Variation of PAI-1 and oxidant-antioxidant status in Four Human Cancers

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ABSTRACT:

Objective: The present study was disigned to invistigate the effect of plasmin-plasminogen system variable which is plasminogen activator inhibitor-1 (PAI-1) on the occurrence of the development of malignant and benign tumors. In addition to illustrate the oxidative stress effect on the malignant and benign tumors occuring and complication progress. Which involving Glutathione Peroxidase (GPx) and Hydrogen Peroxide H_2O_2. On the other hand, current study amied to nominate the most suitable variant as an effective tumor marker

Design and Methods: The experiments comparing the PAI-1 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 Plasminogen Activation Inhibitor type 1 (PAI-1), Glutathione Peroxidase (GPX) and Hydrogen Peroxide (H_2O_2) was determined by applied ELISA method by certain kit in all study groups individuals.

Results: The result of the statistical analysis in the current study showed a significant decrease ($p < 0.05$) in the level of glutathione 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. Statistically significant high levels of PAI-1 and H_2O_2 in malignant tumor patients compared to benign and healthy controls, but no significant differences between benign and healthy controls. Several conclusions were accomplished from the observed results in the current study, have been included several: firstly to PAI-1variable concentration have played an important role in tumors suscptibility and synthesis of tumor cells in both malignant and benign tumors, secondly tothe oxidative stress has been associated with tumors suscptibility by the evidence of variations levels of oxidative stress biomarkers and could be used as predictive tool for determining the degree of the underlying oxidative stress in malignant and benign tumors, and thirdly to Glutathione are more candidate tumor markers in patients with malignant tumors.

Key Words: PAI-1, GPX, H_2O_2, Human Cancers

INTRODUCTION:

The serine protease inhibitor (SERPIN) family includes PAI-1. It is arguably the most crucial part of the plasminogen-plasmin system in the control of numerous physiological processes and in the etiology of numerous diseases, including cancer. Endothelial cells, liver, adipose tissues, vascular smooth muscle cells, as well as a significant proportion of tumor cells all produce PAI-1. In addition to being a powerful tPA and uPA inhibitor, PAI-1 also directly inhibits plasmin. In the α-granules of platelets, PAI-1 is kept. The majority of PAI-1 in platelets is in a latent state, but when it is released into a thrombus or the ECM, it can have an impact on both fibrinolysis and ECM functions. In healthy persons who are at rest, the plasma level of PAI-1 is around 1 nM, which is two to three times the amount required to block the circulating plasminogen activators [1,2].

Oxidative stress is an important component in linking environmental toxicity to a multi-stage carcinogenic process. In addition, oxidative stress is characterized by the cumulative effect of more than one activity, such as a multistage process (three stages in one cell; onset, rise, and progression), such as the development of cancer. Reactive oxygen species (ROS) are produced in response to endogenous and exogenous stimulation. Reactive oxygen species (ROS) can affect all of these stages of carcinogenesis [3]. For this reason, the term oxidative stress is used to describe an imbalance between cellular levels of oxidants and antioxidants [4]. Cell damage caused by free radicals is thought to play an important role in the progression of the aging process and degenerative diseases associated with ageing (particularly atherosclerosis, cataracts, diabetes, neurodegenerative diseases, immune system disorders, and cancer formation). Oxidative stress has been associated with nearly 50 pathogenetic diseases [5]. Antioxidants are substances that prevent, reduce or delay the oxidation of substances that may be subject to oxidation such as proteins, fats, carbohydrates and DNA in living cells, this is called antioxidant defense. Antioxidants are substances that prevent or delay the damage of free oxygen radicals in their target suits. Antioxidants are classified into two categories, enzymatic and non-enzymatic. The enzymatic antioxidants are superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx), and the non-enzymatic antioxidants are vitamin E, vitamin C, vitamin A, selenium (C), transferrin and lactoferrin. Antioxidants are often intracellular and sometimes extracellular [6]. The present study aims at cheiving a set of tasks that can be summarized as follows: firstly to investigating and evaluating of variable PAI-1 in sera of malignant and benign tumors patients and healthy individuals, secondly to assessment of oxidant-antioxidant status that are presented by evaluating glutathion peroxidase and hydrogene peroxide levels in sera of malignant and benign tumors and healthy individuals, and thirdly to studying the possible relationship between PAI_1 variable, this is on the one hand, and with oxidant-antioxidant on the other side.

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 January to the end of October2022, 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 samples were 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. Plasminogen Activation Inhibitor type 1 (PAI-1), Glutathione Peroxidase (GPX) , and Hydrogen Peroxide (H_2O_2) 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 Plasminogen Activator Inhibitor1 Levels Among The Studied Groups. In the current study, there are significant differences of PAI-1 levels in Malignant tumors Patients compared to benign and healthy controls, same results were observed when comparing malignant with benign and healthy control to gether but threre is no significant differences between bengin and healthy as showen in Table (1)

Table (1) : Comparison of Plasminogen Activator Inhibitor1 Levels Among The Studied Groups

Subjects(n)	PAI-1 Concentration(ng/mL) Mean ± SD	Min-Max PAI-1 Concentration (ng/mL)	p-value
Malignant 80	15.254± 5.334	3.49-23.37	0.040 For1*vs*.2 0.000 For1*vs*.3 0.111 For2*vs*.3
Benign 40	13.117± 5.846	3.77-22.84	
Control 40	11.207± 4.774	3.11-22.36	

1.Malignant 2. Benign 3. Control

There were no significant differences observed when comparing patients with breast malignancies with prostate (p=0.068) and there were no significant differences between prostate patients with lung (p=0.584), but there is a significant difference when comparing patients with breast malignancies with lung and colorectal (p=0.019,p=0.008).There is also a significant difference between patients with malignant tumors of the colorectal with the prostate and lung (p=0.000).

Table (2): Comparison of Plasminogen Activator Inhibitor 1 Levels Among Four Types of Malignant Tumors

Subjects(n)	PAI-1 Conc.(ng /mL) Mean ± SD	Min-Max PAI-1 Conc. (ng/mL)	p-value
Colorectal 20	17.916± 2.950	13.66-23.37	0.008For 1*vs.* 2 0.000For1*vs.* 3 0.000For 1vs.4 0.068For 2vs. 3 0.019For 2vs. 4 0.589For 3vs. 4
Breast 20	14.328± 4.835	3.86-20.32	
Prostate 20	11.903± 4.449	3.49-19.81	
Lung 20	11.193± 4.087	3.49-18.81	

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

Since motile cells focalize uPA on the cell surface through association with uPAR, which is highly expressed on tumor cells, uPA has been considered as the critical trigger for plasmin formation during tumor cell invasion and metastasis. Importantly, by having binding sites other than for uPA, uPAR interacts with vitronectin, integrins, and transmembrane receptors to facilitate intracellular signaling by effector molecules that are involved in cell migration. By binding to vitronectin, PAI-1 prevents the interaction between vitronectin and two cell surface-associated proteins, namely uPAR and avß3 integrin, and as a result represses cell migration on vitronectin in the extracellular matrix (ECM). Apart from directly inhibiting uPA-mediated plasmin formation, PAI-1 also inhibits the activity of the uPA/ uPAR complex by promoting its endocytosis via LRP1, followed by the degradation of uPA and recycling of uPAR. Furthermore, it causes the cell to detach from the ECM. PAI-1 was thus expected to have an anti-tumor Ceffect. Interestingly, ample evidence has been provided for a paradoxical pro-tumorigenic function of PAI-1, being both pro-angiogenic [(7)]and anti-apoptotic [(8)], documented to be dependent on the stage of cancer progression, the cell type, the source (i.e., host or tumor) and on the relative concentration of PAI-1[(9–11)]. By blocking avβ3-mediated endothelial cell migration on vitronectin in the extracellular matrix, PAI-1 was shown to promote angiogenesis by stimulating integrin a5β1-mediated endothelial cell migration toward fibronectin inside tumor tissue [(12)]. Apart from its interaction with vitronectin, PAI-1 can modulate cell migration by binding to surface receptor LRP1 that triggers intracellular signaling. Indeed, uPA and PAI-1 are among the most highly induced proteins in several migratory or invasive tumor cell types. Even though some studies failed to show a correlation between elevated levels of PAI-1 and poor clinical prognosis [(13–15)], PAI-1 has been established as one of the most reliable biomarkers and prognostic markers in many cancer types, including breast [(16–18)], ovarian[(19)], bladder [(20,21)], colon[(22)], renal [(23)]and non-small cell lung cancers[(24)].

The role of oxidative stress (OS) in cancer has been a favored topic for research in the recent years. OS occurs when redox homoeostasis within the cell is altered. This imbalance may be due to either an overproduction of spontaneous reactive oxygen species (ROS) and/or a deficiency in antioxidant systems [(25,26)]. The deficiency of an antioxidant system will induce an accumulation of ROS within the cell. The inhibition of key enzymes involved in the synthesis of glutathione or ROS-scavenging enzymes cause sustained OS [(27)]. Glutathione peroxidase-1 (GPx-1) is an intracellular antioxidant enzyme that enzymatically reduces hydrogen peroxide to water to limit its harmful effects The tripeptide L-γ-glutamyl-cysteinyl-glycine, or glutathione, is present in all mammalian tissues at 1–10 mM concentrations and exists in the thiol-reduced (GSH) and disulfide-oxidized (GSSG) forms. GSH is the predominant form and accounts for >98% of total glutathione [(27)]. In its reduced state, GSH is the most abundant nonprotein sulfhydryl in the cell (ranging from 0.1 to 10 mM) and plays a very important role in maintaining cellular homeostasis and redox balance, representing an essential element of the intracellular defense against ROS. According to ANOVA test, highly significant differences (p= 0.000) were noticed when compared healthy control group with both benign tumors and malignant tumors groups , while; no significant variation was noted

when benign tumors patients group compared with healthy control group (p=0.135), as shown in Table (3)

Table (3): Comparison of Glutathione Peroxidase Levels Among The Studied Groups

Subjects(n)	GPX Concentration(ng/mL) Mean ± SD	Min-Max GPX Concentration (ng/mL)	p-value
Malignant 80	0.971± 0.578	0.12-2.48	0.000 For1*vs.*2 0.000 For1*vs.*3 0.135 For2*vs.*3
Benign 40	1.924± 0.982	0.49-4.53	
Control 40	2.203± 1.062	0.62-5.17	

1.Malignant 2. Benign 3. Control

Statistical non-significant differences (p>0.05) were noticed when the four types of malignant tumors group were compared together using ANOVA test, as shown in Table (4). The present findings have the same opinion with the other study that demonstrated, the type of malignant tumor no effect on serum GPX levels [28].

Table (4) : Comparison of Glutathione Peroxidase Levels Among Four Types of Malignant Tumors

Subjects(n)	GPX Conc.(ng /mL) Mean ± SD	Min-Max GPX Conc. (ng/mL)	p-value
Colorectal 20	1.022 ± 0.654	0.14-2.48	0.578 For 1*vs.* 2 0.357 For1*vs.* 3 0.710 For 1vs.4 0.713 For 2vs. 3 0.354 For 2vs. 4 0.197 For 3vs. 4
Breast 20	0.919 ± 0.380	0.12-1.57	
Prostate 20	0.851 ± 0.462	0.14-1.70	
Lung 20	1.091 ± 0.754	0.14-2.48	

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

Present study recorded decreased in GPx concentration which suggested may be associated with tumor initiation, proliferation, and migration, as a consequence of increased oxidative stress and pro-tumorigenic redox signaling.Low tumor GPx levels can also predict patient outcomes. For example, data from the previous research demonstrate that lowexpression of GPx3 is associated with poor survival in lung adenocarcinoma and low-grade glioma. However, in other tumor tissues GPx3 levels is elevated[29],and high expression is associated with poor patient outcomes in cancers such as stomach and lung squamous cell carcinomas.Another issue to consider is how the extracellular tumor environment contributes to the regulation of GPx3 activity. As such, the availability of GSH may also be an important regulator of GPx3 activity. Interestingly, multidrug resistance proteins, which are often upregulated in cancer, also transport GSH. This suggests that an enhanced efflux of GSH into the extracellular space could contribute to increased GPx3 activity in the tumor microenvironment. As such, one might also speculate that amino acid precursors of GSH synthesis, and compounds such as N-acetylcysteine (NAC) could amplify the activity of GPx3. Given that NAC has been shown to promote metastasis of some tumor types [30,31]. it is of interest to determine if the presence of GPx3 in the tumor microenvironment is necessary for the pro-metastatic actions of NAC.

Accumulating evidence suggests that hydrogen peroxide (H_2O_2) plays an important role in cancer development. Experimental data have shown that cancer cells produce high amounts of

H_2O_2. An increase in the cellular levels of H_2O_2 has been linked to several key alterations in cancer, including DNA alterations, cell proliferation, apoptosis resistance, metastasis, angiogenesis and hypoxia-inducible factor 1 (HIF-1) activation. It has also been observed that the malignant phenotype of cancer cells can be reversed just by decreasing the cellular levels of H_2O_2. On the other hand, there is evidence that H_2O_2 can induce apoptosis in cancer cells selectively and that the activity of several anticancer drugs commonly used in the clinic is mediated, at least in part, by H_2O_2. the high levels of H_2O_2 commonly observed in cancer cells may be essential for cancer development; these high levels, however, seem almost incompatible with cell survival and may make cancer cells more susceptible to H_2O_2-induced cell death than normal cells. An understanding of this dual role of H_2O_2 in cancer might be exploited for the development of cancer chemopreventive and therapeutic strategies [32]. In the current study, there are no significant differences of H_2O_2 levels in Malignant tumors Patients compared to benign while there are significant differences between pateints with malignant tumor and control ,and between benign and control as showen in Table (5).

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

Subjects(n)	H_2O_2 Concentration(ng/mL) Mean ± SD	Min-Max H_2O_2Concentration (ng/mL)	p-value
Malignant 80	1.290± 0.583	0.51-2.59	0.316 For1*vs.*2 0.000 For1*vs.*3 0.008 For2*vs.*3
Benign 40	1.193± 0.485	0.57-2.14	
Control 40	0.894±0.258	0.28-1.37	

1.Malignant 2. Benign 3. Control

There were no significant differences observed when comparing patients with colorectal malignancies with prostate and lung (p=0.459, p=0771, p=0352) and there were no significant differences between breast patients with breast, prostate and lung (p=0.303, p=0.848, p=0.223), but there is a significant difference when comparing patients with prostate malignancies with lung (p=0.223).

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

Subjects(n)	H_2O_2 Conc.(ng /mL) Mean ± SD	Min-Max H_2O_2 Conc. (ng/mL)	p-value
Colorectal 20	1.354± 0.736	0.51-2.59	0.459For 1*vs.* 2 0.771For1*vs.* 3 0.352For 1vs.4 0.303For 2vs. 3 0.848For 2vs. 4 0.223For 3vs. 4
Breast 20	1.216± 0.384	0.71-2.16	
Prostate 20	1.408± 0.728	0.51-2.59	
Lung 20	1.180± 0.395	0.71-2.16	

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

Hydrogen peroxide can be generated by the 2-electron reduction of oxygen by various oxidoreductases, including xanthine oxidase, which, findings suggest, predominately produces hydrogen peroxide [33,34]. previous studies also suggest that NADPH oxidase subtypes NOX4 may preferentially produce hydrogen peroxide rather than superoxide anion, which is the major ROS produced by other NOX isoforms [35] . Hydrogen peroxide has a longer half-life than superoxide, and unlike superoxide, hydrogen peroxide can transfer across lipid membranes by either diffusion or transport through channels, such as aquaporins [36]. Regulation and removal of hydrogen peroxide prevents the formation of the highly reactive

and damaging hydroxyl radical, which can be formed by reaction of hydrogen peroxide with Fe^{2+} (Fenton reaction). Intracellularly, the Fenton reaction is limited, in part, by the lack of free transition metals in cells, but may play a role in oxidative damage after ischemia-reperfusion or under other oxidative stress conditions that involve accumulation of high levels of intracellular hydrogen peroxide and liberation of Fe^{2+} from intracellular storage sites [37,38]. Excess hydrogen peroxide can also lead to oxidation of susceptible cellular protein thiols to sulfenic (SOH) or sulfinic (SO_2H) acid and irreversible oxidation to sulfonic (SO_3H) acid [39]. Low levels of hydrogen peroxide, however, maintain essential modifications of protein thiols including the formation of intra- and intermolecular disulfides (including mixed disulfides with low molecular weight thiols like reduced glutathione [GSH]) [40,41]. Also, at low levels, hydrogen peroxide plays a role as a second messenger in signal transduction by modulating the oxidation state of redox-sensitive cysteines (Cys) to promote kinase function [40,41]. H_2O_2 acts as extracellular and intracellular signaling molecule that mediates multiple effects in biological systems, including recruitment of immune cells to damaged areas and cell migration. H_2O_2 is obtained from mitochondria generated superoxide ions in a process that is catalyzed by the overexpressed superoxide dismutase (SOD). Compared with normal cells, cancer cells show increased generation rate of H_2O_2 (up to 0.5 nmol/10^4 cells/h) [42] , resulting in a higher level of H_2O_2 in the tumor than normal tissues. In addition to its essential role in cellular signaling, overproduced H_2O_2 has been exploited as a major precursor for highly active ROS, such as hydroxyl radical, peroxynitrite, and hydrochlorides. Importantly, researchers have found that H_2O_2 accumulated in tumors can be decomposed to generate O_2 under certain conditions, which is used to supplement the O_2 . Since the accumulation of H_2O_2 can increase the oxidative stress and reflect the development of many diseases, taking H_2O_2 as a cancer diagnostic marker as well as a therapeutic target presents tremendous theranostic potential.

Both of fibrinolytic proteins and oxidative stress parameters are illustrated a significant alterations in their levels as a response to the malignancies alteration effect, In order to verify the changes of the glutathione and hydrogen peroxide with the plasminogen activation inhibitor type 1 (PAI_1) variable. 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. Positively significant correlations were recorded at the relationship were evaluated for PAI-1 to hydrogen peroxide concentration in malignant tumors group ($r = 0.288$ at $p < 0.035$). No such results were noted when the serum PAI-1 concentration of the (malignant tumors group was correlated to their corresponding GPX level, as shown in Table(7) . Negatively significant correlations were recorded at the relationship were evaluated for to H_2O_2 concentration with glutathione activity in the malignant tumors groups ($r = -0.486$ at $p < 0.003$) as shown in Table (7).

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

Variables		PAI-1	H_2O_2	Glutathione
PAI-1	Pearson Correlation (r)	1	0.288*	-0.106-
	Sig. (2-tailed)		0.035	0.351
H_2O_2	Pearson Correlation(r)	0.288*	1	-0.486**
	Sig. (2-tailed)	0.035		0.003
Glutathione	Pearson Correlation(r)	-0.106-	-0.486**	1
	Sig. (2-tailed)	0.351	0.003	

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

In the present work, PAI_1 marker level in benign tumors patients were correlated to the levels of oxidative stress biomarkers in the purpose of monitoring the probable cellular changes concurrent with the rise in the susceptibility and development of benign tumors . No such results were noted when the serum PAI-1 concentration of the (benign tumors group) was correlated to their corresponding GPX level, as shown in Table (8). Negatively significant correlations were recorded at the relationship were evaluated for to H_2O_2 concentration with glutathione activity in the malignant tumors groups ($r = -0.486$ at $p < 0.003$) as shown in Table (8). Negatively insignificant correlations were recorded at the relationship were evaluated for H_2O_2 concentration with GPX activity in the benign tumors groups as shown in Table (8)

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

Variables		PAI	H_2O_2	Glutathione
PAI	Pearson Correlation	1	0.093	-0.078
	Sig. (2-tailed)		0.056	0.630
H_2O_2	Pearson Correlation	0.093	1	-0.102
	Sig. (2-tailed)	0.568		0.530
Glutathione	Pearson Correlation	-0.078	-0.102	1
	Sig. (2-tailed)	0.630	0.530	

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

the plasminogen activator inhibitor 1 (PAI-1) is a physiologic inhibitor of plasminogen activators. PAI-1 expression is increased in the fibrotic diseases and in experimental fibrosis models. The deletion of the PAI-1 gene reduces, whereas the overexpression of PAI-1 enhances, the susceptibility of animals to fibrosis induced by different stimuli, indicating an important role of the plasminogen activator/plasmin system development of many types of fibrosis. Many growth factors, including transforming growth factor beta (TGF-β) and tumor necrosis factor alpha (TNF-α), as well as other chemicals/agents, induce plasminogen activator/plasmin system expressions in cultured cells and in vivo. Reactive oxygen and nitrogen species (ROS/RNS) have been shown to mediate the induction plasminogen activator/plasmin system of by many of these stimuli . Table 3-22 summarizes the relationship of plasminogen activator/plasmin system with ROS/RNS and oxidative stress status in the development of many type of fibrosis in the regulation of plasminogen activator/plasmin system expressions during fibrogenesis [43].

present study showed similar results with the above studies. Endogenous H_2O_2 increased PAI-1 production . The results of the conducted present study revealed that oxidative stress in cancerous cells causes a significant increase in PAI-1 and hydrogen peroxide at all of the investigated cancer types [44].Present study attempted to elucidate the molecular signaling mechanisms by which glutathione and H2O2 regulate expression of the respective fibrinolytic factors. Therefore, current thesis tested the protein level of PAI-1 in cancerous cells subjected to oxidative stress. It is found strong correlation between PAI-1, H_2O_2, in the contribution of regulation of fibrinolytic factors . In addition, another study found that the inhibition of PAI-1 synthesis by PAI-1 inhibitor results in markedly increased u-PAR expression levels are involved in this regulation [45]. Reactive oxygen species (ROS) are closely associated with the intracellular signal cascade, thus strongly implicating involvement in tumor progression. However, the mechanism by which ROS are generated and how ROS target downstream molecules to trigger tumor metastasis is unclear.

Receiver operating characteristic (ROC) curves for the analyzed PAI-1 marker and oxidative stress markers. The diagnostic tests (area under the curve, cutoff values, sensitivity, and specificity) of the PAI-1,glutathione and hydrogen peroxide 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 1 , 2 , 3 .

The glutathione peroxidase had specificity 88.8% and sensitivity 60% . The hydrogen peroxide H_2O_2 presented minimum specificity 70%, with a sensitivity of 52.5%.

Table (9) :Diagnostic test of serum tumor PAI 1 variable and oxidative stress markers

Markers	Area under the curve	Cut off value	Sensitivity	Specificity
PAI-1	0.70	13.6116	0.713	0.625
Glutathione	0.791	1.7311	0.888	0.600
H_2O_2	0.856	0.9548	0.700	0.525

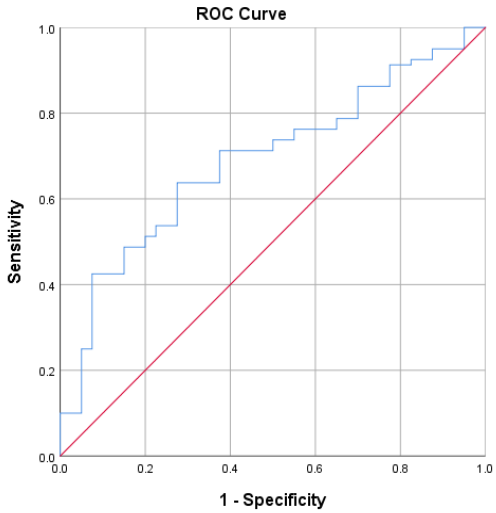

Figure (1) : Reciver Operating Characteristic (ROC) Curve of PAI-1

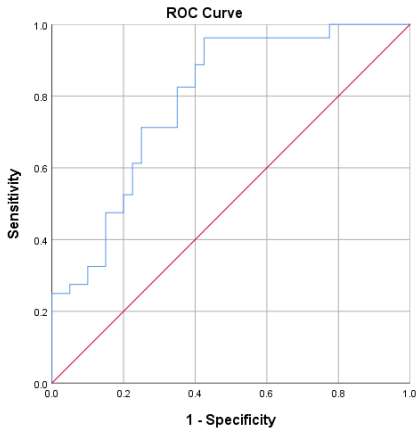

Figure (2): Reciver Operating Characteristic (ROC) Curve of GPX

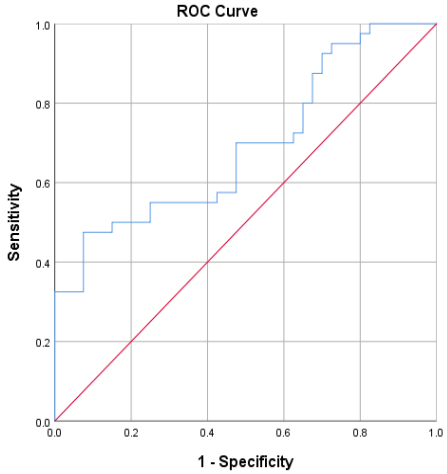

Figure (3): Reciver Operating Characteristic (ROC) Curve of H_2O_2

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" [(46)] . 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" [(47)]. 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.

The second variables which was recorded higher specificity and sensitivity, it was glutathione, which was more reduced in malignant prostate tumors (0.851± 0.462) than other malignant tumors, so can consider glutathione as tumor marker for prostate cancer, because it have higher specificity and sensitivity 88.8% and 60% respectively.

Conclusions

Several conclusions were accomplished from the observed results in the current research, which PAI_1 variable concentration have played an important role in tumors suscptibility and synthesis of tumor cells in both malignant and benign tumors. Breast tumors have been affected by PAI_1 varible variation other than types of tumors .The oxidative stress has been associated with tumors suscptibility by the evidence of variations levels of oxidative stress biomarkers and could be used as predictive tool for determining the degree of the underlying oxidative stress in malignant and benign tumors . PAI-1 is more candidate tumor markers in patients with malignant tumors. The variation in PAI_1 variable are related with variations in oxidant-antioxidant system in malignant tumor patients.

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