesions alongside mammography.

2. Subjects and Methods

2.1. Study Design and Subjects

This study included 271 females divided into five categories according to BI-RADS classification. Only one blood sample was collected from each woman enrolled in the study to detect TK1 concentration, before undergoing mammography and Fine Needle Aspiration Cytology (FNAC) or true cut, from January 2014 to June 2017.

All subjects were categorized as either BI-RADS 1, 2, 3, 4, or 5 at the time of recruitment, as determined by mammography, ultrasound, or combination of both modalities if needed. All BI-RADS 3 subjects were followed for three years with 6 months interval by using imaging techniques and pathological examination if necessary. Each woman who participated in this study signed an informed consent form preceding inclusion in the study protocol. This study was approved by the local Ethical Committee. Control subjects had one mammogram minimally with normal results and no history of cancer, while cancer patients did not have previous cancer nor received treatment before sample collection.

We excluded subjects if they had a bacterial or viral infection at presentation, their follow-up was not possible, or they were reluctant to present written informed consent, or their samples were haemolysed.

Tumor stage was categorized according to the TNM-classification, which was conducted correspondingly to the WHO System [14]. The histopathological grading was classified according to the Bloom and Richardson system classification [15]. The characteristics of the subjects were represented in Tables (1 & 2).

2.2. The Detection of TK1

Blood samples were collected using serum separator tubes and clotted for one hour at room temperature then centrifuged

for 15 minutes at 1000 g then the supernatant sera were fractionated and stored at -80°C.

The concentrations of serum TK1 were measured by using a Thymidine Kinase 1 Enzyme-linked Immunosorbent Assay (ELISA) (commercial kit; MyBiosource, Inc., USA). The procedure was implemented according to the manufacturer's protocol; 100µl standard or sample was added to each well then incubated 2 hours at 37°C. The plate was aspirated then 100µl of Biotin-antibody (1x) was put in each well then incubated 1 hour at 37°C.

Each well was aspirated and washed then 100µl of HRP-avidin (1x) was added and incubated 1 hour at 37°C. Each well was aspirated and washed then 90µl of TMB Substrate was added to each well and incubated for 15-30 minutes at 37°C. 50µl of Stop Solution was put then read at 450 nm using 800™ TS Absorbance Reader (Bio Tek Instruments, Inc., USA). Results were expressed as pg/ml. The analytic sensitivity was 15.6 pg/ml. The coefficients of variation within- and between-assays were <8% and <10%, respectively.

Table 1. Subject characteristics.

Characteristics	No. (%)
Age (Years)	
Min. – Max.	24.0 – 87.0
Mean ± SD.	41.4±10.5
Median	41.0
Menopausal Status	
Premenopausal	189 (69.7)
Perimenopausal	52 (19.2)
Postmenopausal	30 (11.1)
BI-RADS	
1	40 (14.8)
2	41 (15.1)
3	100 (36.9)
4	40 (14.8)
5	50 (18.4)

Table 2. Clinicopathological characteristics of breast cancer patients.

Characteristics	No. (%)
Clinical Stage	
I	19 (17.8)
II	43 (40.2)
III	45 (42.0)
Histological Grade	
I	18 (16.8)
II	47 (43.9)
III	42 (39.3)
Lymph Nodes	
+Ve	79 (73.8)
-Ve	28 (26.2)
Vascular invasion	
+Ve	76 (71.0)
-Ve	31 (29.0)

2.3. Statistical Analysis

IBM SPSS software package version 20.0 was used to analyze data. The Kolmogorov-Smirnov test was utilized to verify the normality of distribution. Quantitative data were described using interquartile range (IQR), median, mean and standard deviation. The significance of the obtained results

was judged at the 5% level. Chi-square test, F-test (ANOVA), Receiver operating characteristic curve (ROC) and Spearman coefficient were used [16, 17].

3. Results

3.1. The Study Participants

An overall of 271 women was volunteered for this study with a mean age of 41.4±10.5 years. The majority of them were in premenopausal status (69.7%) and most breast cancer patients had lymph nodes and vascular invasion (74.8% and 71%, respectively). After three years follow up, there were twenty-eight patients with BI-RADS category 3 developed cancer (28/100), after performing fine-needle aspiration cytology. The total number of BC patients was 107 patients.

The clinicopathological characteristics of all women enrolled in the study, including age, menopausal status, BI-RADS categories, and BC patients involving histologic grade, lymph node metastasis, TNM stage, and vascular invasion are summarized in Tables (1 & 2).

3.2. The Concentration of TK1 in Different BI-RADS Categories

There was a highly statistically significant difference in the concentration of TK1 between all BI-RADS categories (1, 2, 3, 4 and 5) as mean ± SD were 158.7±15.52, 207.6±23.60, 227.8±44.55, 251.3±22.46 and 311.5±43.72 pg/ml, respectively, P<0.001. BI-RADS category 1 represented the control group as it was negative for benign and malignant lesions, Figure 1.

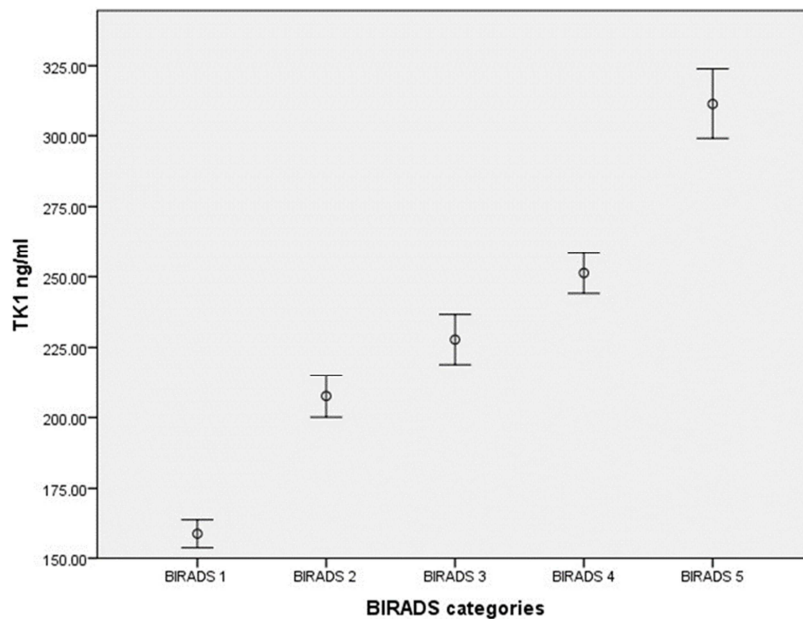


Figure 1. Comparison between different BI-RADS categories dependent on TK1 concentration (mean ± SD).

3.3. The Correlation of TK1 in Patients with Breast Lesions

There was a positive significant correlation between TK1 concentrations and the clinical stage, histological grade, lymph node metastasis and vascular invasion ($r_s=0.321, 0.315, 0.344$ and 0.278 and $P=0.001, P=0.001, P<0.001,$ and $P=0.004,$ respectively). While there was no correlation of TK1 levels versus age and menopausal status ($r_s=0.110$ and 0.087 also, $P=0.070$ and $0.152,$ respectively).

3.4. Receiver Operating Characteristics (ROC) Curve

Area under the curve (AUC) for ROC curve that

differentiated between BI-RADS category 1 and the BI-RADS categories (2, 3, 4 and 5) was 0.973 at a cut off value 175 pg/ml, the sensitivity and the specificity was 91.3% and 87.5%, respectively, Table 3, Figure 2A. While the AUC for the ROC curve that differentiated between benign and malignant breast lesions was 0.968 at a cut off value of 240 pg/ml, the sensitivity and the specificity was 92.5% and 91.2%, respectively, Table 3, Figure 2B.

TK1 concentrations of the 28 cases who developed cancer during follow up in BI-RADS category 3 were higher than the cut off value 240 pg/ml.

Table 3. Results from the ROC curve analysis between different BI-RADS categories dependent on TK1 concentration.

Groups	Youden Index	Cut off (pg/ml)	AUC (CI)	P	Sn	Sp	PPV	NPV	ACC
BI-RADS 1 vs, BI-RADS 2, 3, 4 and 5	0.788	175	0.973* (0.955-0.991)	<0.001*	91.3	87.5	98.17	67.92	92.25
Benign vs, Malignant	0.837	240	0.968* (0.949-0.987)	<0.001*	92.5	91.2	89.19	95.00	92.62

P=probability, Sn=Sensitivity, Sp=Specificity, ACC=Accuracy.

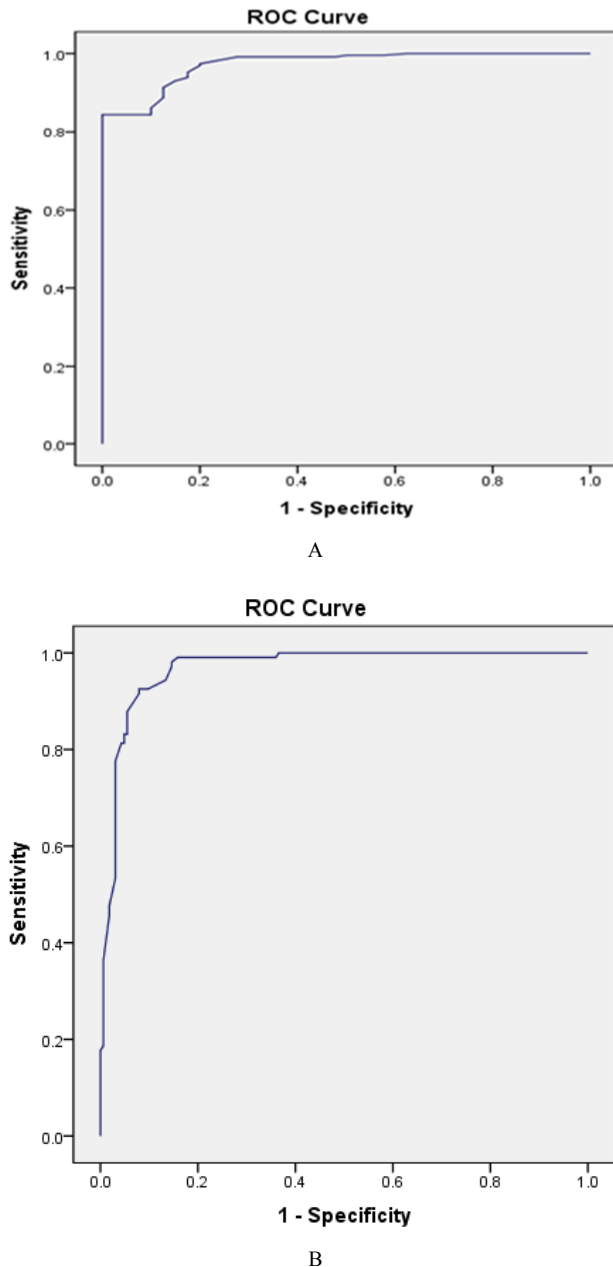


Figure 2. ROC curve analysis for TK1. A. to differentiate between BI-RADS 1 and BI-RADS 2, 3, 4 and 5. B. to differentiate between benign and malignant breast lesions.

4. Discussion

Early detection of BC is a global target for Breast Health Global Initiative (BHGI) and World Health Organization (WHO) particularly in low and middle- income countries to reduce mortality and morbidity also to improve therapeutic and survival outcomes [18, 19].

The non-invasive serological biomarkers may provide data about breast lesions of probable malignancy before the assessment by imaging techniques [20].

Thymidine Kinase 1 is one of the proliferative biomarkers used previously in many screening tests for early detection of cancer. It succeeded in discovering premalignant

transformations of breast cancer before the appearance of any symptoms [20].

In the present study, we observed that the highest levels of TK1 were found in breast cancer patients and the lowest levels were found in the normal individuals. Also, it could discriminate between benign and malignant patients. This was accepted by many previous studies [13, 20, 21].

Our work is the first one to combine between mammography techniques, as a gold standard in the early detection of breast cancer and determination of the concentration of TK1 levels. This simple TK1 test could differentiate between all BI-RADS categories from 1 through 5 including BI-RADS 3 which causes perplexity for radiologists to be assessed [6]. As during follow up of BI-RADS 3, twenty-eight patients who had high TK1 levels developed malignancy later; 82.1% were invasive ductal carcinoma, 14.3% were ductal carcinoma in situ and 3.6% were hepatocellular carcinoma.

Consequently, TK1 could be used in the detection of BC in the premalignant state. Huang S. et. al. [20] and Chen ZH et. al [11]. noticed high TK1 levels in premalignant conditions and the early stages of BC. Previously, TK1 used in screening health programs of cancer and recommended the use of TK1 as a risk predictor biomarker for malignant transformation [11, 21, 22].

Cao X. et. al. [21], Huang S. et. al. [20] Dedicated that TK1 levels were not affected by age of patients and that was in line with our results. Nisman B. et. al. [23] Were found that TK1 levels were not correlated with menopausal status and similarly noticed in this study. Subsequently, it could be used to early assess BC whatever the age of women.

Protein levels of TK1 were positively correlated with lymph node metastasis, clinical stage and histological grade of BC patients which reflected the diagnostic and prognostic value of TK1. A majority of studies to date advocate this correlation where He E. et. al. [24] Confirmed a correlation between tumor and serum TK1 levels with both stage and grade. Kumar J. K. et. al. [25] found elevated TK1 levels in metastatic lymph node patients versus those without these complications.

The mechanism responsible for the discrepancy in TK1 levels among BI-RADS categories and between malignant, benign and normal individuals is still unclear. But there were some possible interpretations; first, due to the presence of a factor in serum modified TK1's stability. Second, normal cells die in the G0 or early G1 in the cell cycle where the levels of TK1 were down, while cancer cells die in the S/G2 phase where TK1 levels were up, consequently liberating more TK1 in serum. Also, there was a difference in the clearance of TK1 between normal and malignant cells [11].

In the present study, AUC, sensitivity, and specificity at a cut off 175 pg/ml were 0.973, 91.3% and 87.5%, respectively, this was in agreement with Chen ZH et. al. [11] that detected AUC and sensitivity were 0.94 and 94.3%, respectively. This indicated that TK1 had high precision in early detecting BC, since $0.9 \geq AUC \leq 1$.

Increasing cut off value to 240 pg/ml to differentiate

between benign and malignant patients resulted in changing of AUC, sensitivity, and specificity as follows 0.968, 92.5% and 91.2%, respectively.

Since BI-RADS 3 was set as a problematic category in BI-RADS Atlas [5] and needed repeating mammography during follow up till assessing upgrading or downgrading of BI-RADS 3 patients, which may cause anxiety/delay in detection, thus adjunction with non-invasive determination of serum TK1 may improve early assessment of BI-RADS 3 and reduce exposure to X-ray.

5. Conclusion

Serum TK1 could detect premalignant transformation before assessment by imaging techniques with a non-invasive, fast, easy, specific and sensitive method. TK1 levels were significantly different among BI-RADS categories. It was strongly positively correlated with clinical stage, histological grade, lymph node metastasis, and vascular invasion. Measuring TK1 in sera of patients that were assigned as BI-RADS 3 may help in upgrading or downgrading this difficult assessment category. These findings suggest the determination of TK1 levels as a risk warning biomarker to improve early detection of BC, enhance accuracy in the assessment of BI-RADS 3 and reduce repeated exposure to X-ray. According to our results, we recommended performing FNAC or true cut for BI-RADS 3 patients with high TK1 levels.

Furthermore, this research needed to be applied to a larger number of BI-RADS 3 cases and follow up women with BI-RADS 1 and 2 who had higher concentrations of TK1 to early detect BC and to reduce their risk to breast cancer. Also, try to study the molecular mechanisms responsible for the elevation of TK1 levels.

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