UPAR Overexpression Could Be a Potential Favorable Prognostic Marker in Invasive Ductal Carcinoma of Breast

Ata Abbasi[1,2], Elnaz Mehdipour[2], Hengameh Mojdeganlou[2]

[1] *Cellular and Molecular Research Center, Cellular and Molecular Medicine Institute, Urmia University of Medical Sciences, Urmia, Iran*

[2] *Department of Pathology, Faculty of Medicine, Urmia University of Medical Sciences, Urmia, Iran*

Received: 23 Jul. 2021; Accepted: 26 Mar. 2022

Abstract- Breast cancer is the most common malignant tumor in women. Some factors, including histological grade, vascular invasion, and metastasis, are known prognostic factors. Many studies have been performed to find more predictive factors such as urokinase plasminogen activator system markers. Here, we tried to evaluate the relationship between Urokinase-type plasminogen activator receptor expression and other histopathologic parameters. 62 malignant breast tumors were enrolled. UPAR immunohistochemistry staining was performed on paraffin blocks. We evaluated the relationship between UPAR expression and histopathologic factors, including tumor size, tumor type, histologic grade, lymph node status, lymphovascular and perineural invasions, and hormone receptors status (ER, PR, and HER2). The patient's mean age was 46.18±10.35 years. We found a positive relationship between UPAR expression and the presence of carcinoma insitu (*P*=0.027). A negative relationship was observed between UPAR expression and lymph node involvement (*P*=0.01), the number of involved lymph nodes (*P*=0.027), and also intensity and percentage of UPAR positivity in the case with lymph node involvement (*P*=0.005 and 0.029, respectively). UPAR expression is associated with lymph node metastasis which is one of the most important predictors of prognosis in breast cancer. So, it could be used as a favorable prognostic factor in breast cancer.

Acta Med Iran 2022;60(5):293-298.

Keywords: Urokinase plasminogen activator (UPAR); Breast cancer; Histologic grade

Introduction

Breast cancer is the most common cancer among women worldwide and represents the leading cause of death among women (1,2). Its incidence and mortality rates are increasing significantly (3). There are a variety of risk factors for breast cancer, including race, genetics, alcohol and exogenous hormone consumption, and parity and age of menarche (4).

Treatment is multimodal and involves a combination of chemotherapy, radiotherapy, surgery, and /or endocrine therapy (5,6). In recent years, investigation for new drugs and targeted therapy have led to advances in the treatment of this cancer (7). They have attracted great attention because they give the possibility of killing cancer cells without significant side effects on other healthy organs. These drugs interact with molecules that are exclusively expressed or overexpressed in tumoral cells (8).

The plasminogen system that includes the urokinase plasminogen activator (uPA) and its receptor (uPAR) is a good candidate for targeted therapy (8).

The urokinase plasminogen activator (uPA) is a serine protease. It converts plasminogen to plasmin and leading to the dissolution of clots. It also controls the process of inflammation, wound healing, cellular apoptosis, and angiogenesis (9). Also, the uPAR expression is significantly increased in cancer in particular conditions such as hypoxia (8).

It has also been known that overexpression of the uPA and its receptor uPAR leads to the aggressive phenotype in some cancers, such as breast, lung, and GI cancers, and it is highly correlated with metastasis (10,11).

Corresponding Author: A. Abbasi
Department of Pathology, Faculty of Medicine, Urmia University of Medical Sciences, Urmia, Iran
Tel: +98 9124397395, Fax: +98 33433854, E-mail address: aabbasi@alumnus.tums.ac.ir

Molecular targets can be used as diagnostic and therapeutic options. Specifically, they provide new treatment options to patients who do not respond to traditional therapies or cannot receive chemotherapy (8).

Taking together all previous data and Based on the importance of uPAR as a molecular target in cancer, we aimed to evaluate the relationship between uPAR expression and histopathologic parameters of breast cancer such as histologic type, tumor grade and size, vascular invasion, lymph node metastasis, and patients age.

Materials and Methods

A total of 62 patients who were diagnosed with invasive carcinoma of the breast and underwent mastectomy were enrolled. With ethical considerations, paraffin blocks were taken from the archive of the pathology department of Urmia University of Medical Sciences (UMSU), Urmia, Iran. Immunohistochemistry (IHC) staining of these samples for hormone profiles, including estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2/neu), and Ki67 were also included.

The prepared glass slides (Hematoxylin and eosin (H and E) and IHC preparations) were reinvestigated by 2 pathologists blinded to the clinicopathological data. Tumor grading and staging were performed according to the Nottingham modification of the Bloom Richardson system and the American Joint Committee on Cancer (AICC) system, respectively.

New sections were made for the broken and poor-quality slides.

Tissue specimen and immunohistochemistry

Four-micrometer thick sections were prepared from the paraffin blocks, and IHC staining for uPAR was performed according to the manufacturer's protocol. A sample from a known case of urinary bladder cancer is used as a positive control with diffuse cytoplasmic staining. Primary antibody was also omitted for negative control.

Histological evaluation

Immunohistochemistry results for uPAR staining were interpreted as follows:

The intensity of staining: None, mild, moderate, or strong.

Proportion (Percentage) of reactivity: Cytoplasmic immunoreactivity in ≥10% of cells was interpreted as positive (regardless of the intensity of staining), and cytoplasmic staining in <10% of cells was interpreted as negative (12,13).

Statistical analysis

Statistical analysis was performed using SPSS version 16.0 (SPSS Inc., Chicago, IL, USA) to investigate the relationships between all findings, and the results are expressed as mean±SD. The qualitative data were determined by χ 2 analysis. $P \leq 0.05$ was considered to be statistically significant.

Results

Study population

Sixty-two cases were enrolled in this study. The patients' mean age was 46.18±10.35 years (Range: 29-72 years). In twenty-seven patients (43.5%), the masses were on the right, and in thirty-five (56.5%) were on the left breast. The mean diameter of the tumor was 4±2.41 cm. of 62 cases, 58 (93.5%) were diagnosed with invasive ductal carcinoma, and 4 (6.5%) were with invasive lobular carcinoma. Two (3.2%) of the evaluated tumors were grade I, 32 (51.6%) were grade II, and 28 (45.2%) were grade III.

ER, PR, and Her2/neu staining

Of 62 cases, 16 (25.8%) were luminal A, 15 (24.2%) were Her2 positive, 22 (35.5%) were luminal B, and 9 (14.5%) Were triple negative.

uPAR staining

IHC staining for the uPAR marker revealed that 38 cases (61.3%) were positive, and 24 cases (38.7%) were negative for this marker (Figure 1). Based on the cytoplasmic staining intensity, 19 cases (30.6%) were weakly positive, 14 cases (22.6%) were moderately positive, and 5 (8.1%) were strongly positive. There was a statistically significant relationship between uPAR expression with axillary lymph node involvement and insitu component (P=0.01 and P=0.027, respectively), as the lower expression of UPAR molecule, the higher frequency of lymph node involvement and in situ component. The relationship between uPAR expression and clinicopathological parameters is shown in (Table 1). Moreover, there was a relationship between uPAR expression and the number of axillary lymph node involvement (P=0.027) (Table 2). A statistically significant relationship between axillary lymph node involvement and intensity and percentage of uPAR expression was also seen (P=0.005 and P=0.029, respectively) (Table 3). However, no relationship was

found between uPAR expression and histological type, histological grade, tumor side, tumor size, Lymph-vascular invasion, perineural invasion, nipple involvement, skin involvement, both estrogen and progesterone receptor, HER 2/neu, and Ki 67 status.

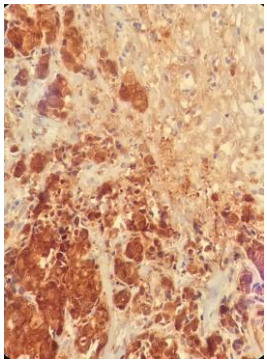

Figure 1. Showing UPAR staining using the immunohistochemistry method (IHC) with strong cytoplasmic staining in tumoral cells (IHC, 40X)

Table 1. Showing histologic parameters and UPAR expression

			UPAR expression		
		N %	**Positive (n=38)**	**Negative (n=24)**	***P***
Histological type	**Invasive Ductal Carcinoma**	58 (93.5%)	35	23	0.56
	Invasive lobular Carcinoma	4 (6.5%)	3	1	
	Medullary carcinoma	0	0	0	
Histologic grade	**Grade I**	2 (3.2%)	1	1	0.75
	Grade II	32 (51.6%)	21	11	
	Grade III	28 (45.2%)	16	12	
Tumor Side	**Right**	27 (43.5%)	13	14	0.06
	Left	35 (56.5%)	25	10	
Tumor Size	**< 2cm**	10(16.1%)	7	3	0.76
	2cm – 5 cm	41(66.1%)	25	16	
	> 5cm	11(17.8%)	6	5	
Lymph-vascular invasion	**present**	46 (74.2%)	29	17	0.63
	Not identified	16 (25.8%)	9	7	
Perineural invasion	**present**	28 (45.2%)	17	11	0.93
	Not identified	34 (54.8)	21	13	
Nipple involvement	**present**	9 (14.5%)	6	3	0.72
	Not identified	53 (85.5%)	32	21	
Skin involvement	**present**	10 (16.1%)	7	3	0.53
	Not identified	52 (83.9%)	31	21	
Axillary lymph node Involvement	**present**	49 (79%)	26	23	0.01*
	Not identified	13 (21%)	12	1	
INSITU component	**present**	39 (62.9%)	28	11	0.027*
	Not identified	23 (37.1%)	10	13	
Estrogen receptor	**Positive**	33 (53.2%)	23	10	0.147
	Negative	29 (36.8%)	15	14	
Progesterone Receptor	**Positive**	35 (56.5%)	24	11	0.18
	Negative	27 (43.3%)	14	13	
HER 2	**Positive**	22 (35.5%)	13	9	0.86
	Negative	40 (64.5%)	25	15	
Molecular Subtype	**Luminal A**	16 (25.8%)	11	5	0.28
	Luminal B	22 (35.5%)	15	7	
	HER2 / neu	15 (24.2%)	9	6	
	Triple Negative	9 (14.5%)	3	6	
Ki 67	**Ki67 ≤ 14 %**	13 (20.9%)	6	7	0.23
	Ki67 >14%	15 (24.1%)	8	7	
	undefined	34 (55%)	24	10	

*$P<0.05$ is considered as significant

Table 2. Shows the relationship between UPAR expression and the number of involved axillary lymph nodes

			uPAR expression		
		N %	Positive (n=38)	Negative (n=24)	*P*
Number of Axillary lymph node Involvement	**1-3**	20 (32.3%)	8	12	0.027*
	4-9	14 (22.6%)	9	5	
	≥10	15 (24.2%)	9	6	

*P< 0.05 is considered as significant

Table 3. Shows the relationship between the intensity of UPAR expression and axillary lymph node involvement

			Axillary lymph node Involvement		
		N %	Present (n=49)	Not identified (n=13)	*P*
intensity of uPAR expression	**Negative**	24 (38.7%)	23	1	0.005*
	Weakly Positive	19 (30.6%)	16	3	
	Moderately positive	14 (22.6%)	8	6	
	Strongly positive	5 (8.1%)	2	3	
uPAR expression	**Negative (<10%)**	26 (41.9%)	24	2	0.029*
	Positive (≥10%)	36 (58.1%)	25	11	

*P< 0.05 is considered significant.

Discussion

Breast cancer is the most common cancer among women worldwide and also the leading cause of death among women (1,2). Although the incidence of this cancer is lower in Iran compared to other countries, its incidence and mortality rates are increasing significantly (3,14,15).

Today employment of the biomarkers gained great interest in the treatment of breast cancer and many studies have been done to find the different aspects of these biomarkers in the treatment and prognosis of the cancers. Among these biomarkers is the urokinase of plasminogen activator that overexpression of its receptor (uPAR) has been found in many cancers, and it correlates with aggressive phenotypes (10).

Studies have shown that the expression of uPAR correlates with the invasive course of the tumor and shorter overall survival and Relapse-free survival (13).

Our study revealed a statistically significant relationship between uPAR expression and the presence of carcinoma insitu component and lack of axillary lymph node metastasis. So that there was a reverse relation between uPAR expression and the number of involved lymph nodes. When there was lymph node involvement, we found a reduction in the percentage and intensity of immunostaining.

Breast cancer metastasizes to the lymph nodes through the lymphovascular system. It is notable that lymph node is one of the most important predictors of breast cancer. And the number of involved lymph nodes is associated with poor survival (16). So, lots of studies focused on the relationship between lymph node status and breast cancer prognosis.

In our study, no significant relationship has been found between uPAR expression and tumor histologic type and grade, molecular type, tumor size or site, lymphovascular and perineural invasion, skin or nipple involvement, and estrogen and progesterone receptors and HER2. A similar study by Seth B. Sereff has shown that the expression of uPA and uPAR was unrelated to HER2 status. But Sereff has found that Primary ER/PR status was related to uPA, uPAR, or PAI-1 levels that ER/PR negative cancers expressed elevated uPA and uPAR in comparison with ER/PR positive tumors (9). That is inconsistent with our experiment.

A consistent study by Andres *et al*., in the U.S., has shown that the expression of uPAR, uPA and PAI-1 had no relationship with age, menopause, tumor grade, and hormone receptors expression. But they found neither a relationship between uPAR, uPA, and PAI-1 expression and lymph node involvement which is not similar to our findings (17).

In the study of Kim *et al*., in South Korea, the

number of lymph node metastasis is significantly higher in patients with high uPAR expression than in patients with low levels of uPAR expression, which is inconsistent with our study (18).

A study by Maja Lampelj *et al.*, in Slovenia, has shown that there was a relationship between tumor size and grade, histologic type and lymphovascular invasion, and uPAR and plasminogen activator inhibitor-1 (PAI-1) expression; there was also a reverse relationship between positive hormone receptors and uPA (19). However, no significant relationship was found between patients' age and axillary lymph node involvement and uPA and PAI-1. None of the above relationships have been found in our study.

In Ukraine, Jelisavac-cosic S *et al*., have found a statistically significant relationship between histologic grade, tumor size, Nottingham index, and uPA and PAI-1 expression, which is inconsistent with our study (20).

In conclusion, we found that uPAR expression in breast cancer is associated with the presence of carcinoma insitu components and lower axillary lymph node metastasis. Lymph node metastasis is an important prognostic factor in breast cancer and based on our findings, uPAR expression could be a predictive marker for patient prognosis in breast cancer.

Since this is a novel subject and only a little research was done, controversial findings do exist regarding this topic. Therefore, more detailed studies, especially on the relationship of this marker with different cancer parameters, can lead to new insights into patient treatment and prognosis of breast cancer.

Acknowledgments

This research has been supported by Urmia University of Medical Sciences research grants. The authors declare that there is no conflict of interest.

References

1. Wörmann B. Breast cancer: basics, screening, diagnostics and treatment. Med Monatsschr Pharm 2017;40:55-64.
2. Fisusi FA, Akala EO. Drug Combinations in Breast Cancer Therapy. Pharm Nanotechnol 2019;7:3-23.
3. Anastasiadi Z, Lianos GD, Ignatiadou E, Harissis HV, Mitsis M. Breast cancer in young women: an overview. Updates Surg 2017;69:313-7.
4. Coughlin SS. Epidemiology of Breast Cancer in Women. Adv Exp Med Biol 2019;1152:9-29.
5. Chew HK. Adjuvant therapy for breast cancer: who should get what? West J Med 2001;174:284-7.
6. de Matteis A, Nuzzo F, D'Aiuto G, Labonia V, Landi G, Rossi E, et al. Docetaxel plus epidoxorubicin as neoadjuvant treatment in patients with large operable or locally advanced carcinoma of the breast: a single-center, phase II study. Cancer 2002;94:895-901.
7. Gu G, Dustin D, Fuqua SA. Targeted therapy for breast cancer and molecular mechanisms of resistance to treatment. Curr Opin Pharmacol 2016;31:97-103.
8. Zuppone S, Assalini C, Minici C, Bertagnoli S, Branduardi P, Degano M, et al. The anti-tumoral potential of the saporin-based uPAR-targeting chimera ATF-SAP. Sci Rep 2020;10:2521.
9. Sereff SB, Daniels MW, Wittliff JL. Relationships of protein biomarkers of the urokinase plasminogen activator system with expression of their cognate genes in primary breast carcinomas. J Clin Lab Anal 2019;33:e22982.
10. Harel ET, Drake PM, Barfield RM, Lui I, Farr-Jones S, Van't Veer L, et al. Antibody-Drug Conjugates Targeting the Urokinase Receptor (uPAR) as a Possible Treatment of Aggressive Breast Cancer. Antibodies (Basel) 2019;8:54.
11. Fisher JL, Field CL, Zhou H, Harris TL, Henderson MA, Choong PF. Urokinase plasminogen activator system gene expression is increased in human breast carcinoma and its bone metastases--a comparison of normal breast tissue, non-invasive and invasive carcinoma and osseous metastases. Breast Cancer Res Treat 2000;61:1-12.
12. Christensen A, Kiss K, Lelkaitis G, Juhl K, Persson M, Charabi BW, et al. Urokinase-type plasminogen activator receptor (uPAR), tissue factor (TF) and epidermal growth factor receptor (EGFR): tumor expression patterns and prognostic value in oral cancer. BMC Cancer 2017;17:572.
13. Hildenbrand R, Schaaf A, Dorn-Beineke A, Allgayer H, Sütterlin M, Marx A, et al. Tumor stroma is the predominant uPA-, uPAR-, PAI-1-expressing tissue in human breast cancer: prognostic impact. Histol Histopathol 2009;24:869-77.
14. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015. CA Cancer J Clin 2015;65:5-29.
15. Ghoncheh M, Pournamdar Z, Salehiniya H. Incidence and mortality and epidemiology of breast cancer in the world. Asian Pac J Cancer Prev 2016;17:43-6.
16. Liu D, Chen Y, Deng M, Xie G, Wang J, Zhang L, et al. Lymph node ratio and breast cancer prognosis: a meta-analysis. Breast Cancer 2014;21:1-9.
17. Andres SA, Edwards AB, Wittliff JL. Expression of urokinase-type plasminogen activator (uPA), its receptor (uPAR), and inhibitor (PAI-1) in human breast carcinomas and their clinical relevance. J Clin Lab Anal

2012;26:93-103.

18. Kim EY, Do SI, Hyun K, Park YL, Kim DH, Chae SW, et al. High Expression of Urokinase-Type Plasminogen Activator Is Associated with Lymph Node Metastasis of Invasive Ductal Carcinoma of the Breast. J Breast Cancer 2016;19:156-62.
19. Lampelj M, Arko D, Cas-Sikosek N, Kavalar R, Ravnik M, Jezersek-Novakovic B, et al. Urokinase plasminogen activator (uPA) and plasminogen activator inhibitor type-1 (PAI-1) in breast cancer-correlation with traditional prognostic factors. Radiol Oncol 2015;49:357-64.
20. Jelisavac-Cosic S, Sirotkovic-Skerlev M, Kulic A, Jakic-Razumovic J, Kovac Z, Vrbanec D. Prognostic significance of urokinase-type plasminogen activator (uPA) and plasminogen activator inhibitor (PAI-1) in patients with primary invasive ductal breast carcinoma - a 7.5-year follow-up study. Tumori 2011;97:532-9.