# Evaluation of Thymidine Kinase-1 as a potential biomarker and its association with CA 15-3 in patients with non-metastatic breast cancer



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**Abstract:**

Breast cancer has been more common among Iraqi women in recent years. Monitoring proteins in human bodily fluids for early stage detection and prognosis of breast cancer is the best way and the first step toward effective therapy, delaying metastases and lowering mortality. In this study, we aimed to evaluate the value of Thymidine kinase-1 as a diagnostic and prognostic marker in non-metastatic breast cancer patients and to analyze the associations between TK1 and standard CA 15-3 biomarker, as well as clinicopathological parameters. This study included 60 newly diagnosed patients, 49 receiving adjuvant hormone therapy, and 50 women who had a benign breast tumour as controls. We measured the serum levels by ELISA technique of TK1 and CA 15-3. The results showed there were a significant difference in the mean serum TK1 level amongst study groups ($p < 0.001$), and showed no significant correlation with serum CA 15-3 in newly diagnosed group, nor in treated group, $p = 0.429$ and $p = 0.545$, respectively. It also showed there were no significant correlations of serum TK1 and CA 15-3 with menopause status, age, BMI, tumor size, node status, grade, tumor type, ER, PR and Her2. Serum TK1 and CA-15-3 were excellent predictors by ROC, 90% and 88.3%, respectively, and the combination of the TK1 ELISA and CA 15-3 biomarkers increased sensitivity to 98.3%.

**Keywords:** Breast cancer, Thymidine Kinase-1, CA 15-3, Tumor.

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## Introduction

Breast cancer (BC) is the most common type of malignant tumors diagnosed in females. In 2020, it was surpassed lung cancer as the most commonly diagnosed malignant, with an estimated 2.3 million new cases, constituting around 11.7% of all cancers in the worldwide (Sung *et al.*, 2021). This cancer has a high ability to metastasis which leading to high mortality. Early detection leads to improvement of its outcome and raised survival rate (Ragab *et al.*, 2018). The use of molecular biomarkers ensures that BC patients receive appropriate therapy. Biomarkers; such as estrogen receptor (ER), progesterone receptor (PR) and HER2 play important roles in predicting the prognosis and determine the specific treatment for each patient (Tarighati *et al*., 2022). About 70% of breast cancer diagnoses are hormone receptor (HR)-positive, and are candidates for hormonal therapy (ET), including tamoxifen and aromatase inhibitors (Robertson *et al.*, 2021).

Treatment decisions are essential for breast cancer patients; Thymidine kinase (TK) is one of the most controversially debated markers. Thymidine kinase (TK) is an enzyme, a phosphotransferase, 2'-deoxythymi-dine kinase, ATP-thymidine 5'-phosphotransferase (Hanousková *et al.*, 2020), that catalyzes the phosphorylation of thymidine by ATP into thymidine monophosphate, synthesizing deoxy-thymidine triphosphate through the salvage pathway of DNA precursor synthesis (McCartney *et al.*, 2019).

There are two isoenzymes of human TK in cell : a cytoplasmic thymidine kinase 1 (TK1) and a mitochondrial one TK2 (Bolayirli *et al.*, 2013). Researchers found differences between the TK isoenzymes in highly proliferative fetal tissue and adult tissue. They identified two isoenzymes that were termed fetal TK (TK1) and adult TK (TK2) (Bitter *et al.*, 2020). TK-1 activity is cell-cycle dependent and shows a different manner in normal proliferating cells compared with tumor cells. In normal proliferating cells, TK1 is low or absent in resting cells, rising at late G1/early S phase and peaking in the S phase then dramatically reduced to undetectable level by the end of M phase (Bagegni *et al.*, 2017)(McCartney and Malorni, 2020). However, TK-1 activity may remain high in G2 and M phases of cell cycle in tumor cell (Jagarlamudi and Shaw, 2018).

TK-2 activity is independent on cell cycle regulation, so remain stable and without any variations during the cell cycle. it is located and needed in mitochondria for DNA synthesis (Jagarlamudi and Shaw, 2018).

In connection with death of proliferated cells, Thymidine Kinase-1 has been proposed as a marker for cell proliferation (Larsson *et al.*, 2020). It is released into blood circulating; hence increased activity or concentrations of sTK-1 have been found in malignancies patients (Tribukait, 2020), hematological and solid tumors including breast, lung, colorectal cancer, among others. Serum TK-1 can be also used as a prognostic marker for monitoring the responses to chemotherapy or surgery (Weagel *et al.*, 2018).

CA 15-3 is the MUC1 gene's protein product. It is a membrane associated glycoprotein that is mostly expressed on the apical side of glandular epithelial cells in alveoli and mammary ducts in normal mammary tissue. The MUC1 gene is usually highly overexpressed in the tissue of malignant breast cancers. Its expression has been shown to be more than 10 times higher in malignant breast carcinomas than in neighbouring normal glandular epithelium (Pedersen *et al.*, 2013; Coppola *et al.*, 2021).

CA 15-3 is a significant breast cancer biomarker that plays a critical role in breast cancer screening and has been widely adopted into clinical routine (Saadati *et al.*, 2019; Li *et al.*, 2020), However, the correlation between this indicator and breast cancer prognosis has not been conclusively established (Li *et al.*, 2020). The concentration of serum carcinoma antigen 15-3 gives valuable information for monitoring therapy, as well as for predicting the early diagnosis of breast cancer recurrence following curative surgery(Hasanzadeh *et al.*, 2018; Hassanpour *et al.*, 2019), especially in patients who cannot be evaluated using radiological methods (Saadati *et al.*, 2019). Since elevated levels of this tumour biomarker in serum tend to rise breast cancer severity or tumour size (Abed *et al.*, 2020).

In conjunction with imaging, disease history, and clinical course, an aberrant level or increase of CA-15.3 helps to diagnose tumour progression in metastatic breast cancer (Clatot *et al.*, 2020; Attia *et al.*, 2021), only, according to current the American Society of Clinical Oncology guidelines (Choudhury and Agarwala, 2018; Li *et al.*, 2020). In contrast, The European Group on Tumor Markers has suggested using CA15-3 and CEA to assess a patient's likely disease course, diagnose disease progression early, and select therapy options in breast cancer (Li *et al.*, 2020). Despite its relationship with BC cells, an increase in CA 15-3 can also be observed in benign illnesses such as liver disease and benign breast, lung, or ovarian disease; hence, it is not regarded a diagnostically specific biomarker for BC (Coppola *et al.*, 2021). Prior to now, there was insufficient evidence supporting the use of CA-15.3 instead of traditional follow-up.

The lack of predictive markers leaves the selection of administering endocrine therapy alone or in combination with other agent to empirical considerations. Given that a large part of HR+/HER2-neg patients will receive extended benefit from (ET) alone (Bonechi *et al.*, 2018), as well as, diagnoses and therapies are incomplete and many patients die due to recurrence; thus, improved diagnosis and prognosis using novel biomarkers is essential for improve the diagnosis and treatment of breast cancer (Ragab *et al.*, 2018).

The aims of this study were to evaluate the values of serum TK1, and CA15-3 as prognostic markers by ELISA in breast cancer patients to analyze the associations between TK1 and other biomarkers in non-metastatic breast cancer patients, and to compareTK1 and CA 15-3 to histological grading.

## Material and methods

From 16 October 2019 to 30 June 2021, this case-control study was done on 159 female patients with breast tumors, both malignant and benign. Women patients were recruited Baghdad Medical City. The practical part was conducted in the Research Laboratories of the College of Medicine/Al-Nahrain University's Department of Chemistry and Biochemistry. These groups consist of the following; (a) **Sixty** newly diagnosed women patients with early stages breast cancer. (b) **Forty-nine** patients with early stages of breast cancer using endocrine therapy for the first time. (c) **Fifty** newly diagnosed women with benign breast tumors as control group. The selection criteria were as follows: (a) All patients were assessed by a consultant physician and diagnosed with early-stages, non-metastatic breast cancer. (b) Absence of metastatic or recurrent breast cancer, as well as no neoadjuvant therapy has been used. (c) Absence of systemic diseases such as hypothalamic or pituitary gland abnormalities, diabetes and hypertension. Chronic inflammatory conditions, hepatic, renal, or cardiac failure. After explaining study objectives and obtaining written informed consent from all patients, baseline demographic and clinical data were collected from participants by interview and recorded using a study questionnaire. Histopathological reports included the patient's age, tumor type, size, grade, nodal status, oestrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor 2 (Her2neu) status. The study was approved by the Institutional Review Board of the College of Medicine at Al-Nahrain University (approval date: 10/14/2019 and approval number: 5/1/52/1571).

## Statistical Analysis

Statistical analysis was performed using SPSS program (version 22). Because all continuous variables were normally distributed, data were presented as mean ± standard deviation (mean±SD) and all statistical comparisons were done using the independent t-test, with a P value of 0.01 regarded statistically significant. Spearman rank correlation was used to analyze the relationship between the variables. Analyses were performed as a two-tailed, and the descriptive level of significance was set at $p< 0.001$. The Receiver Operating Characteristic "ROC" curve technique was used to establish the utility of any parameter as a diagnostic or screening tool for disease, as well as the capacity to calculate the "cut-off value" with the highest sensitivity and specificity for disease diagnosis.

## Results

159 patients were examined during the study period. The patients under study were divided into three groups; 60 newly diagnosed women with breast carcinoma, 49 women with breast carcinoma receiving adjuvant hormonal treatment, and 50 women with benign tumors as control (Table 1.1)

**Table 1.1:** Comparison of mean age and frequency distribution according to age among study groups

| Characteristic | Control group *n* = 50 | Newly diagnosed BC *n* = 60 | Treated BC *n* = 49 | *p* |
|---|---|---|---|---|
| **Age (years)** | | | | |
| Mean ±SD | 45.79 ±10.46 | 50.02 ±10.68 | 47.67 ±10.59 | 0.279 O |
| Range | 22 -70 | 32 -75 | 27 -75 | NS |
| 22-29, *n* (%) | 2 (4 %) | 0 (0.0 %) | 2 (4.1 %) | |
| 30-39, *n* (%) | 16 (32 %) | 10 (16.7 %) | 8 (16.3 %) | |
| 40-49, *n* (%) | 12 (24 %) | 19 (31.7 %) | 17 (34.7 %) | |
| 50-59, *n* (%) | 11 (22 %) | 18 (30.0 %) | 17 (34.7 %) | |
| 60-69, *n* (%) | 8 (16 %) | 11 (18.3 %) | 4 (8.2 %) | |
| 70-75, *n* (%) | 1 (2 %) | 2 (3.3 %) | 1 (2.0 %) | |

***n:** denotes the number of cases; **SD**: is the standard deviation; **BC:** denotes breast carcinoma; **O:** denotes one-way ANOVA; **NS:** denotes not significant at p > 0.05.*

The results showed there no statistically significant differences in mean age between study groups ($p$ = 0.279). The mean age of control group was 45.79 ±10.46 years, that of newly diagnosed

women with breast carcinoma was 50.02 ±10.68 years and that of women with breast carcinoma receiving hormonal treatment was 47.67 ±10.59 years. The frequency distribution according to age showed that the majority of women with breast cancer were between 40 to 49 and 50 to 59 years.

The frequency distribution of breast cancer patients and control women by menopausal status showed that the control group included 28 (56 %) pre-menopausal women and 22 (44 %) post-menopausal women, the group of newly diagnosed breast cancer included 32 (53.3 %) pre-menopausal women and 28 (46.7 %) post-menopausal women, and the group of treated breast carcinoma included 29 (59.2 %) pre-menopausal women and 20 (40.8 %) post-menopausal women. There was no statistically significant difference in the frequency distribution of women with breast cancer and controls according to menopausal status (p = 0.698).

**Evaluation of serum markers levels among study groups**

The serum levels of TK-1and CA-15-3 contrasted among study groups are summarised in Table 2. There was a significant difference in the mean TK-1 and CA 15-3 levels amongst study groups ($p < 0.001$); the level was highest in newly diagnosed group followed by treated group and then by control group.

**Table 2:** The serum levels of TK-1 and CA-15-3 contrasted among study groups

| **Before** | **Control group** ***n*** **= 50** | **Newly diagnosed BC** ***n*** **= 60** | **Treated BC** ***n*** **= 49** | ***p*** |
|---|---|---|---|---|
| **TK-1 (pg/ml)** | | | | |
| Mean ±SD | 1.28 ±0.61 C | 2.86 ±1.05 A | 2.38 ±0.95 B | < 0.001 O ** |
| Range | 0.74 -2.84 | 1.21 -5.41 | 0.58 -4.59 | |
| **CA 15-3 (U/ml)** | | | | |
| Mean ±SD | 10.17 ±3.29 B | 36.06 ±19.83 A | 11.12 ±3.15 B | < 0.001 O ** |
| Range | 5.83 -17.40 | 11.23 -118.33 | 5.15 -18.98 | |

***n***: denotes the number of cases; **BC**: breast carcinoma; **SD**: denotes standard deviation; **O**: denotes one-way ANOVA; **NS**: denotes not significant at $p > 0.05$; **: denotes significant at $p \leq 0.01$; capital letters A, B, and C were used to express level of significance according to the post hoc LSD multiple comparison test; in such a way that different letters indicate significant difference while similar letters indicate no significant difference; and letter A takes the highest mean value followed by B and then C.

**Correlation between serum biomarkers concentrations:**

Serum CA-15-3 showed no significant correlation with serum TK1 in in newly diagnosed group, nor in treated group, p = 0.429 and p = 0.545, respectively.

**Correlations of serum markers with other parameters**

Correlations of serum markers with other parameters in group of newly diagnosed breast cancer are shown in table 3. There were no significant correlations of serum TKI and CA 15-3 with menopause status, age, BMI, tumor size, node status, grade, tumor type, ER, PR and Her2.

**Table 4.11:** Correlations of serum markers with other parameters in group of newly diagnosed breast cancer

| **Characteristic** | **TK-1** | **CA 15-3** |
|---|---|---|

| | *r* | *p* | *r* | *p* |
|---|---|---|---|---|
| menopause status | -0.071 | 0.591 | 0.162 | 0.215 |
| age | -0.113 | 0.388 | 0.180 | 0.170 |
| BMI | -0.007 | 0.957 | 0.090 | 0.492 |
| tumor size | -0.101 | 0.441 | 0.211 | 0.106 |
| node status | 0.037 | 0.777 | -0.208 | 0.110 |
| grade | 0.024 | 0.857 | -0.080 | 0.546 |
| type | 0.030 | 0.822 | 0.175 | 0.182 |
| ER | -0.016 | 0.904 | 0.029 | 0.825 |
| PR | 0.103 | 0.432 | 0.076 | 0.563 |
| Her2 | -0.028 | 0.832 | -0.121 | 0.358 |

**The diagnostic potential of serum markers in detecting breast carcinoma**

A receiver operator characteristics curve analysis was performed for serum markers and the results are displayed in table 4 and 5, and figure 1 and 2. Serum TK-1 and CA-15-3 were showed excellent predictors.

**Table 5:** Receiver operator characteristics (ROC) curve parameters of serum markers

| Characteristic | Serum TK1 | CA 15-3 |
|---|---|---|
| Cutoff | >1.67 | >17.4 |
| AUC | 0.919 | 0.977 |
| 95 % CI | 0.836 to 0.969 | 0.915 to 0.998 |
| *p* | < 0.001** | < 0.001** |
| Sensitivity % | 90.0 | 88.3 |
| Specificity % | 84.2 | 100.0 |
| Accuracy % | 91.9 | 97.7 |

AUC: denotes area under the curve; CI: denotes confidence interval; **NS**: denotes not significant at $p > 0.05$; **: denotes significant at $p \leq 0.01$.

**Table 5:** Sensitivity and specificity of combined markers

| Characteristic | TK1 | CA 15-3 | TK1/CA15-3 |
|---|---|---|---|
| Sensitivity % | 90.0 | 88.3 | 98.3 |
| Specificity % | 84.2 | 100 | 84.2 |
| Accuracy % | 91.9 | 97.7 | 94.9 |

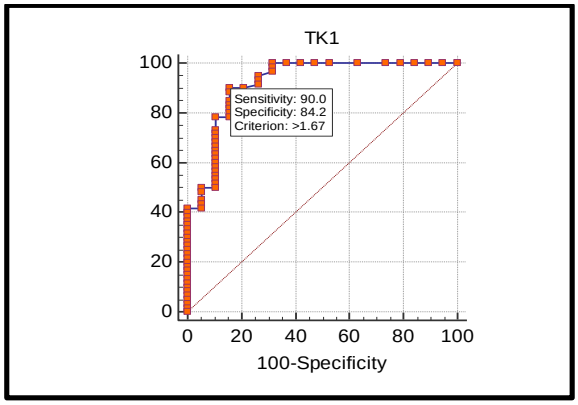


**Figure 1:** A receiver operator characteristic (ROC) curve to find the optimal TK-1 cutoff value for accurately diagnosing breast cancer.

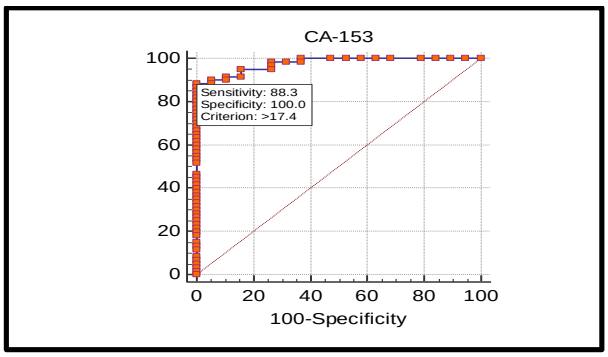


**Figure 3:** A receiver operator characteristic (ROC) curve to find the optimal CA-15.3 cutoff value for accurately diagnosing breast cancer.

## Discussion

Breast cancer is the most prevalent female cancer in the world and the second greatest cause of cancer-related death; therefore, it is essential to identify reliable prognostic markers that may identify patients at high risk of recurrence and select the most appropriate therapy for them (Ragab *et al.*, 2018). Since cancer is characterized by unrestricted cell expansion, cancer could be diagnosed using markers of cellular proliferation (Li, Wei and Song, 2018). As this enzyme plays a crucial role in pyrimidine deoxynucleotide synthesis, elevated levels of TK1 in tumor cells correlate with their proliferation. Due to this property, TK1 has been utilized as a proliferation marker in lymphomas and leukemias for many years in human medicine (Hallek *et al.*, 1999). Several commercial assays, such as TK-REA, TK-Liaison, and DiviTum, are available to evaluate the TK1 activity levels in different malignancies. Despite the fact that TK1 activity levels correlate with the stage of breast cancer, the overall sensitivity of the assays, which is less than 35%, is quite low (Nisman *et al.*, 2013). This restricts the clinical application of TK1 activity tests in patients with breast cancer.



Antibodies developed against the C-terminal portion of TK-1 give an alternate method for determining TK1, and in this situation, TK1 protein levels are tested. Using a dot blot assay, previous investigations demonstrated that TK1 protein levels were considerably elevated in the sera of patients with a variety of solid tumor types (Kumar *et al.*, 2016). A recent study using an immunoaffinity assay revealed that TK1 protein levels are considerably elevated in the sera of patients with early-stage breast and prostate cancer, but not TK1 activity. A possible explanation is that there was a greater proportion of inactive TK1 in the serum of patients with solid tumors than in the sera of leukaemia patients (Jagarlamudi, Hansson and Eriksson, 2015).

The present study showed a significant difference in mean TK-1 level among study groups; the level was highest in newly diagnosed group followed by treated group and then by control group, with excellent predictors, when it carried out by receiver operating characteristics curve (ROC).

Similar to our study, Hei et al. (Hei, 2020) in their meta-analysis , and Nisman et al. (Nisman *et al.*, 2013) reported that serum TK-1 level is significantly elevated in patients with breast cancer in comparing to breast benign tumor group. Under normal cellular decay mechanisms, TK1 is degraded prior to cell classification and its concentration is less than 1 pmol/L. When cancer cells proliferate, predominantly in the S and G phases, considerable levels of intracellular TK1 are released, resulting to a significant rise in its serum concentration (Li, Wei and Song, 2018).

Also the present study showed there was no significant correlation with Clinico-pathological characteristics; tumor size, nodal status, grade, ER/PR, HER2 status and CA 15-3 as standard biomarker. In Contrast, previous studies showed that the TK-1 activity level was positively significant associated with T-stages (Kumar *et al.*, 2016), tumor grade and lack of ER/PgR expression (Nisman *et al.*, 2013), and TK1 activity and TK1

level values with the CA 15-3 values (Bolayirli *et al.*, 2013; Kumar *et al.*, 2016). In our study, number of patients was limited, and patients have been staged into I and II according to TNM system, therefore they are closely similar in their clinco-pathological characteristics, so no statistical significant differences were shown.

Several studies have been conducted to evaluate the clinical use of CA 15-3 as a breast cancer biomarker. CA 15-3 has a limited sensitivity for detecting early-stage tumors (Duffy, 2006), However, it is commonly employed for monitoring therapy in patients with advanced breast cancer (Park *et al.*, 2008). The sensitivity of the CA 15-3 test for breast cancer was up to 50% (Pedersen *et al.*, 2013). A combination of CA 15-3, CEA, and HER 2 enhanced sensitivity by 64% in metastatic patients (Lumachi F., 2004). Recent research indicates that the combination of CEA, CA 15-3, and TK1 protein enhanced sensitivity by 90% (Elfagieh *et al.*, 2012). Other research suggests that the combination of CA 15-3 and TK1 protein increased sensitivity from 63% and 61% for each separately to 76% (Kumar *et al.*, 2016). In the present study, the combination of TK-1 and CA 15-3 biomarkers raised the sensitivity from 90% and 88%, respectively, to 98%.

Consequently, the results of this study clearly imply that a combination of TK-1 ELISA and CA 15-3 will be extremely useful, as the two tests appear to provide complimentary information.

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