Glutathione -S-transferase, Reduce Glutathione and Xanthine Oxidase Activities in Patient with Prostate Cancer

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ABSTRACT:

In the present study, the estimation of free radicals scavenger enzyme (antioxidant enzymes) such as glutathione-S-transferase (GST) and the reduce glutathione (GSH) activities, in sera with prostate cancer patients (n=25)and healthy control(n=23), were found a highly significant decreased in GSH ($p < 0.005$) and significant decreased GST ($p<0.05$).Also we determined the activity of xanthine oxidase (XO), and were found elevation and highly significant at($p<0.00١$) in xanthine oxidase . The correlation between GSH and GST also determined between control and prostate cancer patients, the result show weak positive correlation in comparison with control. Conventional-PAGE profile of crude serum control and prostate cancer for protein show no different between them.

الخلاصة:

تضمنت الدراسة قياس فعالية إنزيم إزالة الجذور الحرة(الأنزيمات المضادة للأكسدة) مثل Reduce Glutathione] [(GSH),glutathione-S-transferase(GST)في مصول مرضى سرطان البروستات (N=25) وأشخاص أصحاء(N=23) وقد تبين من الدراسة إن الإصابة بسرطان البروستات يؤدي إلى استنفاذ الكلوتاثايون من خلال الانخفاض العالي المعنوية في مستوى فعالية[(GSH) و(GST) ($p < 0.005$) و ($p < 0.05$) على التوالي] .كذالك نحن قدرنا فعالية إنزيم الزانثين اوكسيديز(XO) واظهرت النتائج ارتفاع عالي المعنوية في سرطان البروستات($p < 0.001$) . العلاقة بين GSH و GST تم تحديدها للأشخاص الأصحاء ومرضى سرطان البروستات وتبين وجود علاقة موجبة ضعيفة عند المقارنة مع الأصحاء. وبين نموذج الهجرة الكهربائية عدم وجود فروق في حزم البروتينات بين أمصال المرضى والأصحاء.

INTRODUCTION:

Prostate cancer (adenocarcinoma) is now the most common cancer in men [l].The prostate gland is a walnut size stricture which is located around the urethra at the base of the bladder [2]. This gland in the male reproductive system that helps produce semen, the thick fluid that carries sperm cells [3].Beginning about the age of 30 years, the prostate gland undergoes a gradual change where the inner part of the gland begins to enlarge at a very slow rate. Men are generally unaware at this change unless the growing gland presses on the bladder or urethra which may cause a change in urination. This normal enlargement is known as benign prostatic hyperplasia [2].In spite of a significant refinement in early detection of prostate cancer, approximately 10-20 % of patient present with metastasis lesions at the time of diagnosis [4, 5, 6]. In addition, locally advanced cancer is fount in nearly one-half of surgically treated patient with clinically organ-confined tumors [4, 7].

Best known and well characterized markers for prostate cancer diagnosis are the proteins prostate specific antigen [PSA] prostatic acid phosphates [PAP] prostate specific membrane kallikrein 2 (hk2) [8, 9, and 10]. PSA is the most widespread serum marker for prostate cancer but it can not adequately destiny wish between prostate cancer and benign prostatic hyperplasia (BPH), in the detection rang of (4-10) ng/ml since its concentration in serum is elevated in patient with either of these diseases [11]. Therefore, novel tumor associated antigens as additional diagnostic markers and sensitive diagnostic method are required [8].

Prostate cancer is a major public health problem in the developed world. It is the most common human cancer and the second leading cause of cancer death. There is evidence that oxidative damage may play a role in prostate cancer [12,13].

Antioxidants are the body's premier resource for protection against the diverse free radical and other oxidative stressors to which it invariably becomes exposed [14]. The antioxidant defense system is sophisticated and adaptive, and glutathione GSH is a central constituent of this system [15].Glutathione is the major antioxidant produced by the cell, protecting it from free radicals, if left unchecked, will damage or destroy key cell components (e.g. membranes , DNA) [16]. In addition, GSH is a very important detoxifying agent, inability the body to get rid of undesirable toxins pollutant [17,18]. GSH plays a crucial role in maintaining a normal balance between oxidation and antioxidation [19]. So, the GSH concentration in the blood may serve as a useful indicator of disease risk in humans.Glutathione-S-transferases (GST):are ubiquitous multifunction enzymes [20].

GST is thought to play a physiological role in initiating the detoxification of potential alkylating agents, pharmacologically active compounds. These enzymes catalyze the reaction of such compounds with the -SH group of glutathione, thereby neutralizing their electrophilic sites and rendering the products more water-soluble [21].

Xanthine oxidase (XO) is the final enzyme in the degradation of purines. It converts hypoxanthine to xanthine and subsequently to uric acid. Unlike other oxidases. jlmmunohistochemical location of XO has been reported its presence in various tissues from various animal species [22] J Other researcher demonstrated the ubiquitous localization of this cytosolic enzyme in human tissues, such as tongue, esophagus, trachea, sweat glands, mammary glands, small and large intestine, renal tubules skeletal muscle, lung, spleen and liver. It was found that human blood or serum contains XO activity, since high concentration of XO antibodies are present in human sera [23].

XO may play a beneficial role by producing superoxide radicals [24]. Previous studies suggested a role of XO as an antimicrobial agent [25]. Moreover, it was reported that uric acid, in its normal range, serve as an antioxidant. Therefore, XO serves as a defense mechanism by generating uric acid as well [24].

PATEINTS & METHODS:

A-Samples:

Collection the samples: A total of (25) patient with mean age (63) were included in this study, and the entire patients were undergoing treatment in the Merjan hospital, Babylon Governorate. The reference group consisted of (23) healthy person.

B-Estimation of Reduce Glutathione(GSH) Activity :

Serum reduced glutathione (GSH) was measured by reaction with DTNB (Ellmans reagent) [26].

C- Estimation of Glutathione-S-transferase (GST) Activity :

Serum glutathione-S-transferase (GST) activity was estimated according to the method of Habig *et.al.* 1 974 [27] .

D- Estimation of Xanthine Oxidase (XO) Activity :

Serum xanthine oxidase enzyme XO activity was estimated according to the method of Bruder *et.al,* 1989[23].

E-Conventional –Polyacrylamide Gel Electrophoresis(PAGE):

PAGE technique was used to analysis of crude samples of control and prostate cancer patients.

RESULTS & DISCUSSION:

In this study the control variants were determination sera of apparently healthy individuals ,compare with variants in patient group. Then, the determination of different variants was done in sera of patients with prostate cancer. GSH, a ubiquitous cellular antioxidant, play a central role in defense against a variety of disease and both exogenous and endogenous. It function include the detoxification of xenobiotics, carcinogens, free radicals and peroxides; regulation of immune function, and maintenance of protein structure, function, and turnover [28] .

GSH concentration were found to significantly decrease in sera of patient with prostate cancer in comparison to the GSH level of serum in control group ($p < 0.005$),table 1. So the depletion of GSH levels in this study, when compared with healthy control support the hypothesis those conceders GSH a protective factor against the development.

On the other hand the Glutathione -S-transferase (GST) level in the serum of patients with prostate cancer had shown significant decreases in comparison to the enzyme level of serum in control group ($p < 0.05$) , table 2.

The significant decreases in GST level due to the effect of testosterone on GST , because testosterone increase oxidative stress so consumption of more GST [29].

The Xanthine Oxidate (XO) level in the serum of our patient with prostate cancer had show significantly higher in comparison to the XO level of serum in control group ($p < 0.00١$), table 3.

Xanthine oxidase, the last enzyme in the degradation of purine derivative from nucleic acid.It can react with various classes of substrates, but its main role is to oxidize hypoxanthine and xanthine to uric acid .It also oxidizes aldehydes, and purines.XO enzyme is involved in the pathogenesis of reperfusion tissue injury by the production of super oxide radical, and H_2O_2 or both while, the other [30, 31] attributed the increase in the level of XO in the patient with prostate cancer is due to the dehydrogenase from of xanthine oxidase converted to the oxidized from during oxidative stress.

The correlation between GSH and GST in control and patients with prostate cancer show weak positive correlation ($R^2=0.0322$,$R^2=0.0138$ respectively ,figure 1,A,B).That mean appear the oxidative stress in patients with prostate cancer .The observation of conventional -PAGE of total serum samples (control and prostate cancer)showed no different in bands comparing to the control groups (figure 2)

Table 1:Reduced Glutathione(GSH)Activity in Patients with Prostate Cancer in Comparison with the Control.

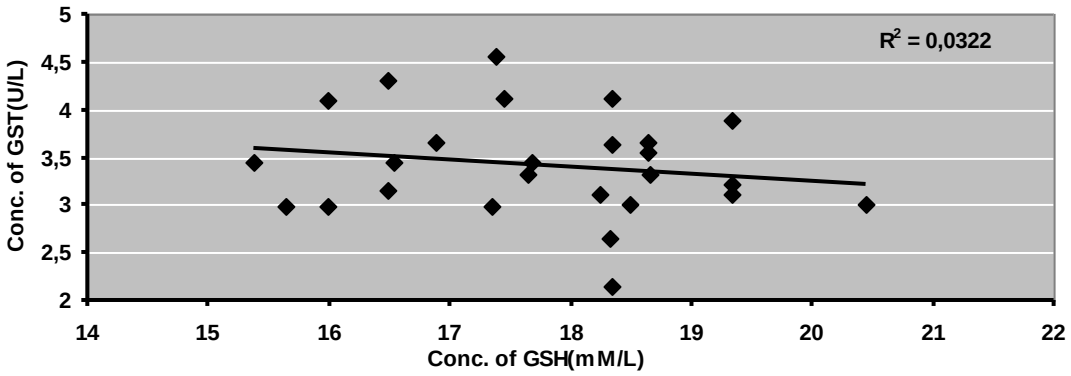
Groups	*No.*	*Mean*	*±SD*	*P Value*	*Statistical*
					Control
GSH	23	18.12	6.61	-	-
					Prostate Cancer
GSH	25	4.21	2.66	0.002	p <0.005

Table 2:Glutathione-S-transferase (GST) Activity in Patients with Prostate Cancer in comparison with the Control.

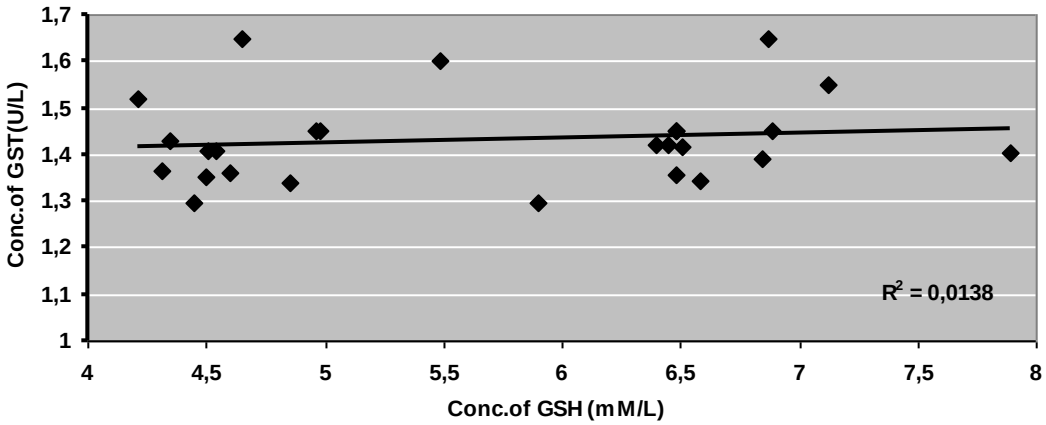
Groups	*No.*	*Mean*	*±SD*	*P Value*	*Statistical*
					Control
GST	23	3.51	1.49	-	-
					Prostate Cancer
GST	25	1.28	0.36	0.03	p <0.05

Table 3:Xanthine Oxidase (OX) Activity in Patients with Prostate Cancer in Comparison with the Control.

Groups	*No.*	*Mean*	*±SD*	*P Value*	*Statistical*
					Control
OX	11	0.001	0.0005	-	-
					Prostate Cancer
OX	13	0.002	0.0009	0.0004	١p <0.00

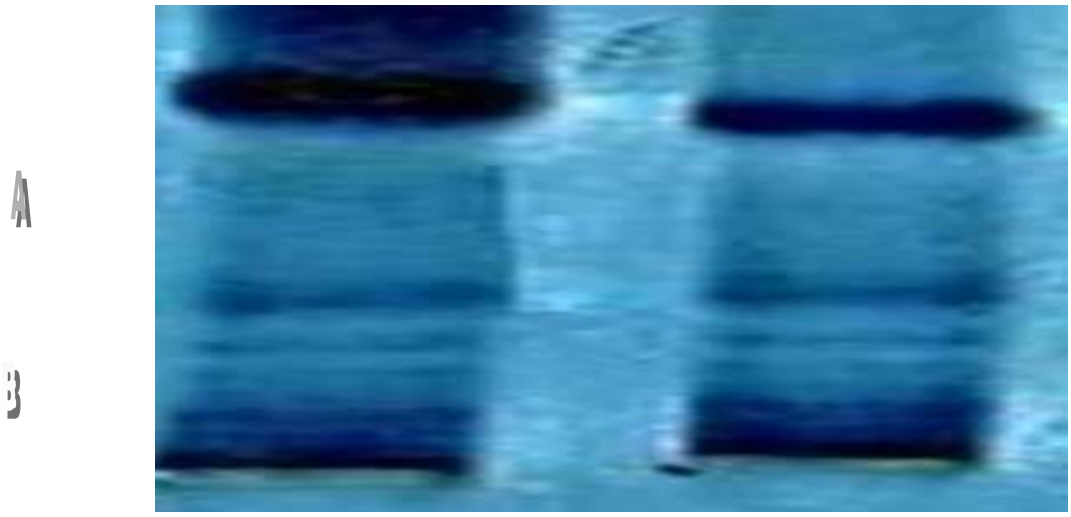


A



B

Figure 1:The Correlation Between the Level of GSH and GST Activity [Control(A) and Prostate Cancer(B)].



Control Prostate cancer

Figure 2:Conventional -PAGE ,Profile of Crude Serum Control(A), Prostate Cancer. The Gel was Stained for Protein with CBB R-250.

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