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Some tumor markers and hormonal receptors as Prognostic Parameters Of Breast Cancer

Faten Zahran Mohamed	Chemistry Department, Faculty of Science , Zagazig University, Zagazig , Egypt
Hossam Darwish	Medical Oncology Department, Damietta Cancer Institue, Damietta, Egypt,
Arafa A.M Belal	Chemistry Department, Faculty of Science ,Port-Said University, Port-Said , Egypt
Waleed Yousef Abd EL-razek	Chemistry Department, Faculty of Science ,Port-Said University, Port-Said , Egypt

ABSTRACT

Breast cancer is the most frequent malignant tumor in women worldwide. Breast cancer is the most common cancer among Egyptian women, it representing 18.9% of total cancer cases (35.1% in women) among the Egypt National Cancer Institute's (NCI) series of 10,556 patients during the year 2001,Breast Self- Examination (BSE) is a simple and easy way to detect any changes in the breast and help in early detection of breast cancer, which is highly recommended in young age started from age of 20 years. It is necessary to find a prognostic and diagnostic markers of breast cancer.
The present study is undertaken to detect any alterations in u-PA , CA15.3 and NO levels in breast cancers patients with different TNM staging to , and to determine the relationship between these parameters and estrogen and progesterone receptor expression, This may improve the patient survival rate with a timely treatment.The individuals under study were taken from Damietta cancer institute.

Our results showed a posi¬tive correlation between urokinase-type plasminogen activator (u-PA) and CA 15.3, they were significantly increased among breast cancer patients compared to healthy controls by (10.9%) & by (73.6%) , by (133.8%) & by (180.8%) , by(324.1%) & by (907.2%) , by (429.5%) & by (1650.5%) ,by (923.9%) and by (7666.6%) , in, T1, T2, T3, T4, and metastasis stages respectively, ($p<0.001$), but NO values was significantly decreased in T2, T3, T4, and metastasis stages by 36.8,75.5, 86.0, and 94.2%, respectively; ($p<0.001$), compared to negative control group.

u-PA and CA15.3 markers may be used as a guide in the diagnosis and treatment of cancer patients with different TNM stages , also these markers are elevated among ER+/PR+ expression compared to normal control .

KEYWORDS breast Cancer, urokinase-type plasminogen activator (u-PA) , nitric Oxide (NO). Estrogen receptor - progestrone receptor -CA15.3.

Introduction:
Breast cancer tumor markers are frequently used among women with early-stage disease and are associated with an increase in both diagnostic procedures and total cost of care. A better understanding of factors driving the use of and the potential benefits and harms of surveillance based tumor marker testing is needed **(Ramsey ,et al 2015)**

Assessment of hormone receptors for clinical management of breast cancer patients is strongly advocated to provide prognostic information and best therapeutic options **(Deepti ,et al 2015)**

The interaction of uPA with its receptor also triggers cellular responses leading to cell migration, proliferation, and expression of specific genes **(Blasi , et al 2010).**

On the other hand, tumour- associated proteolytic factors enable tumour cells to disintegrate the stroma in their immediate vicinity, intravasate into lymphatic or blood vessels, and then spread systemically. Among the key players in the proteolytic cascade leading tumour invasion and metastasis are factors of the plasminogen activation system **(Carmeliet, 2003).**

The urokinase plasminogen activator (u-PA) system is involved in the proteolytic degradation of the basement membrane and extracellular matrix and thus plays a role in the process of tumor invasion and metastasis. Pericellular proteolysis starts with the activation of serine protease uPA, which catalyzes the conversion of plasminogen into plasmin. There are 2 endogenous inhibitors of the uPA system, PAI-1and PAI-2 (plasminogen activator inhibitors 1 and 2). Due to their involvement in tumor progression the components of the plasminogen activation system are candidates for prognostic marker investigation in oncology **(Voorzanger- Rousselot , et al 2007)**.

CA 15-3 is the most widely used serum marker in breast cancer **(Duffy, 2010)**.

CA15-3 is one of the antigens related to breast cancer, can be used to monitor the recurrence and metastasis of breast cancer **(Tarhan, et al 2013).**

Nitric oxide (NO) mediates an array of physiologic and pathologic processes including the formation and function of blood vessels as well as the growth and dissemination of tumors via blood vessels **(Fukumura ,et al 2006)**

Tumor cells interact with adjacent neighboring cells to help them to "escape" more easily from the original tumor, through oxidative stress. Tumor cells develop in the stroma, a niche whose abundance and nature favor their development. In highly-aggressive forms of cancers,tumor cells are exposed to high levels of oxidative stress that they use to transform the stroma and disseminate more easily. Thus, modified stromal cells act as a scaffold, opening the way for tumor cells to move outside their primitive location **(Toullec, et al 2010)**

Breast cancers are defined according to the expression of estrogen receptor (ER), progesterone receptor **(PR)(Sorlie, et al 2003).**

ER is probably the most powerful diagnostic and prognostic marker for breast cancer. About 60% of women below 50 years has been reported with ER positive and is increased up to 80% in women's above 50 years. Estrogen hormone is an established risk factor for breast cancer **(Pankaj Taneja,et al 2010).**

The detection of ER and PR expression plays a key role in judging the prognosis of disease **(Patel, et al 2013)**.

Subjects and Methods :
Individuals
The present study involved seventy five Patients (Breast cancer patients) in aged (33 to 67) with different TNM tumor stages ,have been selected from damietta cancer institute, Damietta, Egypt during the years 2012 and 2013, the metastasis occurred in 24 out of 75 patients. and 15 negative controls (Healthy volunteers) aged (32 – 66) years.

I-Blood sampling
Five ml venous blood was drawn in the morning from fasting patients and controls . After centrifugation at the speed of 1500 r.p.m , the serum sample were collected and preserved in the freezer at -20 according to the method of **(Henry, 1979)**.

II-Tissue sampling
Breast cancer tissue samples of patients group were taken after surgery.Tissue block preparations were formalin-fixed within 4 to 6 . immunohistochemistry was performed, after epitope retrieval, with a polymer-based detection system ,using mouse monoclonal antibodies for ER and PR, ER (1D5; 1:50 dilution), PR (PgR636; 1:400 dilution), and a Herceptin kit according to the method of **(Krisztina,et al 2009)**.

Serum u-PA concentration were measured by the quantitative sandwich enzyme immunoassay technique (American Bosters u-PA ELISA, USA). Each standard and sample were assayed in duplicates according to the method of **(Finckh, et al ,2003)**.

The levels of CA15-3 in serum were detected using Chemiluminescence immunoassay according to method of **(Aziz , 1992)**.

Serum Nitric oxide was measured as the stable end product, according to the method of **(Miranda, et al.2001)** based on the reduction of nitrate by vanadium trichloride combined with detection by acidic Griess reaction.

The immunohistochemistry staining technique was applied to mark the expression of ER and PR in tissue according to method of **(Krisztina,et al 2009)**.

Statistical analysis:
All statistical analyses were done by a statistical for social science package "SPPS" 14.0 for Microsoft Windows, SPPS Inc. **(Levesque, 2007)** and considered statistically significant at a two- sided P <0.05.

Numerical data were expressed as Mean± S.D. The levels of markers were analyzed by ANOVA.The correlations between serum biochemical data in different studied groups were evaluated by Pearson's correlations coefficient,to quantify the relationship between the studied parameters. P value <0.01 was considered significant.

Results:
The present study involved 75 Patients (Breast cancer patients) and 15 negative controls (Healthy volunteers). Breast cancer patients were divided into different Tumor Size relative to Stages as shown in table (1).5 Patients were categorized as T0 by (6.6 %), 5 were T1 by (6.6%), 18 were T2 by (24%), 11 patients were T3 by (14.6%), 12 patients were T4 by (16%), and 24 case were metastasis by (32%) table(1) .

Table (1): Classification of samples according to TNM Staging

Tumor Staging	Number of cases	Ratio (%)
T1	10	13.4
T2	18	24
T3	11	14.6
T4	12	16
T4/Metastasis	24	32

Table (2) summarize the mean values of NO, uPA, and CA 15.3 and their significance in all studied groups.

The mean values of NO, u-PA, and CA 15.3 levels in negative healthy individuals were found to be 17.89±1.4 (µmol/l),65.19±9.4(pg/ml), and 9.9±1.5 (U/l) respectively.

NO values was significantly decreased to be 11.3±1.09 ,4.39±0.97,2.5±0.38, 1.03±0.44 in T2, T3, T4, and metastasis stages by 36.8,75.5, 86.0, and 94.2%, respectively; (p<0.001), compared to negative control group.

While u-PA and CA 15.3 were significantly increased to be 72.3±13.7 by (10.9%) & 17.19±3.0 by (73.6 %), 152.4±27.9 by (133.8%) & 27.8±3.2 by (180.8%) , 276.5±62.6 by (324.1%) & 99.71±9.15 by (907.2%) , 345.2±62.1 (429.5%) & 173.3±23.6 by (1650.5%) , 667.5±60.8 by (923.9) and by 768.9±98.6 by (7666.6%) , in T1, T2, T3, T4, and metastasis stages respectively, (p<0.001),table(2).

Table (2): Mean levels ± S.D of all parameters in all studied groups according to TNM Staging .



The positivity of ER and PR (ER+/ PR+) was increased according to the stages of the tumor table (3).

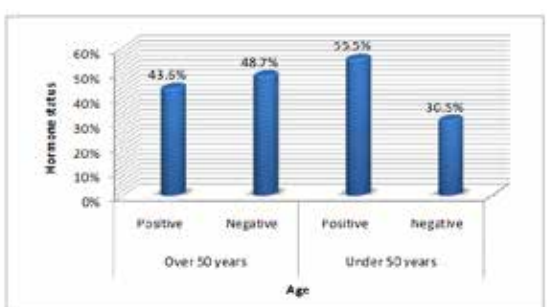
Table (3) summarize the percentage of ER and PR positivty and negativity in all stages of the study.

Breast cancer patient		ER+/PR+	ER-/PR-	ER+/PR-
*TNM staging				
T1	10	0(0%)	10(100%)	0(0%)
T2	18	5(28%)	9(50%)	4(22%)
T3	11	6(55%)	4(36%)	1(9%)
T4	12	8(67%)	2(16.5%)	2(16.5%)
Meta	24	18(75%)	5(21%)	1(4%)

The hormone status was found to be 55.5% and 30.5% as positive and negative respectively, in age over 50 samples. While in samples age under 50, the percent was found to be 43.6%, and 48.7%, as positive and negative hormone status, respectively, as illustrated in fig(1) and table(4).

Table (4) summarize the percentage of ER and PR positivity and negativity with ages (over and under 50 years).

breast cancer patient			ER+/PR+	ER-/PR-	ER+/PR-
*Age (years)					
< 50	36	75 (48 %)	20(55.5%)	11(30.5%)	5(14%)
> 50	39	75 (52%)	17(43.6%)	19(48.7%)	3(7.7%)



Fig(1): relation between age and hormones

Fig(1): relation between age and hormones

Correlations between different Studied Parameters among studied groups:

Table (5) summarizes the correlations between different parameters in this study.

There were significant positive correlations between u-PA , and CA 15.3, while there was negative correlation between NO and (uPA and CA15.3).

Table (5): Pearson's correlations analysis between different studied parameters in all studied groups:

Parameter		NO	u-PA	CA 15.3
NO	r	------	-0.854**	-0.722**
uPA	r	-0.854**	------	0.945**
CA 15.3	r	-0.722**	0.945**	-------

**Correlation is significant at p<0.01

Discussion:

Breast cancer is a major health problem that affects the lives of millions. For the year 2012, it was estimated that 226,870 women in the United States will be diagnosed with breast cancer and that 39,510 women will succumb to it **(Siegel, et al 2012)**

Breast cancers are typically marked by high percentage of aggressive **cells (Ahmad, et al 2012).**In recent years, the incidence of breast cancer is significantly on the rise in the East and West, hence, how to treat breast cancer, effectively assess the therapeutic effect, correctively evaluate the prognosis and find the postoperative recurrence of patients with breast cancer in an early stage have been paid attention by more and more scholars at home and abroad **(Deng ,et al2013).**

In the process of tumorgenesis and development, tumor regenerative capillaries capable of providing nutrients for tumor cells and favorable conditions for distal metastasis are the precondition to induce the local growth, infiltration and distal metastasis of malignant tumors, hence, how to inhibit tumor angiogenesis is a new research hotspot at present **(Thielemann, et al 2013).**

A number of biomarkers have been studied to evaluate malignancies.However, no specific markers for breast cancer have been discovered and those that are currently available lack the sensitivity and specificity for early detection of cancer and timely treatment **(Jawed Akther,et al 2013).**

One of the major causes resulting in the patients with breast cancer death is postoperative tumor recurrence and metastasis. More effective clinical predictors have been explored in medical field for many years, for comprehensively and accurately understanding the probability of postoperative recurrence and metastasis of breast cancer **(Godfrey, et al 2013)**. The tumor-related indicators in all individuals cannot be detected systemically and comprehensively due to limited economics and techniques, thereby, joint detection of specific tumor markers is of great importance **(Lawicki, et al 2013).**

Malignant tumors have a capacity to degrade the extracellular matrix (ECM) by controlled proteolysis. One proteolytic system involved in these processes is the urokinase-type plasminogen activator (u-PA) system **(Han, et al 2005).**

The mean value of u-PA in control group was found to be 65.19±9.4 , this value was significantly increased in T1,T2, T3, T4, and metastasis stages by 10.9% ,133.8% ,324.1% ,429.5% ,923.9% respectively, (p<0.001), these results are in accordance with previous studies**(Konukoglu , et al 2007)** who reported that, u-PA seem to be associated with the angiogenetic and metastatic process of breast cancer.

uPA is involved in regulating breast cancer invasion and metastasis, explained by its ability to facilitate ECM degradation, cell proliferation, angiogenesis, migration and adhesion **(De Cremoux,et al 2009 and Linlin, et al 2013).**

The mean value of CA 15.3 in control group was for to be 9.9±1.5 this value was significantly increased in T1,T2, T3, T4, and metastasis stages by 73.6% ,180.8% ,907.2% ,1650.5% ,7666.6% respectively, (p<0.001), these results are in agreement with **(Tarhan ,et al2013)** who stated that , CA15.3 can be used to monitor the recurrence and metastasis of breast cancer

CA15-3, a variant of mammary epithelial surface glycoprotein and an antigen related to breast cancer, can be considered as the indicator to detect recurrence and metastasis of breast cancer. Studies have confirmed that as a sort of glycoprotein is applied to detect the

recurrence rate of various epithelial tumors and judge the prognosis **(Lawicki ,et al 2013).**

Increased CA 15-3 in serum is reported in about 10% of patients in early breast cancer and is subsequently elevated as the disease progresses. An increase of CA 15-3 five to ten times above normal upper limit can predicts breast cancer; however a low value cannot exclude metastasis **(Michael J. et al,2006)** making this more of prognostic marker rather than diagnostic marker.

NO may participate in the induction of genotoxic lesions as well as promoting angiogenesis, tumor cell growth and invasion **(Lala andChakraborty,2001).**

Our results revealed that NO was significantly decreased in T2, T3, T4, and metastasis stages by 36.8% ,75.5% ,86% ,94.2% respectively, ($p<0.001$) compared to negative healthy controls, These results are in agreement with **(Omar et al, 2011)** , who reported that, serum nitric oxide was lower in metastatic compared with non-metastatic cancer breast.

Nitric oxide (NO) signaling has various oncogenic effects in cancer cells **(Switzer, et al 2011).**

NO activates signaling through epidermanl growth factor receptor (EGFR) and phosphatoinositide-3kinase enzyme(PI3K) **(Glynn, et al 2010)**, NOS2 expression may have deleterious effects in the progression of certain human cancers including ER breast cancer.

ER is an independent prognostic factor of breast cancer with the condition similar to axillary lymph nodes and the same size. As the end product of estrogen, its synthesis has to depend on the launch of estrogen, illustrating that it is indeed active. However, as a special protein capable of regulating the growth and differentiation of breast cancer, its detection with PR can be applied to judge the prognosis of disease and endocrine therapy in clinic**(Godfrey et al., 2013)..**

PR is controlled by estrogen therefore it shows the indication of proper functioning of ER pathway and thus PR assay can be used for prognostic purposes **(Payne, et al 2008)**. Relevant literatures reported that positive ER expression was obviously higher than the negative in postoperative 5-year survival rate of breast cancer, and both recurrence and distal metastatic rate were lower, whereas this advantage was unconspicuous over 5 years **(Dede et al., 2013).** There are also some relevant studies regarding PR in breast cancer **(Lee et al., 2013).**

The incidence of receptor positivity was lower in higher age groups than that reported in earlier studies **(Geeta , et al 2011).**

Our results revealed that u-PA and CA 15.3 were increased with ER+/PR+ , these result are in agreement with **(Schneider, et al 2003)** who indicated that hormone-receptor-positive breast cancers may carry an elevated risk of nodal involvement if accumulation of uPA is shown inside their tumor cells, and also serum CA15-3 was increased with ER+/PR+, So estrogen receptors status is strongly correlated with elevated CA15-3 level and these result is consistent with what's reported by **(Atoum, et al 2012).**

Conclusion:

Detection of u-PA , CA 15.3,NO, ER and PR can complement mutually in the diagnosis, treatment and prognosis of breast cancer, and can be regarded as a crucial reference indicator. The expression of u-PA has an important application value in the aspects of assessing lymph node metastasis, recurrence monitoring and clinical staging of tumors in clinic.

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