

*Research Article***Serum Thymidin Kinase 1 activity as a prognostic factor in breast cancer patients treated with adjuvant chemotherapy****Mohamed S. Allam, MD.* Samer S. Mohammad, MD.*****Mohamed S. Zaghlol, MD.* Ahmed M. Abdullah, M.B.B.Ch, MSc.***

Department of Clinical Pathology , Faculty of Medicine, Al Azhar University, Assiut, Egypt

Abstract

Objective: The aim of this study was to evaluate the changing of TK1 (where TK is thymidine kinase) activity before and after adjuvant chemotherapy as prognostic factor in patients with breast cancer. **Patients and methods:** 40 patients with breast cancer lesion were included in the study and 40 healthy volunteers . The patient's age ranged from 28 to 76 years old with the mean age \pm 38.24 year. The all patients were referred from the Oncology department to Clinical pathology department AT Al-Azhar Assiut University Hospital during the period from November 2017 to October 2018. **Results:** In the current study in the breast cancer group, the highest TK activity was observed than in control group & in the breast cancer group, the highest TK activity was observed pretherapy than post therapy group. **Conclusion:** Based on the results of this study we can conclude the following: This study demonstrates that TK1 is a promising serum marker in breast cancer. High serum TK1 activity, which is associated with aggressive features of primary breast cancer, was found to be associated with a cancer recurrence.

Keywords: Breast cancer lesions, Assessment of TK1 using Human thymidine kinase 1by ELISA This assay employs the quantitative sandwich enzyme immunoassay technique.

Introduction

Cancer is a leading cause of death in the world, and the number of affected individuals is increasing, although different methods of treatment for cancer, for example, surgery, radiotherapy, chemotherapy, and endocrine therapy have been improved tremendously in recent years. Cancer can be treated effectively, if discovered at an early stage. Diagnostic and prognostic markers play a key role in classifying tumors and determining the best treatment plan for a patient⁽¹⁾.

Breast cancer is a malignant tumor that starts in the cells of the breast. A malignant tumor is a group of cancer cells that can grow into (invade) surrounding tissues or spread (metastasize) to distant areas of the body. The disease occurs almost entirely in women, but men can get it, too⁽²⁾.

Thymidine kinase (TK) is a cellular enzyme involved in a salvage pathway for DNA synthesis. There are two isoforms of this enzyme: TK1 and TK2. TK1 is found in the

cytoplasm of dividing cells and is absent in resting cells. TK2 is located in the mitochondria of resting cells. TK1 is a soluble biomarker associated with DNA synthesis which has been used for prognosis and monitoring of treatment of lymphoma and leukemia since 1980, and also to some extent in patients with solid tumors⁽³⁾.

Patients and method

The study included 40 breast cancer patients and 40 healthy volunteers as the control group.

For all patients we did the followings:

- CBC
- Liver function tests
- Renal function tests
- ESR
- CA 15-3
- TK1 activity was measured enzyme immunoassay method.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. Antibody specific for TK1 has been pre-coated onto a microplate.

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. Centrifuge the sample again after thawing before the assay. It is recommended that all samples and standards be assayed in duplicate.

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Refer to the Assay Layout Sheet to determine the number of wells to be used and put any remaining wells and the desiccant back into the pouch and seal the ziploc, store unused wells at 4°C.
3. Add 100µl of standard and sample per well. Cover with the adhesive strip provided. Incubate for 2 hours at 37°C. A plate layout is provided to record standards and samples assayed.
4. Remove the liquid of each well, don't wash.
5. Add 100µl of Biotin-antibody (1x) to each well. Cover with a new adhesive strip. Incubate for 1 hour at 37°C. (Biotin-antibody (1x) may appear cloudy. Warm up to room temperature and mix gently until solution appears uniform.)
6. Aspirate each well and wash, repeating the process two times for a total of three washes. Wash by filling each well with Wash Buffer (200µl) using a squirt bottle, multi-channel pipette, manifold dispenser, or autowasher, and let it stand for 2 minutes, complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
7. Add 100µl of HRP-avidin (1x) to each well. Cover the microtiter plate with a new adhesive strip. Incubate for 1 hour at 37°C.

8. Repeat the aspiration/wash process for five times as in step 6.

9. Add 90µl of TMB Substrate to each well. Incubate for 15-30 minutes at 37°C. Protect from light.

10. Add 50µl of Stop Solution to each well, gently tap the plate to ensure thorough mixing.

11. Determine the optical density of each well within 5 minutes, using a microplate reader set to 450nm. If wavelength correction is available, set to 540 nm or 570nm. Subtract readings at 540 nm or 570 nm from the readings at 450 nm.

This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

Statistical analysis

The collected data were revised, organized, tabulated and statistically analyzed using statistical package for social sciences (SPSS) version 23.0 for windows. Data are presented as the Mean \pm standard deviation (SD), frequency, and percentage. Categorical variables were compared using the chi-square (χ^2) and Fisher's exact tests (if required). Continuous variables were compared by the Student t test (two-tailed) and one – way ANOVA test for parametric data with Bonferroni post hoc test to detect differences between subgroups.

Results

The study included 40 breast cancer patients and 40 controls .

Table (1): Comparison of baseline serum TK of studied cases n=40 and healthy controls n=40

Parameters	Cases N=40		Controls N=40		P value
	Mean	SD	Mean	SD	
TK (Du/L)	107.5	37.14	56.175	8.4	<0.001 S

+Data are presented as mean \pm standard deviation.

*Independent sample t-test was used

+p-value is significant if $p < 0.05$.

Table (1) and figure (1) clarify that in the breast cancer group, the highest TK activity was observed than in control group (107.5 \pm 37.14 VS 56.175 \pm 8.4; $P < 0.001$).

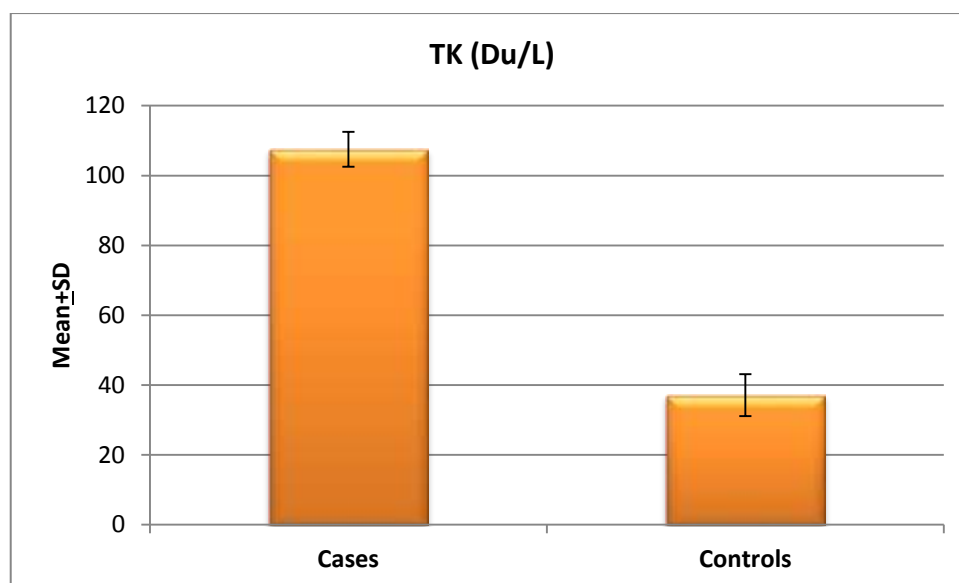


Fig. (1): Comparison of baseline serum TK of studied cases and healthy controls

Discussion

Cancer is a leading cause of death in the world, and the number of affected individuals is increasing, although different methods of treatment for cancer, for example, surgery, radiotherapy, chemotherapy, and endocrine therapy have been improved tremendously in recent years. Cancer can be treated effectively, if discovered at an early stage. Diagnostic and prognostic markers play a key role in classifying tumors and determining the best treatment plan for a patient⁽⁴⁾.

Breast carcinoma (BC) is the most frequent carcinoma and the second commonest cause of death from malignant disease among women in the world. Disease-free interval (DFI) and overall survival (OS) have been obtained with the extensive use of adjuvant systemic therapies. The possibility of having strong prognostic and/or predictive markers is of the utmost importance for clinicians in order to identify patients at higher risk of relapse and to select the most appropriate systemic treatment for an individual patient. Prognostic factors are those that predict the risk of recurrence or of death from BC independently of treatment. Predictive factors are those that distinguish between patients who are more or less likely to respond to a given therapy⁽⁵⁾.

The function and role of TK1 in neoplastic disease has therefore been extensively studied, primarily as a diagnostic biomarker for a variety of cancer types. As a biomarker, higher serum TK1 activity levels correlate with a more advanced cancer stage and grade⁽⁶⁾.

Serum TK1 levels also show prognostic potential, as their levels help predict future relapse at the time of primary diagnosis in breast and colorectal cancer patients⁽⁷⁾.

Conclusion

Based on the results and discussion of this study we can conclude the following:

This study demonstrates that TK1 is a promising serum marker in BC. High serum TK1 activity, which is associated with aggressive features of primary breast cancer, was found to be associated with a cancer recurrence.

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