Plasmin Activator System in Malignant Tumors Implications for Invasion and Metastasis Management

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Abstract
Objective: firstly to Investigating and evaluating of plasmin- plasminogen markers that include; uPA, tPA, plasmin, in sera of malignant and benign tumors patients and compare them markers levels to their corresponding healthy controls, secondly to studying the possible relationship between plasmin-plasminogen system variables, and thirdly to Prediction which one of plasmin-plasminogen system variables is more specificity and sensitivity to choose it to be good tumor marker. Design and Methods: The experiments comparing the plasmin activator in 160 individuals subdivided to three groups: first group consisted from 80 patients with malignant tumors between the ages of (30-63) years while the second group comprised from 40 patients with benign tumors between the ages of (30-60)years, and third group consisted from 40 subjects who appeared in healthy characterization(control group) between the ages of (27-63)years. All participants underwent to the medical examinations to make sure they have tumors. The activity of plasmin, urokinase-type plasminogen activator (u-PA) and tissue plasminogen activator (t-PA) was determined by applied ELISA method by certain kit in all study groups individuals. Results: The results of the statistical analysis in the current study showed a significant decrease in the levels of ($p < 0.05$) uPA , tPA ,and plasmin ($p = 0.000$) in patients with malignant tumors compared to benign and healthy controls, and the same results were observed when comparing the healthy and benign control together.
Key Words: uPA, tPA, Plasmin, Malignant tumors

Introduction

Cancer (malignancy) is a heterogeneous disorder characterized by different cellular genetic alterations and diverse clinical behaviors resulting from uncontrolled division leading to loss of control over cell growth [1,2].Malignant tumor develops antigenic potential which is the ability to form new blood vessels and capillaries, therefore, malignant tumors can generate their own blood supply to bring in oxygen and nutrients [3]. In order to continue to spread rapidly and counteract the hostile environment observed in tumors, cells must increase the rate of metabolic reactions to provide adenosine triphosphate (ATP), lipids, nucleotides, and amino acids needed to produce daughter cells[4]. An increase in the rate of glycolysis (conversion of glucose to pyruvate) is one of the most necessary modifications in metabolism, as well as aerobic glycolysis (lactate production) which is a universal phenotype of tumors even in the presence of O_2[5].

In fact, extracellular matrix lysis (ECM) is one of the most important steps involved in local invasion and remote metastatic diffusion [6] . The critical intermediary for the degradation of fibrin is plasmin - a strong serine protein. Plasmin also plays a key role in ECM degradation directly and indirectly through activation of several proteases that act on matrix degradation. Plasminogen is generated by its precursor zymogen, plasminogen, via the cleavage of the peptide bond Arg561 -Val562 by tissue plasminogen activator (tPA) and plasma activator urukinase type (uPA) .The liver produces and excretes plasminogen [7] . In processes, such as wound healing and tissue remodeling occurring in TME, uPA is the predominant form of plasminogen activator, while tPA is the main circulatory activator. While tPA is found in the ECM of most tissues, uPA is localized on cell surfaces by its receptor uPAR. The activities of both tPA and uPA are regulated by plasminogen activator inhibitors (PAI) -1[8] and PAI-2 [9] , while the activity of plasmin itself is inhibited by a 2-antiplasmin [10] and a2-macromatic globulin [11]. Plasmin function in the tumor microenvironment in the past two decades, the main focus of the plasminogen activation system has been on the various components specifically activators, inhibitors and receptors. However, the effects of proteolytic plasmin on cancer development are not well understood. Plasmin can cleave a wide variety of ECM substrates, growth factors, and cytokines. Plasmin-mediated function in TME can be classified into two categories - proteolytic processing of substrates and proteins and plasmin signaling at the cell surface. Plasmin has been indirectly implicated in the development and progression of tumors by activating various growth factors, such as TGFβ, FGF-2 and HGF. TGFB and uPA are coordinated and tightly regulated in cancer progression. TGFB regulates uPA expression, which enhances plasmin generation and activates TGFβ which contributes to tumor progression through its effect on EMT, invasion and metastasis [12,13].

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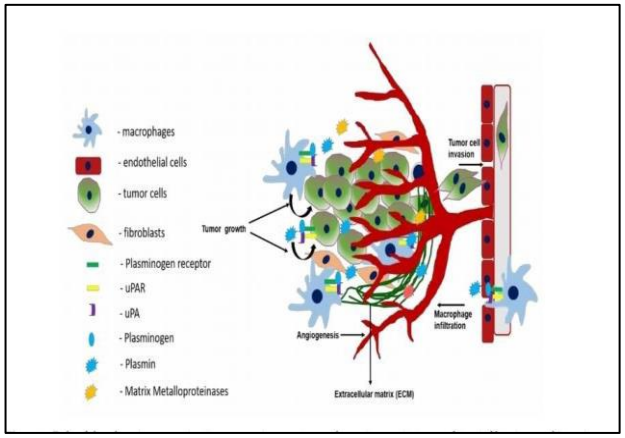

Figure (1): Role of the plasminogen activation system in tumor growth , angiogenesis , macrophage infiltration , and invasion [14] .

Materials and Methods

Study Design

After receiving the required officials approvals, the present study is designed as a case control study conducted on Iraqi citizens in Al-Najaf governorate, Iraq. During the extended period from the beginning of January2022 until the end of October, blood samples are collected from 80 participants with malignant tumors ages ranged from 30 to 63 years and 40 participants with benign tumors ages ranged from 30 to 60 years at the middle Eupharates tumor center and Gastrointestinal and liver diseases and surgery, both patients groups are comprises to 40 subjects were enrolled in the present work according to critical criteria, to be healthy subject group that age ranged from 27_63 years.

Blood Biochemistry Analysis

All blood sampling was carried out under sterile conditions. Blood glucose measurements which included fasting blood glucose, using certain kits from Spinract, Spain , fasting insulin levels using ELISA kit from Calbiotech, USA then determined HOMA-IR by certain equation. urokinase-type plasminogen activator (uPA),tissue-type plasminogen activator (t-PA), and plasmin were measured using ELISA method by Melsin, China kit.

Statistical Analysis:

The statistical analysis was achieved by the statistical package for the social science (SPSS) software for windows, version 23.0. the result were represented as mean ± standard deviation (Mean ± SD**)**. Two - way Analysis of variance was used to compare variables in different studied groups. Pearson's correlation was applied to determine the relation among the measurable factors of the present study, significant was determined regression. The confidence interval was set at 95%, thus p values less than 5% ($p < 0.05$) were considered statistically significant.

Result and Discussion

the total of samples was 160 samples divided in three groups ,first group consisted of 80 malignant tumor patients, second group consisted from 40 patients with benign tumors, the last group consisted of 40 healthy individuals, the malignant tumor group sub divided in to four group recording to type of tumors, first sub group which colorectal tumors included 20 patients, while second subgroup contained 20 samples of breast tumors. The third subgroups included 20 patients with prostate tumors, finally the fourth subgroups contained 20 patients with lung tumors. Table (1) Comparison of urokinase Plasminogen Activator Levels Among The Studied Groups. In the present study there were significant decreasing of uPA levels in Malignant tumors Patients compared to benign and healthy controls, same results were observed when comparing benign with healthy control togather as showen in Table (1)

Table (1): Comparison of urokinase Plasminogen Activator Levels Among The Studied Groups

Subjects(n)	uPA Concentration(pg/mL) Mean ± SD	Min-Max uPA Concentration (pg/mL)	p-value
Malignant 80	150.918 ± 54.690	86.46-291.51	0.049For1*vs.*2
Benign 40	173.115± 65.901	124.30-292.79	0.000 For1*vs.*3
Control 40	200.539 ± 54.730	146.28 -345.60	0.035 For2*vs.*3

1.Malignant 2. Benign 3. Control

Table (2) demonstrated, There were no significant differences observed when comparing patients with colorectal malignancies with other types of malignant tumors (p=0.077,p=0.060) respectively, inaddition to there were no significant differences between breast patients with prostate and lung (p=0.083,p=0.910) and also it was not observed significant difference between patients with malignant tumors of the prostate and lung (p = 0.104) but there is a significant difference between patients with colorectal malignant tumors when compared with the prostate malignant tumors (p=0.001). Data obtained from current study record lower Mean ± SD (121.5573±26.971**)** of uPA levels in prostate malignant tumors than other types of malignant tumors for this, present study suggest that uPA has important role in susceptibility and development of colorectal malignant tumors.

Table (2): Comparison of Urokinase Plasminogen Activator Levels Among Four Types of Malignant Tumors

Subjects(n)	uPA Conc.(pg/mL) Mean ± SD	Min-Max uPA Conc. (pg/mL)	p-value
Colorectal 20	163.619± 45.267	103.05-264.98	0.077 For 1*vs.* 2 0.001For1*vs.* 3 0.060For 1vs.4 0.083 For 2vs. 3 0.910For 2vs. 4 0.104 For 3vs. 4
Breast 20	142.372±40.036	89.93-208.86	
Prostate 20	121.557±26.971	86.46-192.17	
Lung 20	141.037±34.991	87.36-203.95	

1.Colorectal 2. Breast 3. Prostate 4. Lung

Previous study indicated approximately 20 different groups have shown that low levels of uPA in tumor tissues predict poor outcome as prognostic marker in various cancer, uPA provides information that is independent of traditionally used factors such as tumor size, tumor grade, axillary node status and estrogen receptor status in breast tumors. Furthermore, uPA in clinical trial is currently under way to assess whether uPA and its inhibitor, plasminogen activator inhibitor-1, can differentiate between the majority of node-negative breast cancer patients who are cured by surgery from the minority who might benefit from adjuvant therapy. uPA is also prognostic in other malignancies, such as gastric, colorectal, esophageal, renal, endometrial, and ovarian cancers. uPA may thus be a prognostic indicator for multiple types of adenocarcinoma. uPA is involved in regulating breast cancer invasion and metastasis, explained by its ability to facilitate ECM degradation, cell proliferation, angiogenesis, migration and adhesion [15,16]. uPA play a key role in selecting appropriate therapies for patients with breast cancer.

Tissue-type plasminogen activator (tPA) is a serine protease that cleaves plasminogen into active plasmin. In plasma, the primary function of plasmin is the digestion of fibrin, and therefore, tPA is used as a thrombolytic agent for acute treatment of ischemic stroke. However, apart from its fibrinolytic action, tPA is widely distributed in the central nervous system (CNS) and involved in mechanisms of synaptic regulation, synaptic plasticity, and neural injury [17].

The statistical analysis of the tPA result showed the significant decrease (p= 0.000) in the tPA levels of Malignant tumors Patients compared with benign and healthy controls, same results were obtained when the benign tumors group compared with healthy control group as showen in Table (3)

Table (3) : Comparison of Tissue Plasminogen Activator Levels Among The Studied Groups

Subjects(n)	TPA Concentration(ng/mL) Mean ± SD	Min-Max TPA Concentration (ng/mL)	p-value
Malignant 80	1.933± 0.491	1.11-3.23	0.000 For1*vs.*2
Benign 40	2.429 ± 0.575	1.47-3.95	0.000 For1*vs.*3
Control 40	2.696 ±0.812	1.60-4.28	0.050For2*vs.*3

1.Malignant 2. Benign 3. Control

Gender was observed to have no effect on tPA levels in the three study groups, while results of the present work showed significant decrease ($p < 0.05$) of tPA levels in the samples of malignant female patients comparison to benign female patients, with same manner, significant variations were noted when the same gender were compared at the malignant females vs. healthy female and benign female with healthy female (p=0.001) and (p=0.035). As well as same results for males when compared theme tPA levels in three groups.

Results of the recent study agreed with the finding of the present work in the fact of female subgroups showed higher tPA levels comparison to male subgroups

Data from current study recorded lower mean ± SD (1.3452± 0.43197) of tPA levels in prostate tumor patients than other types of malignant tumors for this, present study suggest that uPA has important role in susceptibility and development of colorectal malignant tumors.

Present study recorded that, no significant differences in the tPA levels when the colorectal malignancies, prostate malignant tumors and lung malignant tumors compared together (p=0.067), (p=0.189) respectively. However, there are significant differences when compared colorectal malignant tumors with both breast and lung malignant tumors (p=0.000), as well as there are significant differences between breast tumors patients and prostate malignant tumors also prostate malignant tumors patients and lung malignant tumors (p=0.000) as showen in the Table 4.

Table (4): Comparison of Tissue Plasminogen Activator Levels Among Four Types of Malignant Tumors

Subjects(n)	TPA Conc.(pg/mL) Mean ± SD	Min-Max TPA Conc. (pg/mL)	p-value
Colorectal 20	1.822± 0.404	1.16-2.49	0.000For 1*vs.* 2
Breast 20	2.693± 0.466	1.93-3.72	0.067For1*vs.* 3
Prostate 20	1.545± 0.431	1.05-2.34	0.000For 1vs.4
Lung 20	2.492± 0.573	1.72-3.81	0.000For 2vs. 3 0.189For 2vs. 4 0.000For 3vs. 4

1.Colorectal 2. Breast 3. Prostate 4. Lung

Colorectal cancer is the second most common cancer after lung cancer in men and after breast cancer in women [18]. In addition, despite advances in early diagnosis and treatment, the relative survival of colon and rectal cancer patients has not changed appreciably in recent decades [19,20]. This is due to the fact that colrectal cancer is a heterogeneous disease and its prognosis varies widely despite of the use of prognostic factors such as tumor invasion and lymph node involvement. Consequently, additional prognostic factors are necessary for outcome prediction and treatment planning of subgroups of patients with colorectal cancer. Proteolytic enzymes are involved in cancer invasion and metastasis by virtue of their ability to degrade basement membranes and extracellular matrix proteins surrounding normal tissue [21,22]. Breast cancers with high tPA levels tend to have a better prognosis than those with low levels[23–25]. Colorectal adenoma formation is

associated with marked changes. In a system of plasminogen activation at the tissue level a simultaneous decrease in the level of tPA in terms of antigen and activity in adjacent normal mucosa in patients with colorectal cancer is significantly associated with decreased overall survival [26,27].

Plasmin is a broad-spectrum, highly potent, serine protease whose activation from its precursor plasminogen is tightly regulated by the activators (uPA, uPAR, and tPA), the inhibitors (PAI-1, PAI-2), and plasminogen receptors. Collectively, this system is called the plasminogen activation system. The expression of the components of the plasminogen activation system by malignant cells and the surrounding stromal cells modulates the TME resulting in sustained cancer progression signals.

Levels of serum plasmin concentration were measured in the three study groups; (malignant tumor patients as well as benign tumor patients and healthy controls subjects). Table (5) shows a significant decrease (p = 0.000) of serum plasmin levels in malignant tumor patients group when compared with those of healthy control subjects, with the same manner significant variation was observed when the comparison carried out between benign tumors and healthy control groups; when significant decrease in the levels of plasmin was noted in the benign tumors group compared to those in the healthy subjects. While no significant decrease when compared serum plasmin levels in malignant tumor patients group with those of benign tumor patients.

Table (5): Comparison of Plasmin Levels Among The Studied Groups

Subjects(n)	PlasminConcentration(ng/mL)Mean ± SD	Min-Max PlasminConcentration (ng/mL)	p-value
Malignant80	2.188± 0.400	1.45-2.99	0.588 For1*vs.*20.000 For1*vs.*30.000For2*vs.* 3
Benign40	2.245 ± 0.583	1.30-3.99	
Control40	2.737 ± 0.714	1.57-4.63	

1.Malignant 2.Benign 3. Control

Gender was observed to have no effect on serum plasmin levels in the malignant and benign tumors groups as well as healthy control groups, while results of the present work showed significant decrease ($p < 0.05$) in the plasmin levels of the female malignant tumors patients samples comparison to healthy female, with the same manner; significant variations were noted when the malignant tumors male were compared with both benign tumors male (p = 0.026) and control group male (p = 0.000), respectively.

According to ANOVA test, highly significant differences ($p< 0.05$) were noticed when compared colorectal malignant group with breast (0.000) ,prostate malignant tumors group (0.004) and lung malignant tumors group(0.000), while; no significant variation was noted when breast tumors group compared with both prostate tumors and lung tumors as well as same result obtained when compare prostate tumors with lung tumors as shown in Table (6).

Table (6): Comparison of Plasmin Levels Among Four Types of Malignant Tumors

Subjects(n)	Plasmin Conc. (ng /mL) Mean ± SD	Min-Max Plasmin Conc. (ng/mL)	p-value
Colorectal 20	1.745± 0.305	1.11-2.37	0.000For 1*vs.* 2 0.004For1*vs.* 3 0.000For 1vs.4 0.326For 2vs. 3 0.753For 2vs. 4 0.196For 3vs. 4
Breast 20	2.245± 0.377	1.57-2.99	
Prostate 20	2.120± 0.409	1.52-2.73	
Lung 20	2.285± 0.484	1.45-3.14	

1.Colorectal 2. Breast 3. Prostate 4. Lung

Both of fibrinolytic proteins are illustrated a significant alterations in their levels as a response to the malignancies alteration effect, so, it is necessary to examine the combined effect of these plasmin – plasminogen system parameters on each other during the malignancies and proliferations process occurrence. In order to verify the changes of the plasmin-plasminogen system variables as well as between system variables themselves, linear regression analysis was applied to study the relationship among these parameters in the malignant and benign tumors study groups. Pearson's correlation was used to analyze the results. The correlations of uPA levels to the activity of , tPA, were not statistically clear when the comparison was carried out in the malignant tumors group, as illustrated in Table (7) . While Positively significant correlations were recorded at the relationship were evaluated for uPA to plasmin activity in malignant tumors group ($r = 0.510$ at $p < 0.001$). Person's coefficient demonstrated insignificant relationship between tPA, uPA, in the malignant tumors group, while Positively significant correlations were recorded at the relationship were evaluated for tPA to plasmin concentration in malignant tumors group ($r = 0.275$ at $p < 0.014$).

Table (7) : The Person Correlation (r) Between Studied Variables in Malignant Tumor Patients Group

Variables		uPA	tPA	Plasmin
uPA	Pearson Correlation (r)	1	0.170	0.510**
	Sig. (2-tailed)		0.133	0.001
tPA	Pearson Correlation(r)	0.170	1	0.275*
	Sig. (2-tailed)	0.133		0.014
Plasmin	Pearson Correlation(r)	0.510**	0.275*	1
	Sig. (2-tailed)	0.001	0.014	

*. Correlation is significant at the 0.05 level (2-tailed).**. Correlation is significant at the 0.01 level (2-tailed).

The plasmin- plasminogen system consists of the uPA, tPA and two specific inhibitors, the plasminogen activator inhibitor 1 (PAI-1) and 2 (PAI-2). The uPA converts the proenzyme plasminogen in the serine protease plasmin, involved in a number of physiopathological processes requiring basement membrane (BM) and/or extracellular matrix (ECM) remodelling [28], including tumor progression and metastasis. Data accumulated over the past years have made increasingly clear that the uPA has a multifunctional task in the neoplastic evolution, affecting tumor angiogenesis, malignant cell proliferation, adhesion and migration, intravasation and growth at the metastatic site [29]. In agreement with their role in cancer progression and metastasis, an increased expression of uPA, tPA and PAI-1 has been documented in several malignant tumors, and a positive correlation between the levels of one or more plasmin- plasminogen system plmembers and a poor prognosis has been frequently reported . This is particularly evident in breast cancer, for which uPA has been demonstrated to be the most potent independent prognostic factor described to date. The involvement of the uPAS in cancer progression identifies its components as suitable targets for anti-cancer therapy [30]. In recent studies applying Several therapeutically approaches aimed at inhibiting the uPA/uPAR functions have been shown to possess anti-tumor effects in xenograft models, including selective inhibitors of uPA activity, antagonist peptides, monoclonal antibodies able to prevent uPA binding to uPAR and gene therapy techniques silencing uPA/uPAR expression. All these strategies, however, although promising, need definitive confirmation in humans as, up to now, only few uPA inhibitors entered clinical trial [31]. The overexpression of uPA has been detected in various malignancies, including breast [32,33] and colon cancers [34]. Some dates have shown that a high level of uPA in tumors is associated with a rapid disease progression and a poor prognosis [35,36].

The correlations of uPA levels to the activity of tPA were not statistically clear when the comparison was carried out in the benign tumors group, as illustrated in Table 8 . While Positively significant correlations were recorded at the relationship were evaluated for uPA to plasmin activity in benign tumors group ($r = 0.296$ at $p < 0.015$). Person's coefficient demonstrated insignificant relationship between tPA, uPA, in the malignant tumors group, while Positively significant correlations were recorded at the relationship were evaluated for tPA to plasmin concentration in malignant tumors group ($r = 0.275$ at $p < 0.014$). Person's coefficient demonstrated insignificant relationship between tPA, uPA, in the benign tumors group.

Table (8) : The Person Correlation (r) Between Studied Variables in Benign Tumor Patients Group

Variables		uPA	tPA	Plasmin
uPA	Pearson Correlation	1	0.002	0.296*
	Sig. (2-tailed)		0.990	0.015
tPA	Pearson Correlation	0.002	1	0.010
	Sig. (2-tailed)	0.990		0.952
Plasmin	Pearson Correlation	-0.296*	0.010	1
	Sig. (2-tailed)	0.015	0.952	

Correlation is significant at the 0.05 level (2-tailed).**. Correlation is significant at the 0.01 level (2-tailed).

During activation of the fibrinolytic system plasminogen is converted to plasmin by tissue plasminogen activator (t-PA) or urokinase-type plasminogen activator (u-PA). t-PA is predominantly released from endothelial cells, u-PA primarily by renal parenchymal cells. The activation of plasminogen is regulated by plasminogen activator inhibitor-1 (PAI-1), plasmin is controlled by alpha 2-plasmin inhibitor. The fibrinolytic system is not only involved in the intravascular dissolution of fibrin (thrombi), it also plays a vital role in normal physiologic reproduction, wound repair, angiogenesis, and tissue remodeling. Fibrinolysis is also a vital component in the pathogenesis of neoplastic disease. It is essential in releasing cells from their primary site of origin, providing nutrition for neoplastic cell growth and promoting cell mobility and motility. In neoplastic cells the degradation of the extracellular matrix proteins is facilitated by reduced expression of u-PA, t-PA, and u-PAR. In many forms of carcinoma increased expression of u-PAR and u-PA in first stage of carcinoma, its associated with significantly shorter survival. Lower expression of u-PA in breast cancer cells, for example, is associated with shorter survival and increased relapse rate. Progressively aggressive neoplastic cells evidence low expression of u-PA and u-PAR activities, variable expression of t-PA, and enhanced PAI-1 and PAI-2 activities. In acute nonlymphocytic leukemias, poor outcome correlates with low t-PA levels. In acute progranulocytic leukemia there is a high incidence. In contrast the neoplastic prostatic tissue expresses high u-PA activity and the more aggressive the cell line, the greater the number of u-PAR and the higher the u-PA activity, as well as In gynecologic malignancies, a greater expression of u-PA in combination with cathepsin D is associated with widespread disease and poor prognosis. High u-PA values were also seen in patients with brain, gastric, and hepatic malignancies. It is evident that the plasminogen-plasmin system is a vital component in the biology of neoplastic disease [37]. Receiver operating characteristic (ROC) curves for the analyzed plasmin-plasminogen system markers. The diagnostic tests (area under the curve, cutoff values, sensitivity, and specificity) of the uPA, tPA, plasmin levels were studied, and their respective receiver operating characteristic (ROC) curves were obtained.

These cutoff points, with the results of the diagnostic tests, are shown in Table (9) and Figure (2).

The uPA had maximum specificity 88.8% and sensitivity 70% , tPA had highest specificity 87.5% but the sensitivity was low 50% .

Table (9) :Diagnostic test of serum tumor markers plasmin- plasminogen system variables				
Specificity	Sensitivity	Cut off value	Area under the curve	Markers
0.7	0.888	210.1693	0.855	uPA
0.5	0.875	2.4402	0.749	tPA
0.5	0.800	2.4962	0.632	Plasmin

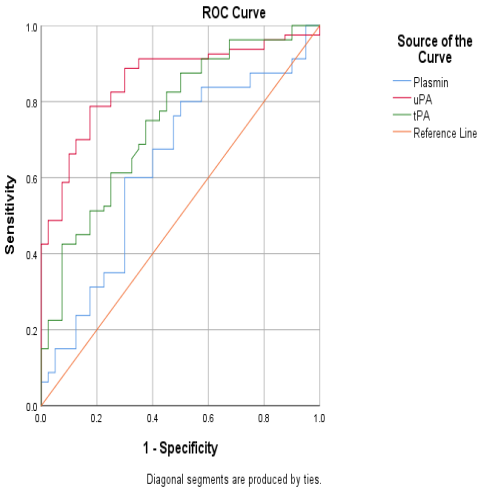

Figure (1) : Reciver Operating Characteristic (ROC) Curve of uPA ,tPA, and Plasmin

The identification of easily determined biochemical molecules for their use as clinical markers of diseases continues to be a topic of great interest in recent researches, especially when cancer is considered. This fact is especially important in the case of many types of tumors where there is a need to have sufficiently reproducible, sensitive, and specific markers that meet clinical expectations. The World Health Organization (WHO), in coordination with the United Nations and the International Labor Organization, defined a biomarker as "any substance, structure, or process that can be measured in the body or its products and influence or predict the incidence of outcome or disease" [38] . At the same time, the NIH defined this term as a "characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention" [39]. To be a predictor of disease, a biomarker must be validated. Validation criteria include intrinsic qualities such as specificity, sensitivity, and knowledge of the confounding and modifying factors. In addition, the characteristics of the sampling and analytical procedures are of relevance when considering the constraints and non-invasiveness of sampling, stability of potential biomarkers, and the simplicity and speed of the analytical method. Present study recorded higher specificity 90% and higher sensitivity 70% for uPA marker, for this may be uPA as candidate tumor marker, when compared uPA levels in four type of malignant tumors found that prostate tumors have low levels (mean ± SD =121.557±26.971) of uPA than other types of malignant tumors, as a result can consider uPA as tumor marker for prostate malignant tumors.

Conclusions

Several conclusions were accomplished from the observed results in the current research, which Plasmin- plasminogen variables concentrations have played an important role in tumors susceptibility and synthesis of tumor cells in both malignant and benign tumors and brostate tumors have been affected by plasmin-plasminogen variables variations other than types of tumors. In addition to that uPA is more candidate tumor markers in patients with malignant tumors.

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