Journal of Basic and Environmental Sciences

ISSN
Online:2356-6388
Print:2536-9202

Research Paper Open Access

The prognostic value of P53 and CA15.3 tumor markers in breast cancer patient

[1]Noha B.E Tohamy,[2] Nassr Allah Abd El-Hameid, [3]Reem El Shenawy, [4]Loay Kassem, [2]Mohamed N. Seddek

[1] Radioisotopes Department, Nuclear Research Center, Egyptian Atomic Energy Authority, Egypt.
[2] Department Zoology Faculty of Sciences, Benha University, Egypt
[3] Departments of Biotechnology Research Institute, Microbial Biotechnology Division, National Research Centre, 12622, Cairo, Egypt
[4] Clinical Oncology Department, Kasr Alainy School of Medicine, Cairo University Cairo, Egypt. Medical Oncology Unit, Cairo Oncology Center, Cairo, Egypt

Abstract

Breast cancer is the second most prevalent malignancy among women. The cancer antigen CA 15-3 has been used as a putative serum marker of occult and recurring breast carcinoma .The p53 gene is mutated in human cancer. Immunohistochemistry reveals that the mutant p53 protein is more stable and has a longer half-life compared to the wild type. This study aims to estimate the role of the p53 protein in diagnosing and confirming breast cancer compared to cancer antigen 15-3 (CA15-3.The current research involved 68 women diagnosed with breast cancer who were aged 30-60 years old. The control group consisted of 10 healthy women. Before surgery, sera samples were collected. The Sandwich-ELISA technique measures the amount of p53 protein in sera. An immunoradiometric assay (IRMA) kit was used to measure the sera CA 15-3 levels. The study found a higher level of CA15-3 In breast cancer patients (186.7±218) compared to healthy control groups (8.88±3.7). There are no significant associations between p53 protein levels and patient clinical features such as age and grade. Furthermore, the p53 protein value did not correlate with serum CA 15-3.

Key words: Breast cancer, Tumor marker, CA15-3, p53 protein.

1. Introduction

Breast cancer is one of the most prevalent human tumors, accounting for roughly 25% of all malignancies in females globally. Breast cancer is the most prevalent cancer among women in Egypt, accounting for 38.8% of all cancer cases. The death rate from breast cancer is thought to be roughly 11% **Alorabi et al. [1].** Breast cancer may develop in any of the mammary gland's cells and has a wide

range of morphological traits, diverse immunohistochemistry profiles, and distinct histological subtypes with varying clinical courses and outcomes **Makki [2].** Infiltrating duct carcinoma account for 83.2% of all tumors histologists, followed by infiltrating lobular carcinoma (9.1%) and medullary carcinoma (3.2%). The triple-negative subtype is the second most common subtype (28.5%), followed by the Her2-expressing subtype (19.4%), luminal A subtype (41.2%), and luminal B subtype (13.9%) **Gany et al. [3]**

Breast cancer often presents with no symptoms in its early stages, leading to poor treatment outcomes and a poor prognosis. Early detection through non-invasive methods is crucial for improving clinical outcomes and lowering mortality rates **Abas et al. [4].**

Breast cancer has become extremely difficult to treat; it requires the use of biomarkers to predict, screen, and identify treatment as early as possible. Biomarkers determine prognosis without other considerations. These markers have a direct impact on illness recurrence and mortality. Markers can predict patient responses to therapy **Zidan et al. [5].**

Tumor markers are substances that are produced by tumors or other cells in the body in response to cancer or other benign conditions. Normal cells also produce these markers, but malignant cells produce them in higher amounts. These markers are used to assess the patient's response to treatment as well as to detect metastases or recurrence. CA15-3 and p53 are tumor markers that are commonly seen in breast cancer patients. In breast cancer patients, they serve an important role in diagnosis, monitoring response to therapy, early detection of metastases, and determining recurrence **Yang et al. [6].**

The carbohydrate antigen 15-3 (CA15-3) is the most common blood marker for breast cancer.CA15-3 is a glycoprotein found on the cell surface, produced from the MUC1 gene. This protein is found on the surface of many epithelial cell types and is overexpressed in 90% of breast cancers. The raised level of CA15-3 is used to predict relapse in breast cancer patients and evaluate therapy response at late stages **Hasan [7].** CA15-3 can be used to detect recurring diseases and track how well patients are responding to treatment, although it has limited sensitivity in the early stages of breast cancer diagnosis.

The TP53 gene, found on the short arm of chromosome 17 (17pl3.1), encodes p53. It is a complex that contains 393 amino acids and seven domains. Normal cells contain minuscule amounts of it **Synnott et al. [8]**. P53, also known as protein 53 or tumor protein P53 is a nuclear protein that governs cell cycle progression and functions as a tumor suppressor, impeding cancer development. The cell cycle upon DNA damage identification and the induction of death if the DNA damage is irreparable **Zhao and Sanyal [9].**

P53 mutations are common in breast cancer, with rates ranging from 15-50% depending on the TNM staging of the disease and the technique used for detection. Non-invasive or less progressed breast cancer has a lower rate of changes; for in situ disease, the incidence of mutation is about 15%; for invasive and metastatic disease, it is 2 to 3 times greater **Duffy et al.[10].**

2. *Materials and Methods*

2.1. Sample Collection

This study was performed at the Kasr Al Ainy Centre of Clinical Oncology and Nuclear Medicine (NEMROCK) Cairo University Egypt. Every subject (patients and controls) had three milliliters of their blood drawn were transferred to sanitized test tubes. The samples were then centrifuged at 4000 rpm for 15 minutes to separate it, and the sera were then extracted and stored at -20 °C until it was examined.

Ethical Statement: This work was approved by Faculty of Sciences, Benha University Ethics Committee. Approval number and date: BUFS-REC-2024-185 ZOO.

2.2. Determination of serum CA 15-3

An Immunoradiometric Assay (IRMA) kit (Diasource, Belgium) was used to measure the blood CA15-3 level by the manufacturer's instructions. Briefly, serum was introduced to a plastic tube coated with Mab1, the capture antibody, and incubated it at room temperature for 90 minutes. Following washing, the reaction tubes were shaken for ninety minutes at room temperature before 125 Iodine-labeled anti-CA15-3 reagent (Mab2) was added. Following washing, radioactivity of each tube was counted for 60 seconds using a gamma counter. We applied computer-assisted data reduction to simplify the computations. We determined the concentrations of CA15-3 in every serum sample using the 5-parameter logistic function curve

2.3. Determination of serum p53 levels

The levels p53 in sera were determined using the Sandwich ELISA kit (Elabascience, USA) The ELISA plate that was provided in this kit has been pre-coated with an antibody specific to HumanTP53. Standards or samples are added to the ELISA plate wells and mixed with the appropriate antibody. Then, a biotinylated detection antibody specific for HumanTP53 and an Avidin-Horseradish Peroxidase (HRP) conjugate were added to each microplate well and incubated. Free components are swept away. The substrate solution is applied to each well. Only wells containing HumanTP53, biotinylated detection antibody, and Avidin-HRP conjugate will be blue. The addition of stop solution terminates the enzyme-substrate reaction and causes the color to turn yellow. Optical density (OD) is measured spectrophotometrically at 450 nm ± 2 nm. P53 serum concentrations were determined using standard curves.

2.4. Statistical Analysis

The study data were entered and coded using the Prism Graph Pad version 9. Data was summarized using mean and standard deviation. Comparisons between groups were done using Mann-Whitney test. ROC curve was constructed with area under curve analysis performed to detect best cutoff value of P53 and CA15-3 in breast cancer patients. *P*-values less than 0.05 were considered as statistically significant.

3. Results

This study was conducted on 68 with clinical Grade II , Grade III and metastasis breast cancer prepared to receive preoperative neoadjuvant chemotherapy with an association of Paclitaxe & Docetaxel .The normal healthy group served as the negative group (n=10), whereas breast cancer

patients who had not received therapy were employed as the positive group (n=5).

Clinicopathological characteristics of the 68 patients are shown in **Table 1**. The median age of the patients was 51.5 years (range, 30-60 years). A total of 68 patients had invasive ductal carcinoma (IDC).

Table 1: Clinicopathological characteristics patients

		Number of cases	%
Ag	< 45	25	36.8%
	> 45	43	63.2%
Menopausal status	Post	27	39.7%
	Pre	41	60.3%
Tumor size	T0-T1	15	22%
	T2	22	32.3%
	T3	20	29.4%
	T4	11	16.2%
Lymph node	N0	16	23.5%
	N1	30	44.1%
	N2	14	20.5%
	N3	8	11.7%
Metastasis	Yes	15	22.1%
	No	53	77.9%
Tumor grade	G1	0	0%
	G2	34	50%
	G3	19	27.9%
Estrogen receptor (ER)	Positive	42	61.7%
	Negative	26	38.3%
Progesterone receptor (PR)	Positive	45	66.2%
	Negative	23	33.8%
HER2	Positive	22	32.3%
	Negative	46	67.7%
Ki67	Low	57	83.8%
	High	11	16.2%

Regarding the evaluation of biomarkers in this study relates to age subgroups of patients, as shown in **Table 2** there is no significant difference in the levels of both tumor markers P53 and CA15.3 among the age of all subgroups in breast cancer patients group.

Table 2: Demonstrates variations in the tumor marker measurement across the age subgroups for patients; values were given as mean ± SD.

Age (subgroup)	P53 Mean±SD	CA 15-3 Mean±SD
30-39 (n=8)	**0.928±1.4**	**219±282**
40-49 (n=24)	**0.875±1.3**	**193.8±202**
50-59 (n=28)	**0.933±1.5**	**187±24.7**
≥60 (n=8)	**0.647±0.5**	**122.7±46.2**
P value	**0.964**	**0.853**

However, the data from the present study highlighted differences in the assessments of serum tumor markers P53 and CA15-3 between the negative group and the BC patients group. The study found a significant rise (P<0.0001*) in serum levels of CA15-3 in BC patients group BC patients compared to the negative control group. The study found that P53 levels in serum were not significant, as shown in **Table 3**.

Table 3: the measurement s of serum tumor markers between negative group and BC patients, values were expressed Mean ±SD

	Control n=10	Patients n=68	P value
P53	**0.429±0.14**	**0.879±1.36**	**0.6241**
CA15-3	**8.88±3.7**	**186.7±218**	**0.0001***

* Significance was considered at *p*-value <0.05

Regarding the TNM staging of breast tumors, the results revealed a clearance difference in the evaluation of tumors markers between patient groups classified according to Stage of breast cancer, and there is high significance in the level of P53 among all stages of BC patients. Also, the current study was revealed that there is no significant difference in the level of CA15-3 tumor marker among all TNM stage of BC, as shown in **Table 4**.

Table 4: Variation in tumor markers measurement among patient subgroups according to TNM staging of breast cancer

	Stage I Mean±SD	Stage II Mean±SD	Stage III Mean±SD	Stage IV Mean±SD	P value
P53	**0.7±0.4**	**0.66±0.5**	**0.45±0.19**	**0.36±0.3**	**0.030***
CA15-3	**137.3±25**	**216±246**	**192.2±262**	**150.5±64.2**	**0.453**

* Significance was considered at *p*-value <0.05

On the other hand, as shown in **Table 5, the** level of the CA15-3 tumor marker is highly significant among all grades of BC patients. Also, the current study revealed that there is no significant difference in the level of P53 among all grades of BC.

Table 5: Variation in tumor markers measurement among patient subgroups according to grade of breast cancer

	Grade II	Grade III	P value
P53	**0.96±1.5**	**0.793±1.2**	**0.325**
CA15-3	**213±224**	**158.7±210**	**0.0022***

* Significance was considered at *p*-value <0.05

The result documented in **Table 6** revealed that, there wasn't significant correlation between p53 expression and CA15-3 tumor marker protein level in breast cancer patients *p*=0.5403

Table 6: Correlation between p53 and CA15-3 expression

	R	**95% confidence interval**	**P value**
P53 vs CA 15-3	**0.07556**	**-0.1728 to 0.3149**	**0.5403**

* Significance was considered at *p*-value <0.05.

The cut-off values of p53 and CA15-3 for predicting breast cancer were assessed by ROC analysis as shown in **Table 7**. The cut-off value was determined at 0.389 ng/ml for p53 with a sensitivity of 56.52%, specificity of 60%, (AUC 0.5493) and p value 0.6162. The cut-off value for CA15-3 was determined at > 15.95 U/ml with a sensitivity of 97.07%, specificity y of 100 %,(AUC 0.9838) and p value <0.0001* as shown in Figure 1&2.

Table 7: The Area under the ROC curves, sensitivity, and specificity for serum p53 protein and CA 15-3 in breast cancer patients

	AUC**	**Cut- off value**	**Sensitivity%**	**95% CI**	**Specificity%**	**P value**
Circulating p53	**0.5493**	**0.389**	**56.52%**	**44.79-67.57**	**60%**	**0.6162**
Circulating CA15-3	**0.9838**	**> 15.95**	**97.07%**	**89.90-99.48**	**100%**	**<0.0001***

*Significance was considered as P<0.05; **AUC: Area under the curve

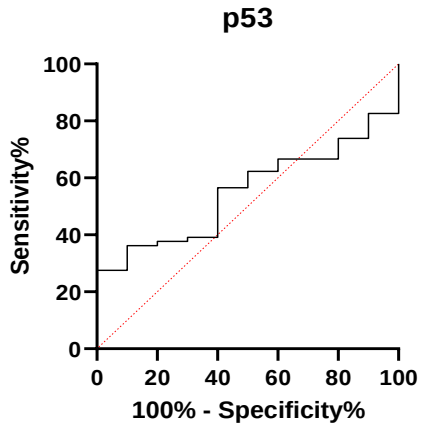

Figure1. ROC curve for serum P53 in breast cancer patients

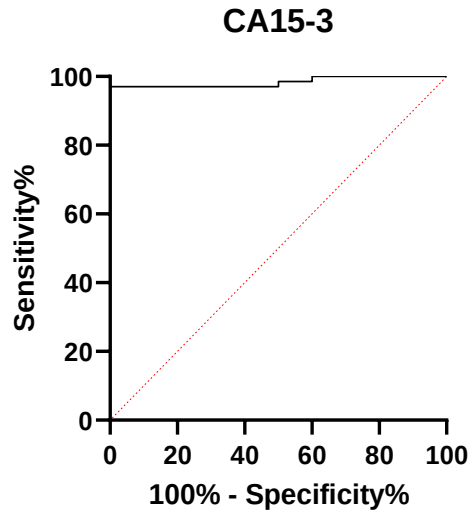

Figure2. ROC curve for serum CA 15-3 in breast cancer patients

4. *Discussion*

Breast cancer has a wide range of aetiologies, with some individuals experiencing gradual growth and a good prognosis, while others experience rapid progression. The stage of diagnosis and the biological properties of the tumor influence the chance of recurrence and prognosis. An important goal of breast cancer diagnosis is to increase the accuracy of biomarkers for early disease detection **Abas et al. [4].**

The most recent research has focused on the role of apoptosis in cancer, specifically breast cancer. Researchers are presently studying apoptotic indicators to identify cancer progression and response to various chemotherapeutic treatments. The most common genetic alteration in human tumors is the P53 mutation. Many types of human malignancies have mutations in the p53 tumor suppressor gene. Breast cancer with a p53 mutation is more aggressive and has a worse overall survival rate. Compared to other solid tumors, breast cancer has a lower frequency of p53 mutations **Arora et al. [11].**

Regarding the measures of tumor markers in this study related to subgroups of the age of patients there is no significant difference in the levels of both tumor markers p53and CA15.3 in patients compared to all age subgroups. This result agrees with **Homaei Shandiz et al.[12]** demonstrated that CA15.3 is a well-known tumor marker, considering that increased levels of this marker can be a significant risk, proving that the expression of p53 was not age-related.

The expression level p53 in normal cells is measured in minutes, and its half-life is extremely brief due to proteasome destruction and ubiquitination. The study revealed no significant difference in the serum levels of P53 between the negative control group and BC Patients. Conversely, the results showed a considerable increase in the levels of CA15-3 in the blood serum of BC patients who tested positive, compared to the negative control group.

With regard to the TNM staging of breast tumor, the results showed that there are clearance differences in the measurement of tumor markers between

patient groups categorized according to the TNM staging of breast cancer; The current study found a significant difference in P53 levels across all BC stages; This result agrees with **Khadhum et al. [13],** except that there appeared to be no significant difference in the levels of tumor markers CA15.3.

On the other hand, the results showed that there are clearance differences in the measurement of tumor markers between patient groups categorized according to the grade of breast cancer, except that there were no significant differences in the serum level of P53 among all degrees this is consistent with **Arora et al. [11]**. However, the CA15.3 tumor marker is highly significant among the grades.

The diagnostic values of CA 15-3 and p53 were compared using receiver operating characteristic curve analysis based on the area under the ROC curve (AUC). The higher AUC corresponds to a better diagnostic test. Serum p53 showed a non-significant AUC (0.5493, P 0.61) with 56.52% sensitivity and 60%specificity at a cut-off value of 0.389U/ml. Serum CA 15-3 showed a significant AUC (0.9838, P<0.0001*) with 97.07% sensitivity and100% specificity at a cut-off value of > 15.95 IU/ml.

5. Conclusion

The current study concludes that p53-protien levels were significant over all BC TNM stages. The findings are inconclusive as to whether detectable p53 protein expression is a random outcome of dedifferentiation or an essential aspect of the malignant phenotype that plays a role in tumor activity. P53 protein may indicate a poor prognosis and a higher probability of tumor relapse, making it a helpful perspective tumor marker in patient monitoring during therapy follow-up.

Acknowledgments

The author acknowledges the National research centre, represented by Dr Sally Farouk researcher in Microbial Bioecology in National Research centre for well performing all statistical analysis of data.

6.References:

1. Alorabi, M. O. H. A. M. E. D., El-Ghazawy, H. A. G. A. R., Alorabi, M., & Elghazawy, H. (2021). Cancer Control in Egypt: Investing in Health. *The ASCO Post*.
2. Makki, J. (2015). Diversity of breast carcinoma: histological subtypes and clinical relevance. *Clinical medicine insights: Pathology*, *8*, CPath-S31563.
3. Gany, F., Ayash, C., Raad, N., Wu, M., Roberts-Eversley, N., Mahmoud, H., & El-Shinawi, M. (2020). Financial and food security challenges of Egyptian women undergoing breast cancer treatment. *Supportive Care in Cancer*, *28*, 5787-5794.
4. Abas, A. S. M., Sherif, M. H., & Farag, S. A. E. (2022). Diagnostic and prognostic role of serum omentin and NGAL levels in Egyptian breast cancer patients. *International Journal of s*
5. Zidan, H. E., Karam, R. A., El-Seifi, O. S., & Abd Elrahman, T.

M. (2018). Circulating long non-coding RNA MALAT1 expression as molecular biomarker in Egyptian patients with breast cancer. *Cancer genetics*, *220*, 32-37.

6. Yang, Y., Zhang, H., Zhang, M., Meng, Q., Cai, L., & Zhang, Q. (2017). Elevation of serum CEA and CA15-3 levels during antitumor therapy predicts poor therapeutic response in advanced breast cancer patients. *Oncology Letters*, *14*(6), 7549-7556
7. Hasan, D. (2022). Diagnostic impact of CEA and CA 15-3 on chemotherapy monitoring of breast cancer patients. *Journal of Circulating Biomarkers*, *11*, 57.
8. Synnott, N. C., Murray, A., McGowan, P. M., Kiely, M., Kiely, P. A., O'donovan, N., ... & Duffy, M. J. (2017). Mutant p53: a novel target for the treatment of patients with triple-negative breast cancer. *International journal of cancer*, *140*(1), 234-246.
9. Zhao, L., & Sanyal, S. (2022). P53 isoforms as cancer biomarkers and therapeutic targets. *Cancers*, *14*(13), 3145.
10. Duffy, M. J., Synnott, N. C., & Crown, J. (2018). Mutant p53 in breast cancer: potential as a therapeutic target and biomarker. *Breast cancer research and treatment*, *170*, 213-219.
11. Arora, K., Khandelwal, R., & Pant, H. (2020). Evaluation of p53 in breast cancer and its correlation with various histological prognostic factors. *Indian J Pathol Oncol*, *7*(3), 392-398.
12. Homaei Shandiz, F., Afzaljavan, F., Tajbakhsh, A., Rivadeh, M., Sharifi, N., Shakeri, M. T., & Pasdar, A. (2021). Age-related variation in expression of breast cancer tumour markers in Iranian patients. *Middle East Journal of Cancer*, *12*(2), 269-275.
13. Khadhum, H. S., Ameen, A. A., & Thuwaini, M. M. (2022). Assessment of CA 15-3 and P53 biomarkers in diagnosis of breast cancer. *Assessment*, *140*(01)..