Urokinase – type Plasminogen Activator System in Human Breast Cancer

Reyadh Salim Al-Jubouri[1*], Faeza Aftan Al-Rawi[2], Ali Hussien Al-Khafaji[3]
[1]Department of Biology, College of Science, Tikrit University, Salah Al-Din, Iraq. [2]Ibn-Sina College of Medicine, Al-Iraqia University, Baghdad, Iraq, [3]Central Public Health Laboratory, Ministry of Health.

Abstract

Urokinase plasminogen activator (uPA), urokinase plasminogen activator receptor (uPAR) and plasminogen activator inhibitor-1(PAI-1) are essential for metastasis, and overexpression of these molecules is strongly correlated with poor prognosis in a variety of malignant tumors. This study revealed direct correlation between immunohistochemical expression of uPA with pathological stage. No significant association of immunohistochemical expressions of uPA, uPAR and PAI-1 with immunohistochemical expressions for estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor -2 (HER-2/*neu*), and direct association between immunohistochemical expressions of (uPA and uPAR) as well as between immunohistochemical expressions of (uPA and PAI-1).

Keyword: Breast cancer, uPA, uPAR ,PAI-1, Tissue microarray

نظام منشط البلازمينوجين نوع يوروكاينيز في أورام الثدي

رياض سالم الجبوري[1*]، فائزه عفتان الراوي[2]، علي حسين الخفاجي[3]
[1]قسم علوم الحياة، كلية العلوم، جامعة تكريت، [2] كلية طب ابن سينا، الجامعة العراقية، [3]مختبر الصحة العامة المركزي، وزارة الصحة، بغداد، العراق.

الخلاصة:

يعتبر منشط البلازمينوجين نوع يوروكاينيز ومستقبل منشط بلازمينوجين نوع يوروكاينيز ومثبط منشط بلازمينوجين نوع 1 ذات اهمية لانتشار مرض السرطان وان التعبير الكيميائي النسيجي المناعي لهذه البروتينات له ارتباط وثيق في التكهن السيء للأورام الخبيثة، اذ توصلت هذه الدراسة الى وجود علاقة مباشرة بين التعبير الكيميائي النسيجي المناعي لمنشط البلازمينوجين نوع يوروكاينيز مع المرحلة السريرية للمرض. فضلا عن ذلك اظهرت هذه الدراسة عدم وجود علاقة معنوية بين التعبير الكيميائي النسيجي المناعي لكل من منشط بلازمينوجين نوع يوروكاينيز ومثبطه ومنشطه مع مستقبلات الاستروجين والبروجستيرون ومتلقي عامل النمو البشري النوع الثاني، وكانت هناك علاقة معنوية بين التعبير الكيميائي النسيجي المناعي لكلاً من منشط بلازمينوجين نوع يوروكاينيز ومستقبل منشط بلازمينوجين نوع يوروكاينيز وكذلك وجود علاقة معنوية بين التعبير الكيميائي النسيجي المناعي لمنشط بلازمينوجين نوع يوروكاينيز ومثبط منشط بلازمينوجين نوع يوروكاينيز.

Introduction

Breast cancer is the most common cause of cancer-related deaths in women [1]. It is continues to rank as one of the top killers of women [2].

In 2009, there were 2987 cases of Iraqi breast cancer in both genders accounting for 19.59% of all newly diagnosed cancer cases. Of them 2906 cases were among females and 81 cases among males. It ranks the first in all the years from

E- mail: reyadhsalim81@gmail.com

1986-2009. It is also the most common cancer among females with incidence rate about 18.45 per 100,000 female population in 2009, compared to 16.65 per 100,000 female population in 2008 [3].

The predominant cause of death in patients with malignant solid tumors is the ability of cancer cells to invade surrounding tissues and form distant metastases. The spread of cancer cells from the primary site to a distant location is known to follow a sequence that requires their detachment from the primary site, migration through the local stroma, invasion into and then extravasation from the vascular tree, before finally migrating toward, adhering to and proliferating at a distant site to form a metastatic tumor [4]. Invasion is accomplished by secretion of a variety of matrix-degrading enzymes including matrix metalloproteinases and plasminogen activator. Urokinase plasminogen activator (uPA) is a serine protease that is involved in extracellular matrix degradation, cancer invasion and metastasis by regulating the plasminogen / plasmin system [5]. Active uPA cleaves inactive plasminogen to generate active plasmin, which can degrade a variety of ECM proteins. Besides, plasmin and uPA can also activate several types of MMPs which, in turn, degrade ECM. Therefore, uPA amplifies proteolytic cascades in ECM degradation which is crucial for cancer invasion. uPA exerts its effect by binding to the urokinase plasminogen activator receptor (uPAR), which localizes uPA on the cell surface, enhancing its plasminogen activation capability [6]. uPA expression has been shown to be up regulated in many cancers, correlated with invasion and metastasis [7]. As well as, plasminogen activator inhibitor-1 (PAI-1), a member of the serine protease inhibitor superfamily, is a key regulator of extracellular matrix homeostasis, protecting the extracellular matrix from excessive degradation [8]. PAI-1 also interacts with the extracellular matrix component vitronectin and thus is believed to be a molecular switch that governs cell adhesion and migration [9]. From these biological properties, it is hypothesized that PAI-1 may play an important role in cancer invasion and metastasis [8]. In support of this hypothesis, high tissue levels of PAI-1 have been reported to predict poor prognosis in several types of human cancers, including breast cancer [10]. Urokinase plasminogen activator (uPA) and plasminogen activator inhibitor 1(PAI-1) tests are tumor markers for breast cancer. High levels may indicate aggressive cancer with high risk of recurrence [11].

Materials and Methods

From March 2011 to Feburary 2012, fifty four paraffin blocks of breast tumors were randomly selected from archive files of Histopathology and Cytology Unit in Tikreet Teaching Hospital-Salah Al-Din-Iraq and Private lab, in Baghdad-Iraq. Forty seven were malignant breast tumors and seven were benign.

For each case, an initial hematoxilin and eosin stained control section was reviewed to confirm an adequate tissue in donor block for transfer to the tissue microarray (TMA) block and to select and mark the location points for cores to be taken. Beecher TMA instrument (Beecher Instrument, Sun Prairie, WI 53590) was used to remove 2 cores of 0.6 mm from each donor block and transferred them to a recipient block. Cores were arranged in sectors, each containing 12 rows with 12 cores per row, the distance between each two cores 1mm and each two rows 1mm. TMA block was cut at a thickness of 5μm on a microtome cutter (Leica RM2135). Sections were placed on poly-L-lysine (PLL) coated slides (polysine, Thermo Fisher) and heated at 58°C for 24 hours after that the melting paraffin wax was added on the top of TMA section to prevent loss of cores. Slides were deparaffinized and rehydrated in graded alcohols, heat-induced epitope retrieval were done by immersing them in a 0.01-mol/L concentration of citrate buffer (pH 6.0) preheated to more than 90°C and left for 20 minutes, followed by 20-minutes cool down period at 25-28 °C. Then slides were incubated with uPA, uPAR, PAI-1, Estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER-2) antibodies markers.

Scoring system of IHC

Cut off values of HER2 receptor were done according to Dako scoring system, using the following categories: 0, negative result or membrane staining in <10% of the tumor cells; 1+; weak and incomplete membrane staining in >10% of the tumor cells; 2+; weak or moderate, complete membrane staining in >10% of the tumor cells; 3+; strong complete membrane staining in >10% of the tumor cells). Score +1 considered negative.

Scoring for ERα and PR was done according to Allred *et al*. [12]. Semi-quantitative system that takes into consideration the proportion of positive cells (scored on a scale of 0-5) and

staining intensity (scored on a scale of 0-3). Every tumor was given a score which represents the outcome of the summation of the intensity of the staining (intensity of score IS) (no staining = 0; weak = +1; intermediate staining = +2; strong staining = +3) with the percentage of stained cells (proportion score PS) (0% = 0); (1% = 1); (2-10% =2); (11-33% = 3); (34-66%=4); (67-100%=5). The proportion and intensity were then summed to produce total scores of 0 or 2 through 8. A score of 0-2 was regarded as negative while 3-8 as positive. The maximum score according to this system was 8.

uPA, uPAR and PAI-1 scoring was employed according to Minisini *et al*. [13] and Dublin *et al*. [14]. It is semi-quantitive system and two parameters evaluated; (percentage of tumor cell stained and the intensity of stain), as a following formula;

Σ(%Positive Cells)×(Staining Score)× 100

The stain intensity negative =0; weak =+1 and strong= +2, cut off value 0%, from 0-10% was weak positive and more than 10% was strong positive.

Chi square, Fisher's Exact test, and ANOVA were used. $P < 0.05$ was considered as significant.

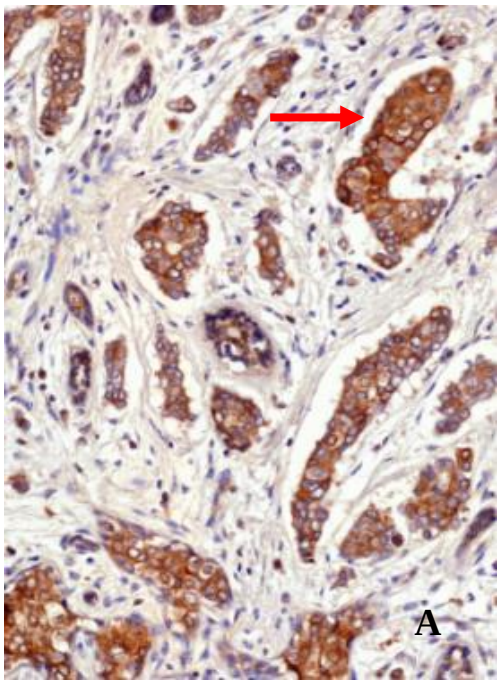
Results

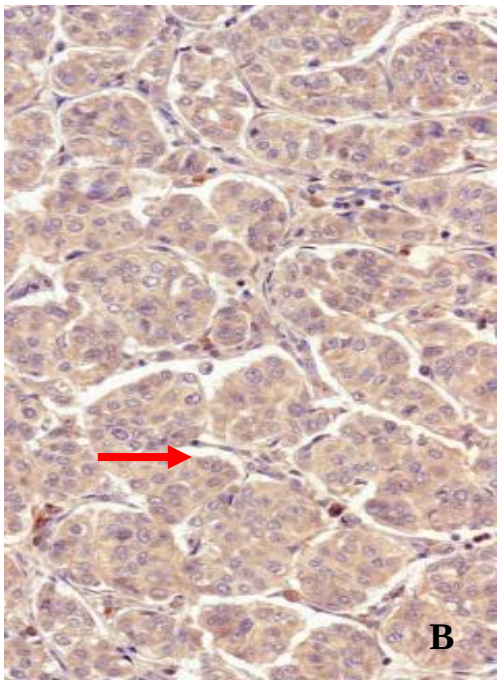
Patient's age ranged from 29-85 years with a mean of 50.7±11.8 years. The peak age frequency was in the age category 40-49 years. All those cases were invasive ductal carcinoma. Of the tissue specimens, 3 (6.4%) were equal or less than 2 cm in largest diameter and 44 (93.6%) were more than 2cm, positive lymph node metastasis were in 32(68.1%). Grade I infiltrative ductal carcinoma formed 6 (12.8%) grade II 32 (68.1%) and grade III 9 (19.1%). 3 (6.4%) were stage I, 20 (42.5%) were stage II and 24 (51.1%) were stage III.

From 7 benign cases 71.4%, 57.1%, 85.7% and 42.9% revealed positive expression for ER, uPA, uPAR and PAI-1 respectively, and these cases were negative for PR and HER-2.

From 47 infiltrative ductal carcinoma 63.8%, 23.4%,25.5%,76.6%,78.7% and 89.3% revealed positive expression for ER, PR, HER-2, uPA, uPAR and PAI-1 respectively. Triple negative breast cancer was showed in 12 out of 47 (25.5%).

Positive immunostaining with uPA, uPAR and PAI-1 was observed in the cytoplasm of tumor cells as a brown diffusion pigmentation figure1. Association of uPA with pathological stage was significant table1, as well this study revealed that indirect association of uPA, uPAR and PAI-1 with ER, PR and HER-2 table2. Significant association of uPA with (uPAR and PAI-1) table3.





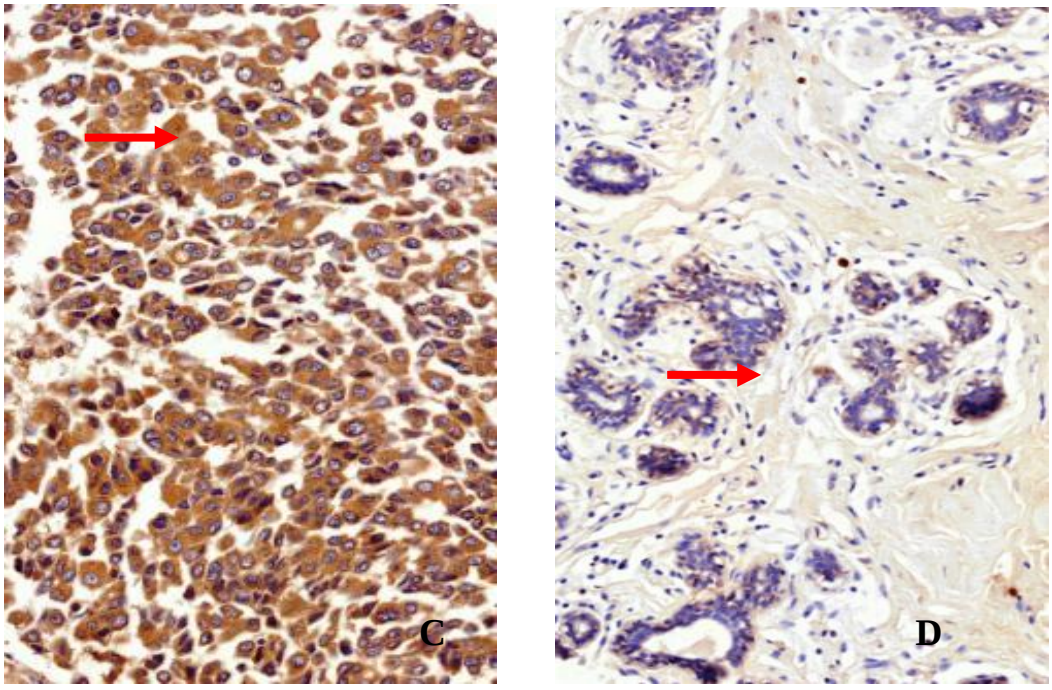


Figure 1- Immunohistochemical staining of uPA, uPAR and PAI-1 in breast tumor. (A) Strong uPA expression in a grade II invasive ductal carcinoma; (B) strong uPAR expression in a grade III invasive ductal carcinoma; (C) strong PAI-1 expression in a grade II invasive ductal carcinoma; (D) and weak uPA expression in the epithelial cells of a fibroadenoma. Original magnification, X10. Red arrows indicated for stained cells.

Table 1- Association of uPA, uPAR and PAI-1expression with clinicopahlogical features

	uPA +	uPA-	uPAR+	uPAR-	PAI-1+	PAI-1-
Tumor largest diameter						
≤ 2cm	3(100%)	0(0%)	1(33.3%)	2(66.7%)	3(1000%)	0(0%)
> 2cm	33(75%)	11(25%)	36(81.8%)	8(18.2%)	39(88.6%)	5(11.4%)
P value	0.4		0.2		0.2	
Nodal status						
Negative	11(73.3%)	4(26.7%)	11(73.3%)	4(26.7%)	14(93.3%)	1(6.7%)
Positive	25(78.1%)	7(21.9%)	26(18.2%)	6(18.8%)	28(87.5%)	4(12.5%)
P value	0.4		0.6		1.0	
Histological grade						
I	3(50%)	3(50%)	5(83.3%)	1(16.7%)	6(100%)	0(0%)
II	27(84.4%)	5(15.6%)	25(78.1%)	7(21.9%)	27 (84.4%)	5(15.6%)
III	6(66.7%)	3(33.3%)	7(77.8%)	2(22.2%)	9(100%)	0(0%)
P value	0.3		0.6		0.4	
Pathological stage						
I	3(100%)	0(0%)	1(33.3%)	2(66.7%)	3(100%)	0(0%)
IIA	5(55.6%)	4(44.4%)	7(77.8%)	2(22.2%)	8(88.9%)	1(11.1%)
IIB	11(100%)	0(0%)	9(81.8%)	2(18.2%)	10(90.9%)	1(9.1%)
IIIA	11(64.7%)	6(34.3%)	13(76.5%)	4(23.5%)	14(82.4%)	3(17.6%)
IIIB	6(85.7%)	1(14.3%)	7(100%)	0(0%)	7(100%)	0(0%)
P Value	0.024**		0.2		0.4	

** significant

Table 2- Association of uPA, uPAR and PAI-1 with ER, PR and HER-2/*neu* expression

	uPA+	uPA-	uPAR+	uPAR-	PAI-1+	PAI-1-
ER+	25(83.3%)	5(16.7%)	23(76.7%)	7(23.3%)	26(86.7%)	4(13.3%)
ER-	11(64.7%)	6(35.3%)	14(82.4%)	3(17.6%)	16(94.1%)	1(5.9%)
P value	0.3		0.2		0.3	
PR+	11(100%)	0(0%)	9(81.8%)	2(18.2%)	10(90.9%)	1(9.1%)
PR-	25(69.4%)	11(30.6%)	28(77.8%)	8(22.2%)	32(88.9%)	4(11.1%)
P value	0.3		0.7		0.9	
HER-2+	9(75%)	3(25%)	9(75%)	3(25%)	12(100%)	0(0%)
HER-2-	27(77.1%)	8(22.9%)	28(80%)	7(20%)	30(85.7%)	5(14.3%)
P value	0.8		0.4		0.1	

Table 3- Association between uPA, uPAR andPAI-1 expressions

	uPA+	uPA-	uPAR+	uPAR-	PAI-1+	PAI-1-
uPA+			27(75%)	9(25%)	32(88.9%)	4(11.1%)
uPA-			10(90.9%)	1(9.1%)	10(90.9%)	1(9.1%)
P value			0.003**		0.005**	
uPAR+					34(91.9%)	3(8.1%)
uPAR-					8(80%)	2(20%)
P value					0.05	

** significant

Discussion

The current study showed no significant association between (uPA, uPAR and PAI-1) with patient's age, tumor largest diameter, lymph nodes status and histological grade. Our results agreed with that of [15] who reported no significant association between uPA, uPAR and PAI-1 expression and patient's age, tumor nodal status, histological grade. Hurd *et al*. determined that uPA, uPAR and PAI-1 were expressed in both high and low grade *in situ* ducal carcinoma and the co-expressed of uPA and uPAR may improve identification of *in situ* ductal carcinoma with increased potential for invasion [16]. Kennedy *et al*. [17] found no significant relationship between uPA and uPAR levels with tumor size and nodal status. Han *et al*. [18] revealed no significant correlation between uPA with tumor size and age, while significant correlation with nodal status. Other studies revealed adverse correlation between uPA/PAI-1 complex and histological grade, as well as found high level of uPA and PAI-1 with lymph node negative primary invasion breast cancer [19,20].Other study found significant correlation between uPA and PAI-1 with histological grade while not significant with uPAR. Additionally, no significant correlation between uPA, uPAR, PAI-1 with tumor size and nodal status and reported that uPA expression breast carcinoma was more common in invasive ductal carcinoma than in intraductal carcinoma [14].

An Italian study, reported positive expression of uPA and PAI-1 in 92% and 91% respectively, also this study reported that PAI-1 expression was not associated with other classical predictive and prognostic factors in breast cancer [13].

Wolff *et al*. [21] found that PAI-1 expression correlated with nodal stage, but not with other patient's parameters and no correlation between uPA and grade. Furthermore, Manders *et al*. [19] reported complexes of uPA and PAI-1 in high-grade, node-negative invasive breast carcinoma indicate a worse prognosis. Thus, tumor grade and expression of these analytes appear interrelated. Jahkola *et al*. [22] revealed no correlation between uPA, PAI-1 with age, tumor size and histological grade.

This study revealed a significant association between uPA positive expression and stage, whereas no relation between positive expression

of uPAR and PAI-1 and stage. Han *et al*. [18] found a significant association between uPA expression and stage. Kotzasch *et al*. [23] revealed that no significant association between uPAR positive expression and tumor (stage, node involvement and age of patients).

This study revealed significant association between uPA (uPAR and PAI-1) this concordance with [14, 24]. Other study revealed a significant association between (uPA and uPAR) and no significant association between (uPA and uPAR) and ER status this is agree with our results [17]. Minisini *et al*. [13] revealed that a significant association between (uPA and PAI-1) and showed direct correlation between uPA and ER. The current results found no significant association between (uPA, uPAR and PAI-1) and HER-2. Jahkola *et al*. [22] revealed no significant association between (uPA and PAI-1) and HER-2. Wollf *et al*. [21] revealed no relation between uPA and ER. Herbeck *et al*. [25] reported that uPA and PAI-1 are particularly useful in classifying high risk breast cancer for treatment with adjuvant chemotherapy. However, they did not find these analytes useful to select patients for endocrine therapy, which is consistent with our findings that uPA, uPAR and PAI-1 expression in breast cancer where unrelated to ER.

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