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UROKINASE PLASMINOGEN ACTIVATOR RECEPTOR, PLASMINOGEN ACTIVATOR INHIBITOR-1,EXTRACELLULAR MATRIX METALLOPROTEINASE PROTEIN INDUCER AND CA 15-3ASPOTENTIAL BIOMARKERS FOR DIAGNOSIS AND PROGNOSIS OF PRIMARY BREAST CANCER

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ABSTRACT

Breast cancer is one of the most important leading causes of cancer deathin the less developed countries.The identification of markers that could assist in diagnosis, evaluation of therapeutic response, detection of recurrence and metastasis is a useful tool. The present study is undertaken to provide insights about the role of urokinase plasminogen activator receptor (uPAR), plasminogen activator inhibitor-1 (PAI-1), extracellular matrix metalloproteinase protein inducer (EMMPRIN), cancer antigen (CA) 15-3 in diagnosis and/or prognosis of breast cancer, evaluate the possible correlations between these biomarkers and the clinico-pathological status of breast cancer and compare between validity of these biomarkers with tumor marker (CA 15-3). A total of 75 women whose ages ranged between 30 to70 years and 10 healthy controls with matched age and sex were included. The patients were divided into 4 groups, group I: Included 39 female patients with breast cancer before operation, group II: Included 17 women from group I followed for 6 months after operation, group III: Included 9 women from group I followed for 12 months after operation, group IV: Included 10 female patients with

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benign breast diseases. Estimation of serum uPAR, PAI-1, EMMPRIN and CA 15-3 by ELISA and related clinico-pathological features were assessed. The results revealed higher mean serum levels of uPAR, PAI-1, EMMPRIN and CA 15-3 in breast cancer women before operation when compared to other 4 groups. Patients after 6 and 12 months follow up showed a decrement of uPAR, EMMPRIN, PAI-1 and CA 15-3 levels.There was significant relationbetween uPAR, PAI-1, EMMPRIN, CA 15-3 and clinicopathological characteristic of breast cancer patients. There was a significant positive correlation between serum uPAR, PAI-1 and EMMPRIN ($p<0.001$).In conclusion, High circulating uPAR, PAI-1 andEMMPRINwere significantly associated with breast carcinogenesis and metastasis. Accordingly, estimation of these biomarkers may predict the breast disease behavior and its prognosis.

Key words:Breast cancer,CA 15-3,EMMPRIN, PAI-1, uPAR

INTRODUCTION

Breast cancer is one of the most common cancers with greater than 1,050,000 cases and 400,000 deaths each year worldwide **(Gouri et al., 2016).**Breast cancer ranks second as a cause of cancer death in women after lung cancer**(DeSantis et al., 2016)**. In Egypt, breast cancer represents about 37.7% of total cancer cases among women **(Zeeneldin et al., 2013).**The development of breast cancer involves a progression through intermediate stages until the invasive carcinoma and finally into metastatic disease. Given the variability in clinical progression, the identification of tumor markers that could predict the tumor behavior is particularly important in breast cancer**(Golubnitschaja et al., 2013).**

The plasminogen activator system is a crucial regulator of the tumor microenvironment, and is heavily concerned in the metastatic process in breast and other common cancers**(Leurer and Rabbani, 2015)**.Urokinase plasminogen activator receptoris a cysteine-rich, glycosyl-phosphatidylinositol (GPI)-anchored cell membrane protein of molecular weight 45–60 kDa.Urokinase plasminogen activator receptorarises from proteolytic cleavage of the GPI anchored by various proteases and determined in body fluids**.**When urokinase plasminogen activator (uPA) is bound to it,

uPAR catalyzes cell surface plasminogen into plasmin, starting a proteolytic cascade including matrix metalloproteinases, such as MMP-2 and MMP-9, and breaks down fibrin and otherextracellular matrix (ECM) constituents **(Tang and Han, 2013).**

Plasminogen activator inhibitor-1(PAI-1)is a member of serine proteinase inhibitors superfamily.PAI-1 is the key inhibitor of plasminogen activators, such as tissue-type plasminogen activator and urokinase-type plasminogen activator, and a chief regulator of the fibrinolytic system **(Declerck and Gils, 2013)**.Although, PAI-1 is an inhibitor of uPA and expected to stop invasion, it interacts with matrix protein vitronectin and inhibitsits interaction to cell-bound adhesion receptors as integrin thus directing a stepwise cell migration. It is also possible that PAI-1 has a direct influence on the development of the disease **(Rouch et al., 2015)**.

Extracellular matrix metalloproteinase protein inducer (EMMPRIN) or cluster of differentiation 147 (CD147) is a single pass integral membrane protein, belonging to the immunoglobulin superfamily. It is usually expressed at low levels in most normal tissues, but is highly upregulated throughout dynamic cellular events, such as tissue remodeling and cancer development **(Tian et al., 2015)**.EMMPRIN was originally identified as a cell surface factor that induces several MMP productions in tumor cells themselves as well as in stromal cells.Soluble EMMPRIN release is linked with the degree of EMMPRIN expression in tumor cells**(Wu et al., 2014).**

The cancer antigen (CA) 15-3 is tumor associated antigen which detects soluble forms of mucin-1 (MUC-1) protein. Mucin-1 is expressed in the duct and aciniof normal breast tissue, but with neoplastic transformation tissue architecture is disrupted leading to shedding of MUC-1 in the blood **(Gautam et al., 2015)**.

The aim of the present study was to:

1-Clarify the possible role of urokinase plasminogen activator receptor,plasminogen activator inhibitor-1, Extracellular matrix metalloproteinase protein inducer and CA 15-3as diagnosticand/or prognostic markers in breast cancer and evaluate the correlations between these biomarkers and the clinico-pathological status of breast cancer. 2- Compare between validity of these biomarkers with breast tumor marker CA 15-3 level.

MATERIAL AND METHODS

Subjects:

The current study included 75 female patients who were selected from the South Egypt Cancer Institute, Surgery Department, Assiut University, from December, 2013 to March, 2015. Their ages ranged between 30-70 years, with a mean ± SD(50.7 ±10.3 years).In addition to 10 age and sex matched healthy controls. All participants signed a written informed consent for participation in the study. The study was approved by the Faculty of Medicine, Assiut University ethical committee in accordance with Helsinki declaration (1975).

Patients were divided into 4 groups as follows:GroupI: Included 39 female patients with breast cancer before operation.Group II:Included 17 women from group I followed for 6 months after the surgical removal of their breast cancers.Group III:Included 9 women from group I followed for 12 months after the surgical removal of their breast cancers. Group IV: Included 10 female patients with benign breast diseases. Three pathological types of benign breast diseases were selected: 7 cases had fibroadenoma (70%), 2 cases had fibrocystic diseases (20%) and 1 case had phylloid tumor (10%).

Group I,II,III women received treatment after surgery in the form of chemotherapy which included fluorouracil 500 mg/m2, epirubicin 100 mg/ m2 or Adriamycin 50 mg/ m2 and cyclophosphamide 500 mg/ m2(FEC orFAC) every 3 weeks for 6 cycles, then radiotherapy for 1.5 months followed by hormonal therapy (tamoxifen 20 mg/day) for patients whose tumors were positive for estrogen and/or progesterone receptors and to be continued for 3-5 years.

The following was done for all participants including demographic characteristics, family history of breast cancer, smoking habits, therapeutic history and personal obstetric history.Imaging studies including mammography and ultrasound were done. Body mass index was calculated for each female.

Exclusion criteria included: the presence of any previous tumors, getting neoadjuvant chemotherapyor undergoing surgical operations for tumor resection, Patients with chronic medical diseases and patients who are unfit for surgery.

Biochemical analysis:

Six ml venous blood was drawn by venipuncture, blood samples were collected without anticoagulant, kept at room temperature for 20 to 30

minutes and then centrifuged at 3000 r.p.m for 15 minutes. Serum was separated and divided into aliquots and stored at −70°C to allow batch analysis of the biochemical markers.

The levels of serum urokinase plasminogen activator receptor, plasminogen activator inhibitor-1 and extracellular matrix metalloproteinase protein inducer were measured using an ELISA kits, supplied by Elabscience Biotechnology Co., Ltd (Wuhan, China)according to the methods of **Sier et al. (1999), Jiang et al. (2010)andIacono et al. (2007),** respectively. Serum Cancer antigen 15-3 was measured using an ELISA kit, supplied by BioTina GmbH (Freiburg, Germany) according to **Duffy et al. (2010).**

Statistical analysis

Data collected were analyzed by computer program SPSS" ver. 20" Chicago. USA. Data expressed as mean, standard deviation and percentage. Whereas the cut-off point, sensitivity and specificity were made using MedCalc program. Prior to analysis the variables were tested for normal distribution using the Shapiro-Wilk Wtest. Student t-test was used for normally distributed data and Mann-Whitney was used for skewed data for the purpose of identifying differences between the tested groups.ANOVA test was used for comparison between different groups. Differences were considered significant at $p \leq 0.05$. Chi Square ($\chi 2$) was used to determine significance for categorical variables. Spearman correlation was used for correlations between groups.

RESULTS

The demographic and clinical data of patients and controls of the current study were clarified in table (1). The age of breast cancer disease group was significantly higher than benign breast diseases. The age at menarche and age at menopause were not relevant to either benign breast diseases or breast cancer diseases groups.The mean levels of BMI were significantly higher in benign and breast cancer groups in comparison to those of controls. Family history of breast cancer and history of contraception in patients and controls were shown in tables (1).

Table (2) showed the levels of various studied parameters in patients and controls. The current study showed a high significant difference ($p<0.001$) in levels of uPAR, PAI-1, EMMPRIN, CA 15-

3between breast cancer patients before operation and control groups and between breast cancer patients before operation and benign breast diseases groups. The mean±SD levels of serum uPAR and EMMPRIN inbreast cancer patients showed a decrement towards normalization of levels 6 and 12 months after the surgical removal of their tumors and after they received medical treatment.

A similar trend was observed in the mean levels of PAI-1, although there were significantly higher levels in breast cancer women,six and twelve months after treatment, their levels were decreased but did not reach normalization. Regarding the mean serum CA 15-3 levels, they were significantly increased in breast cancer women in comparison to those of the controls. Also, their levels were changed towards normalization, 12 months after treatment.

The result of the current study showed significant relation between uPAR, PAI-1, EMMPRIN and clinicopathological characteristic of breast cancer patients before operation as regarding TNM stage,tumor size, axillary lymph node involvement, distant metastasis and lymphovascular invasion (table 3). Interestingly, only CA 15-3's correlation with lymphovascular invasion was statistically significant.

The sensitivity and specificity based on the cut-off levels for each studied variable in breast cancer patients were demonstrated in tables (4).

A significant positive correlation was found between serum uPAR levels and each of serum PAI-1($p<0.001$, $r=0.731$), EMMPRIN($p<0.001$, $r=0.810$) and CA 15-3($p<0.05$, $r=0.368$) levels. Also, significant positive correlation between serum PAI-1 levels and each of EMMPRIN($p<0.001$, $r=0.764$), CA 15-3 ($p<0.01$, $r=0.482$) levelswere observedin breast cancer patients before operation. In addition, a significant positive correlation between serum uPAR levels and CA 15-3 ($p<0.05$, $r=0.569$) levels was observed in breast cancer patients 6 months after surgery. The correlationbetween serum uPAR levels and each of serum PAI-1and EMMPRINin breast cancer group were shown in figures (1) and (2), as examples.

Table (1): The demographic and the clinical data of patients and controls.

Groups / Variables	Controls (n=10)	Benign breast diseases group (n=10)	Breast cancer (group I) (n=39)
Age (years) mean ± SD	44.20±10.81	36.30±9.14 P_1 NS	50.67±10.32 P_1 NS $P_2 < 0.01$
Age at menarche (years) mean ± SD	13.10±1.28	13.30 ±1.25 P_1 NS	13.46±1.62 P_1 NS P_2 NS
Ageatmenopause (years) mean ± SD	52.25±3.20	48.50±2.12 P_1 NS	48.74±3.4 P_1 NS P_2 NS
Body mass index (kg /m^2) mean ± SD	29.20±2.66	32.97±6.93 $P_1 < 0.05$	32.84± 5.20 $P_1 < 0.05$ P_2 NS
Family history of breast cancer N (%) -Yes -No	 1 (10.0%) 9 (90.0%)	 1 (10.0%) 9 (90.0%)	 4 (10.3%) 35 (89.7%)
History of using contraceptives N (%) -Yes -No	 6 (60.0%) 4 (40.0%)	 4 (40.0%) 6 (60.0%)	 21 (53.8%) 18 (46.2%)

Group I:Breast cancerpatients at diagnosis.
P_1:Compared to controls, P_2:Compared to benign breast diseases group, NS: non-significant.

Table (2): The levels of various studied parameters in patients and controls.

Groups / Variables	Controls (n=10)	Benign breast diseases group (n=10)	Group I (n=39)	Group II (n=17)	Group III (n=9)	ANOVA p-value
Serum urokinase plasminogen activator receptor (ng/ml)						
range	0.9-1.2	0.6-1.7	4-13.3	0.7-3.2	0.8-4	
mean ± SD	1.03± 0.12	0.9± 0.4 *a***c	8.5±2.5 ***ab	1.4± 0.7 *b***c	1.8±1.3 *b**c	< 0.001
Serum plasminogen activator inhibitor-1(ng/ml)						
range	3.5-16.29	11-20.4	12.98-75.56	9.53-24.43	10-19.90	
mean ± SD	9.3±4.5	15.2± 3 **a***c	37.66±18.7 ***ab	15.48±4.86 **a***c	14.62±3.6 *a**c	< 0.001
Serum extracellular matrix metalloprotease protein inducer (ng/ml)						
range	0.9-1	0.9-4.2	9.3-18.9	0.5-1	0.7-1	
mean ± SD	0.9± 0.05	2.1±1 ***ac	15.4±2.4 ***ab	0.9±0.2 ***bc	0.9±0.1 **bc	< 0.001
Serum cancer antigen15-3 (U/ml)						
range	14-40	16-42	22.34-100	10.57-62	14-50	
mean ± SD	25.2± 7.5	24.20 ±8.91 ***c	57.1±24.6 ***ab	33.05±15.5 **c	30.60±11.2 **c	< 0.001

Group I, II, III correspond tobreast cancerpatients at diagnosis, 6 months after treatment,12 months after treatment respectively.
a: Compared to controls, b: Compared to benign breast diseases group, c: Compared to breast cancer group at diagnosis, *p<0.05, **p<0.01, ***p<0.001

Table (3): Statistical relation between the studied biochemical parameters and clinicopathological characteristics of the breast cancer patients before operation.

Variables	Number of patients	uPAR	PAI-1	EMMPRIN	CA 15-3
Grade					
- G1 + G2	**26**	**8.5±2.3**	**36.3±18.3**	**15.3±2.3**	**56±23.5**
- G3	**13**	**8.5±3.2**	**41.1±20.6**	**15.6±2.7**	**59.4±27.6**
TNM stage					
-I + II	**24**	**7±1.6 [a]**	**27.3±11.6 [a]**	**14.2±2.2 [a]**	**51.5±23.7**
-III + VI	**15**	**11.3±1.3**	**57.5±12.7**	**17.6±0.8**	**66 ±24.2**
Tumor size					
-$T_{1+}T_2$	**29**	**7.6±2 [a]**	**31.6±14.8[a]**	**14.6±2.2 [a]**	**54.5±22.7**
-$T_{3+}T_4$	**10**	**11.6±1.5**	**58±16.7**	**17.9±0.8**	**64.6±29.7**
Axillary lymph node involvement	**10**	**6.6±1.6 [b]**	**24±9.4[b]**	**13.9±2.4[c]**	**50±24**
-Negative	**29**	**9.3±2.5**	**43±18.9**	**15.9±2.2**	**59±24**
-Positive					
Pathological tumor type					
- Ductal invasive	**37**	**8.7±2.5**	**38.9±18.5**	**15.5±2.3**	**57.7±24.9**
- Other tumor type	**2**	**5.5±0.5**	**15.5±1.5**	**12.9±3.8**	**47±22**
Distant metastasis					
- Yes	**6**	**12±2.4 [b]**	**58.8±19.4 [c]**	**17.8±1.8 [c]**	**68±31.4**
- No	**33**	**8±2.2**	**34.9±17.2**	**15±2.3**	**55±23.3**
Age					
- ≤50 years	**19**	**8±2.2**	**33.6±16.7**	**15±1.9**	**52±20.3**
- >50 years	**20**	**8.9±2.9**	**41.5±20.3**	**15.6±2.8**	**61.8±27.9**
Lymphovascular invasion					
- Yes	**25**	**9.3±2.6[c]**	**45.9±18 [a]**	**15.9±2.4**	**64.9±23.9[b]**
- No	**14**	**7±1.8**	**23±9.6**	**14.4±2.2**	**43.3±20.2**
Menopausal status					
-Premenopausal	**23**	**8.3±2.2**	**36.8±16.6**	**15.5±1.7**	**51.9±21**
-Postmenopausal	**16**	**8.7±2.8**	**38.2±20.5**	**15.3±2.8**	**61.2±26.9**
Estrogen Receptor					
-Positive	**20**	**8.4±2.8**	**35.9±17.9**	**15.3±2.7**	**56±24.5**
-Negative	**18**	**8.7±2.2**	**39.7±20**	**15.5±2**	**58±25.5**
-Not available	**1**				
Progesterone Receptor					
-Positive	**14**	**8.2±2.8**	**35±17**	**15±2.5**	**58±27**
-Negative	**24**	**8.8±2.4**	**40±19.6**	**15±2.4**	**57.6±23.7**
-Not available	**1**				
HER_2\ neu					
-Positive	**8**	**8.4±2.2**	**32.2±19.2**	**15±1.7**	**51.7±22.8**
-Negative	**14**	**9±2.2**	**42.7±18**	**15.8±2**	**60.8±25**
-Not available	**17**	**8.2±2.9**	**36.6±19**	**15.2±2.9**	**56.6±26**

a**:** p< 0.001b**:**p< 0.01c: p< 0.05

Table (4): Cut-off points, sensitivity, specificity and area under ROC curve of different studied parameters in breast cancer patients.

Variables	Cut-off (discriminant analysis)	Sensitivity %	Specificity %	Area under ROC curve
Urokinase plasminogen activator receptor (ng/ml)	**1.22**	**100**	**90**	**1.000**
Plasminogen activator inhibitor-1(ng/ml)	**16.3**	**88.57**	**100**	**0.981**
Extracellular matrix metalloprotease protein inducer (ng/ml)	**1**	**100**	**90**	**1.000**
CA 15-3 (U/ml)	**30.2**	**87.18**	**80**	**0.926**

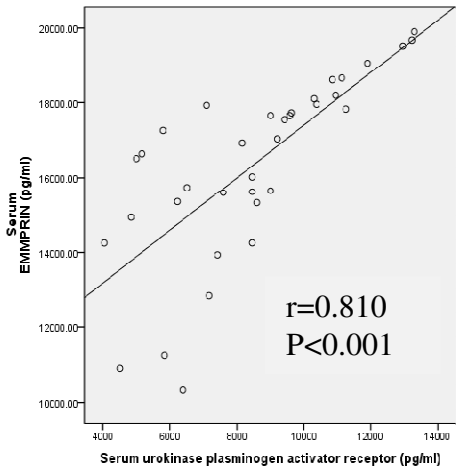

Figure (1): Positive correlation between uPAR and EMMPRIN before operation in breast cancer group.

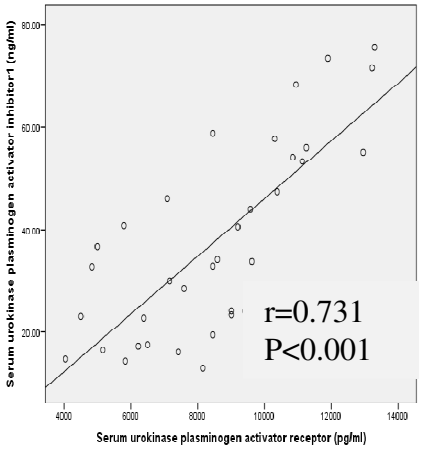

Figure (2):Positive correlation between uPAR and PAI-1 before operation in breast cancer group.

r=0.731
P<0.001

DISCUSSION

Breast cancer detection depends mostly on mammography, which has been associated with decreased breast cancer mortality **(Gøtzsche and Jørgensen, 2013).** However, mammography screening has created controversy due to the risks of false-positive results and over diagnosis of indolent disease **(Welch et al., 2016).** There is thus an urgent need to understand the biochemical bases of tumorigenesis, invasion and metastasis. The addition of a blood-based tumor marker test may raise patient compliance as blood testing is more suitable and would also circumvent the problems related with imaging high-density breast tissue**(Kazarian et al., 2017).**

Binding of uPA to uPAR triggers the alteration of plasminogen to plasmin and the subsequent activation of metalloproteinases such as the MMP2. These events degrade the components of the surrounding extracellular matrix proteins such as laminin, thus contributing to tumor cell invasion and metastasis. Also, uPAR interacts with vitronectin and diverse transmembrane proteins including integrins and G protein-coupled receptors.These interactions are proven to be critical for the role of uPAR in tumorigenesis **(Pavón et al., 2016).**

In the present study, serum uPAR levels were significantly higher in women with breast cancer as compared to control values. These findings are in agreement with that of **Soydinc et al. (2012)** and **Thielemann et al. (2013).** The mean serum level decreased significantly after 6 and 12 months of operation and receiving treatment(table 2).The high standard deviation found after 12 months may be due to difference in response of patients to treatment (range 0.8-4ng/ml). Also, serum uPAR levels were significantly higher in late versus early tumors stages(table 3)as secondaries may synthesize uPAR(**Barajas-Castañeda et al., 2016**).These findings are inaccordance with **(Almasi et al., 2011 and Hao and Friedman, 2016).**An important prognostic factor in the survivaland treatment selection for patients with breast cancer isthe status of the axillary lymph nodes**(Gottwald et al., 2012).** The results of the current study showed a statistically significant difference between levels of uPAR in patients with the lymph nodes involvement and its level in patients without involvement ($p<0.01$). These results are in agreement with those of **Thurison et al. (2016**).

A possible mechanism by which PAI-1 promotes cancer onset and progression is by enhancing angiogenesis and by blocking tumor cell apoptosis**(Duffy et al., 2014).** This blockage of apoptosis was found to be dependent on uPA-mediated activation of plasmin and the interaction of factor of apoptotic signal ligand(FasL) with Fas **(Placencio et al., 2015).**The results of the present study showed significantdifference in level of PAI-1between healthy controls and patients with breast cancer before operation. These levels decreased significantly after 6 months and one year of operation and treatment(table 2). These results are in accordance with those of the previous investigators**(Ma& Zhang, 2012 and Ferroni et al., 2014).** Plasma or serum PAI-1 as well as tumor tissue PAI-1 could be a prognostic factor for breast cancer **(Ma and Zhang, 2012).** High levels of PAI-1 in primary tumor tissue negatively affect the outcome of breast cancer **(Lampelj et al., 2015).**In contrary to the results of the present study, **Kim et al. (2009)** reported reduced serum concentrations in cases versus controls.

The results of the current study showed significant relation between serum PAI-1 and lymph nodes involvement, TNM stage, tumor size, distant metastasis, and lymphovascular invasion (table3). These results are in agreement with those of**(Ferroni et al., 2014and Märkl et al., 2017).** In addition to having a prognostic impact in breast cancer**(Kim et al., 2016)**, PAI-1 measurement in breast cancer seem to possess therapy predictive value, especially in predicting benefit from cyclophosphamide-methotrexate- 5-fluorouracil (CMF) in the adjuvant set (**Harbeck et al., 2013)**.High levels of PAI-1 in breast cancer patients predicted poor outcome**(Duffy et al., 2014)**.

Extracellular matrix metalloproteinase protein inducer can promote tumor cell invasion via activation of urokinase-type plasminogen activator, facilitate secretion of MMP-1, MMP-3, MMP-9 and membrane-type 1-MMP from cancer cells leading to degradation of basement membrane and extracellular matrix **(Zhao et al., 2013)**. In addition, it stimulates tumor angiogenesis by elevating vascular endothelial cell growth factor **(Pinheiro et al., 2015)**, and causes multi-drug resistance in tumor cells via hyaluronan mediated up-regulation**(Gao et al., 2014).** It is implicated in metastasis byinhibiting apoptosis and release of vascular endothelial growth factor that contributed in osteolytic lesions **(Liao et al., 2016)**.

The mean serum EMMPRIN levels in breast cancer women at diagnosis were significantly higher than those of control and benign group values (table2). These finding are in agreement with those of**(Wu et al., 2014 and Tian et al., 2015). Kong et al. (2014)** had shown that EMMPRINexpression significantly rises in breast cancer tissues compared with adjacent normal tissues from 50 cases of breast cancer patients.The present study resultsare not matched with the results of **Moonsom et al (2010)** who published a trial, showing that the EMMPRIN level was significantly lower in cancer patient sera compared to that of normal subjects. The discrepancy to results might be due to the difference in the types of cancers examined and different methodology as the study used competitive ELISA to quantifying serum EMMPRIN levels. Six and twelve months after operation, serum EMMPRIN levels were significantly decreased towards normalization(table2). This suggests that EMMPRIN was secreted from tumor cells, possibly by vesicle shedding **(Wu et al., 2014)**. Therefore EMMPRIN levels can used in monitoring the status of cancer after the surgery and during chemotherapy.Astatistically significant relation was found between serum EMMPRIN and lymph nodes involvement,TNM stage, tumor size and distant metastasis (table3). These results are in agreement with those of**(Wu et al., 2014 and Kong et al., 2016)**.

Concerning CA 15-3, the present study found that its preoperative levels in breast cancer patients were significantly higher than control group and benign group (table 2)with no significant difference between control and benign groups. Similar observations were reported by previous studies**(Gautam et al., 2015 and Nieder et al., 2017)**.CA 15-3 levels decreased significantly after 6, 12 months of operation and treatment to be non-significant from control and benign group levels. These findings are in line with**(Lee et al., 2012, Gautam et al., 2015 and Khan et al., 2016).**

In the current study, the only clinicopathological feature associated with elevated CA 15-3 levels was lymphovascular invasion. In addition, CA 15-3 levels was found to be increased across different stages (stage II 49±17 U/ml, stage III 73.5±26 U/ml, stage IV 99.2 U/ml) but did not reach a statistically significant level. **Lee et al. (2012)** demonstrated that raised CA 15-3 before surgery was significantly associated with tumor size, axillary node involvement and advanced stage. On the other hand, **Geng et al. (2015)**founda

correlationwith metastatic sites.The result was in agreement with previous studies **(Incoronato et al., 2014 and Śliwowska et al., 2016)**. It plays biological roles in cell adhesion and metastasis **(Moazzezy et al., 2014)**.

The present study noticed a significant positive correlation between uPAR and PAI-1 in women with breast cancer. This was consistent with a study done by**Andres et al. (2012)** that revealed a strong correlation between uPAR and PAI-1 expression status. These findings are consistent with those reported for other types of cancers **(Magnussen et al., 2014)**. Furthermore, significant positive correlationsbetween uPAR and each of EMMPRIN &CA15-3 were found in breast cancer group. This correlation may indicate their related role in modulating tumorigenesis and breast cancer invasion. Also, our results showed a significant positive correlation between PAI and each of EMMPRIN & CA15-3in breast cancer group which indicates a match to the used tumor markers. Besides, significant positive correlations between uPAR & CA15-3 were also observed in breast cancer patients 6 months after treatment.

Conclusion: The current study revealed that high circulating urokinase plasminogen activator receptor, plasminogen activator inhibitor 1, extracellular matrix metalloproteinase protein inducer and CA 15-3 are significantly associated with breast carcinogenesis and play together as a battery for tumor progression and metastasis. Accordingly, estimation of these biomarkers may predict the response of the tumor to treatment and guide the lines of treatment towards aggressive targeting line in those with expected bad prognosis.

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الملخص العربى

المستقبلو المانع لليوروكيناز منشط البلازمينوجين ، البروتين المحفز لفلزية بروتين النسيج الخارجى للخلية والأنتيجن السرطانى ١٥-٣ كدلائل حيويةمحتملة لتشخيص و متابعة سرطان الثدى الأولى

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يعد سرطان الثدى أحد أهم أسباب الوفيات من السرطان فى البلدان الاقل تطورالذلك هناك حاجة إلى دلائل موثوق بها تساعد فى التشخيص ، تقييم الأستجابة للعلاج ،الكشف عن عودة المرض وانتشاره.وتهدف هذه الدراسة الى تقديم رؤى حول الدور الذي يمكن أن يكون لبعض الدلائل الحيوية (المستقبلوالمانع لليوروكيناز منشط البلازمينوجين ، البروتين المحفز لفلزية بروتين النسيج الخارجى للخلية ، الأنتيجنالسرطانى ١٥-٣) فى تشخيص و متابعةسرطان الثدى،تقييم الارتباطات الممكنة بين هذه الدلائل الحيوية والحالة الطبية السريرية المرضية لسرطان الثديومقارنة صلاحية و صحة هذه المؤشرات الحيوية مع احد دلائل اورام الثدى(الأنتيجن السرطانى١٥-٣). إشتملت هذه الدراسة على عدد ٧٥ من السيدات وكانت أعمارهم تتراوح بين ٣٠-٧٠ سنة بالإضافة إلى ١٠ من السيدات الأصحاء من نفس المرحلة العمرية وتم تقسيم المرضى إلى أربعة مجموعات وهى : (مجموعة ١) وتشمل ٣٩ من السيدات المصابات بسرطان الثدى،(مجموعة ٢) وتشمل ١٧ من السيدات اللائى تم متابعتهم لمدة ٦ أشهر، (مجموعة ٣) وتشمل ٩ من السيدات اللائى تم متابعتهم لمدة ١٢ شهرا بعد العلاج الطبى و(المجموعة ٤) وتشمل السيدات المصابات بأورام حميدة فى الثدى. ولقد تم قياس معدل كلا من المستقبلوالمانع لليوروكيناز منشط البلازمينوجين ،البروتين المحفز لفلزية بروتين النسيج الخارجى للخلية، الأنتيجن السرطانى١٥-٣فى مصل دم المرضى بطريقة الإليزا.هذا بالاضافة الى تقييم الحالة الطبية السريرية للمرضى.

وقد أوضحت نتائج هذه الدراسة زيادة ذو دلالة إحصائية فى مستويات كلا من المانع والمستقبل لليوروكيناز منشط البلازمينوجين ، البروتين المحفز لفلزية بروتين النسيج الخارجى للخلية،الأنتيجن السرطانى ١٥-٣ فمرضى سرطان الثدى قبل التدخل الجراحى مقارنة بباقى المجموعات الاربعة ووجدت أيضا أن مستويات كلا من المستقبل والمانع لليوروكيناز منشط البلازمينوجين ، البروتين المحفز لفلزية بروتين النسيج الخارجى للخلية ،الأنتيجن السرطانى ١٥-٣ قد إقتربت من المعدل الطبيعى بعد ستة و اثني عشر شهرا من العلاج. بالأضافة إلى ذلك وجدت علاقة ذو دلالة إحصائية بين هذه المؤشرات الحيوية والحالة الطبية السريرية المرضية لسرطان الثدي. و من ناحية اخرى وجد هناك علاقة طردية ذات دلالة إحصائية بين مستويات المستقبل والمانع لليوروكيناز منشط البلازمينوجينوالبروتين المحفز لفلزية بروتين النسيج الخارجى للخلية.

و نستخلص من هذه الدراسة: أن إرتفاع مستوي كلا من المستقبلوالمانع لليوروكيناز منشط البلازمينوجين ،البروتين المحفز لفلزية بروتين النسيج الخارجى للخلية يرتبط ارتباطا وثيقا ذو دلالة إحصائية مع حدوث مرض سرطان الثدى و إنتشاره و على هذا فقياس مستويات هذه الدلالات البيوكيميائية قد تتنبئ بامكانية حدوث المرض و تطوراته.