

Study of some Antioxidants in plasma of patients with bladder cancer

Dr.Israa G. Zainal* , Dr.Mhammed AL-Dori**
& Abdul-Nasser M. Abdul a**

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Abstract

The objective of this study was to determine the catalase activity (CAT), ceruloplasmin activity (CP), glutathione level (GSH) in the plasma of (44) normal donars (22 male, 22 female,) and (66) patients with bladder cancer (22 smoker male, 22 non smoker male, 22 female). Data analysis showed a significant decrease in the mean values of CAT (89.577 ± 17.140 of a smoker male, 92.102 ± 18.898 of non-smoker male, 90.225 ± 12.997 of a female) as compared to normal individuals (155.541 U/mg Hb of a male, 150.797 U/mg Hb of a female) respectively, and GSH (0.109 ± 0.056 of a smoker male, 0.110 ± 0.051 of non-smoker male, 0.084 ± 0.028 of a female) as compared to normal individuals ($0.838 \mu\text{mol/L}$ of a male, $0.809 \mu\text{mol/L}$ of a female) respectively. A significant increase in the mean value of CP in plasma of patients with bladder cancer (56.477 ± 2.671 of a smoker male, 55.602 ± 3.042 of non-smoker, 52.101 ± 3.212 of a female) was recorded as compared to normal individuals (43.908 IU of a male, 41.110 IU of a female) respectively, There is also a negative relationship between CAT & Cp and GSH & Cp levels ($r = - 0.089$, and $r = - 0.104$) respectively, while there is a positive relationship between CAT and GSH ($r = 0.081$) in-patients with bladder cancer.

Key words: catalase , ceruloplasmin , glutathione , bladder cancer.

دراسة بعض مضادات الاكسدة في بلازما مرضى سرطان المثانة

الخلاصة

تهدف هذه الدراسة إلى تحديد فعالية الكاتاليز (CAT) وفعالية السيرولوبلازمين (CP) ومستوى الكلوتاثيون (GSH) في بلازما (44) من عينات السيطرة، (22 ذكور و 22 إناث) و (66) مرضى بسرطان المثانة (22 ذكور مدخنين و 22 ذكور غير مدخنين و 22 إناث). أظهرت النتائج بأن هناك نقص واضح في معدل قيم (CAT) (89.577 ± 17.140) للذكور المدخنين و 92.102 ± 18.898 للذكور غير المدخنين و 90.225 ± 12.997 للإناث) بالمقارنة مع القيم الطبيعية (155.541 U/mg Hb للذكور و 150.797 U/mg Hb للإناث) على التوالي، و GSH (0.109 ± 0.056 للذكور المدخنين، 0.110 ± 0.051 للذكور غير المدخنين، 0.084 ± 0.028 للإناث) بالمقارنة مع القيم الطبيعية ($0.838 \mu\text{mol/L}$ للذكور، $0.809 \mu\text{mol/L}$ للإناث) على التوالي. هناك زيادة واضحة في معدل قيمة السيرولوبلازمين (CP) في بلازما المرضى المصابين بسرطان المثانة (56.477 ± 2.671 للذكور المدخنين، 55.602 ± 3.042 للذكور غير المدخنين، 52.101 ± 3.212 للإناث) مقارنة بالقيم الطبيعية (43.908 IU للذكور، 41.110 IU للإناث) على التوالي، تبين أيضا إن هنالك علاقة سالبة بين CAT و GSH وكذلك بين GSH & Cp ($r = - 0.089$ and $r = - 0.104$) على التوالي، بينما هنالك علاقة موجبة بين CAT و GSH ($r = 0.081$) في مرضى سرطان المثانة.

Introduction:

An antioxidant is a substance, when it presents at low concentrations compared to those of an oxidizable

substrate, significantly delays or inhibits oxidation of that substrate [1]. Antioxidant protects the body from

Chemistry Department, College of Science, Al-Mustansiriya University/Baghdad

** School of Applied Sciences , University of Technology/ Baghdad

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2412-0758/University of Technology-Iraq, Baghdad, Iraq

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the effect of oxidation. During the normal process of converting nutrients to energy (called metabolism), the body creates free radicals; they also enter the body through toxins in food [2]. Antioxidant system in the body includes small molecules such as (1):(vitamins A,E&C),GSH,Uric acid & Flavinoids. (2):enzymes such as ceruloplasmin^[3,6], Super Oxide Dismutase(SOD),Catalase,Peroxidase& Glutathione reductase (3):Trace elements such as Se,Cu,Zn&Mg Antioxidant can act at different levels in an oxidative sequence this can be illustrated by considering one of the many mechanisms by which oxidant stress can cause damage stimulating the free radical chain reaction of lipid peroxidation [7]. Catalase is a metalloprotein present in all oxygen

metabolizing cells .It was first isolated and obtained in crystalline form from liver [8] and later from blood and there sources [9] . Ceruloplasmin is officially known as ferroxidase or iron (II) oxygen oxidoreductase .It is an enzyme (EC:1.16.3.1),belong to the first group of classified enzymes as oxidoreductase [10-12].Ceruloplasmin is a member of the multicopper Oxidase family that couple four one – electron oxidation of a substrate with the four – electron reduction of molecular oxygen to water [13-15]. Glutathione is the important of nonprotein cellular thiol compound, occurs in many different cells of humans. Glutathione is a tripeptide consisting of three amino acid (glycine, cystein, glutamic acid) [16], figure (1).

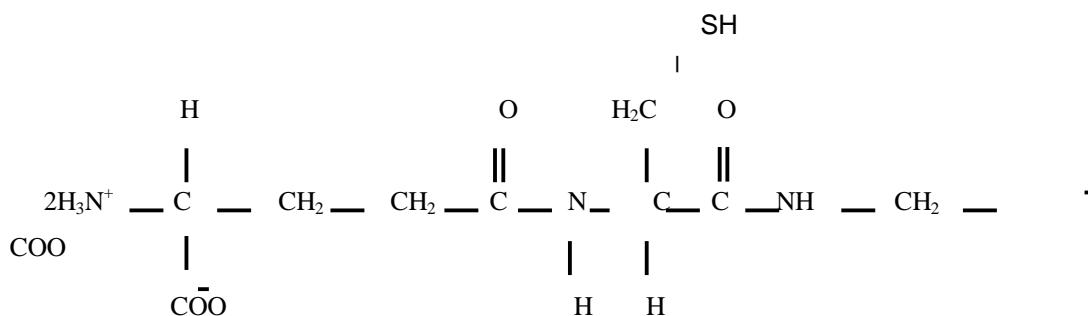


Figure (1): Glutathione (GSH) molecule [16].

The aim of the work includes determining some enzymetic antioxidants like catalase and ceruloplasmin and non-enzymetic antioxidants like glutathione levels in

Materials and Methodes:

All common laboratory chemicals and reagents were of analar grade. Forty-four samples of blood (22) male and (22) females were taken from physically normal volunteers used as controls, age between (35-65) years. Sixty-six samples of blood were taken from

plasma of patients with bladder cancer and normal individuals & Studying the relationship between these parameters .

patients with bladder cancer age (35-75) years after being classified by a specific surgery hospital. In this study these (66) samples were divided into three groups as shown below:

1- Twenty-two patients are males (smokers), age (35-75) years.

2- Twenty-two patients are males (nonsmokers), age (38-70) years.

3- Twenty-two patients are females (nonsmokers), age (36-60) years.

This study was conducted in a specific surgery hospital and Al-karama hospital in Baghdad. Patients suffering from any diseases that may interfere with our study were excluded. 5ml of venous blood samples were collected from these patients in lithium heparin test tubes, the plasma was separated by centrifugation at 1500 x g. for 10 minutes at room temperature, stored

Statistical Analysis:

The results for (CAT), (CP), and (GSH) were analyzed statistically. Values were expressed as a mean+SD. The level of significance was determined by

Results

The catalase activity was estimated in erythrocyte of patients with bladder cancer and normal individuals as clear in figure (1). Results presented in figure (1) revealed that the mean values of catalase activity in erythrocyte of patients with bladder cancer were significantly decreased as compared to normal individuals ($p < 0.01$), figure (1) also shown a decrease in catalase activity of non-smoker patients as compared to normal individuals, and a higher decrease in catalase activity of smoker patients as compared to normal individuals.

Results analysis shows that the catalase activity of female with bladder cancer are less than of a male.

Figure (2) shown that the ceruloplasmin activity increase in patients groups as compared to normal individuals. Figure (3) illustrates the

in an ice bath and used in the same day for enzymatic activity determination. The remainder of each sample was stored frozen until used to estimate other parameters.

The method of Aebi was used to determine the erythrocyte catalase activity^[17]. The activity of ceruloplasmin was measured according to the modified method of Rice using P-phenylene diamine – 2HCl (PPD-2HCl) as a substrate^[18]. Glutathione level was determined according to the Ellman's procedure^[19].

student's t-test- when the P value was equal to or less than 0.05 the difference between the two groups was considered statistically significant^[20].

negative relationship between ceruloplasmin activity and catalase activity in-patients groups this means that the ceruloplasmin activity is oppositely proportional to the catalase activity. The levels of glutathione in the plasma of patients with bladder cancer and normal individuals have been determined as clear in figure (4).

Figure (4) shown that the levels of glutathione decreased in patient groups as compared to normal individuals.

Figure (5) shown the negative relationship between glutathione level with ceruloplasmin activity in patients groups Figure (6) shown the positive relationship between catalase activity with glutathione concentration in patient groups

Discussion

The main finding of the present study is that a significant decrease in the main values of (CAT & GSH) and a significant increase in CP activity in plasma of patients with bladder cancer as compared to normal individuals .

The lower of catalase activity in erythrocyte of patients with bladder cancer may be attributed to :

1-The formation of free radicals and increase of super oxidizing ^[21].

2-The decrease of catalase activity as a result of the tissue damage because of

the inhibiting protection function of (Cat, GSH and SOD) and the loss of

enzymes from cells.

3-Catalase is one of the antioxidant defense that remove free radicals and it

removes the effect of toxic H_2O_2 in the cell ^[22] .

The results in this work for catalase activity are in agreement with Dhalla ^[23] , Dusinovic ^[24]

Increased in ceruloplasmin activity could be attributed to the following reasons:

- Cp is an acute phase reactant that increases in circulation in chronic disease including tumors ^[25] .

conclusions

It is clear from this study that determining the levels of CAT , CP & GSH in patients with bladder cancer may be a good indicator to evaluate this disease.

This study showed that there was a negative relationship between (glutathione level with ceruloplasmin

- Cp is considered as a storage marker of copper in circulation and over 95% of copper is bound to Cp^[26].

The results in this work for CP activity are in agreement with Christine et. al. ^[27] , Barber et al ^[28] , Karaca et al ^[29] they reported a significant increase in plasma of CP activity in ovarian , pancreatic & gastrointestinal tract cancer patients respectively Georgieva et al ^[30] and Yilmaz et al ^[31] found an increase in Cp activity in haematological diseases.

The glutathione cycle operates between GSSG and GSH in the erythrocytes. The Coenzyme, NADPH itself is obtained from the pentose phosphate pathway of glucose metabolism operating in the erythrocytes. However, in bladder cancer, the efficiency of this pathway is lowered with a consequent decrease in the availability of NADPH ^[31]. The serum level of glutathione was approximated 6.4% to that of normal individual. The results in this work show that glutathione level is severely decreased in patients with bladder cancer, and the relationship of glutathione levels with the radicals or related substance may be attributed to the following fact above, and it is an evidence that they are oppositely proportional.

activity) & (ceruloplasmin activity with catalase activity)in patients groups and its an evidence that they are oppositely proportional & a positive relationship between catalase activity with glutathione concentration in patient groups then its an evidence that they are properly proportional.

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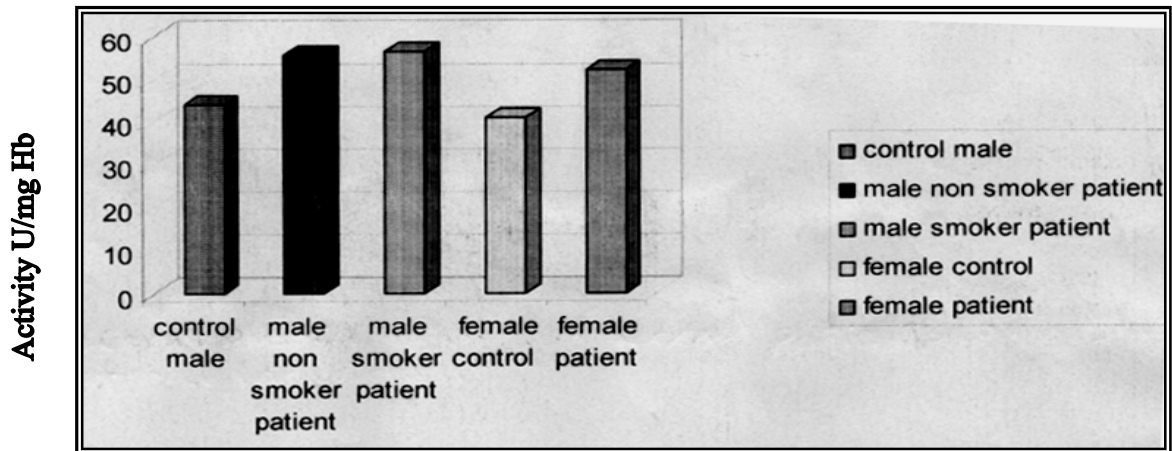


Figure (1) Distribution of the individual and catalase activity in erythrocyte of patients with bladder cancer and normal individuals.

Activity IU

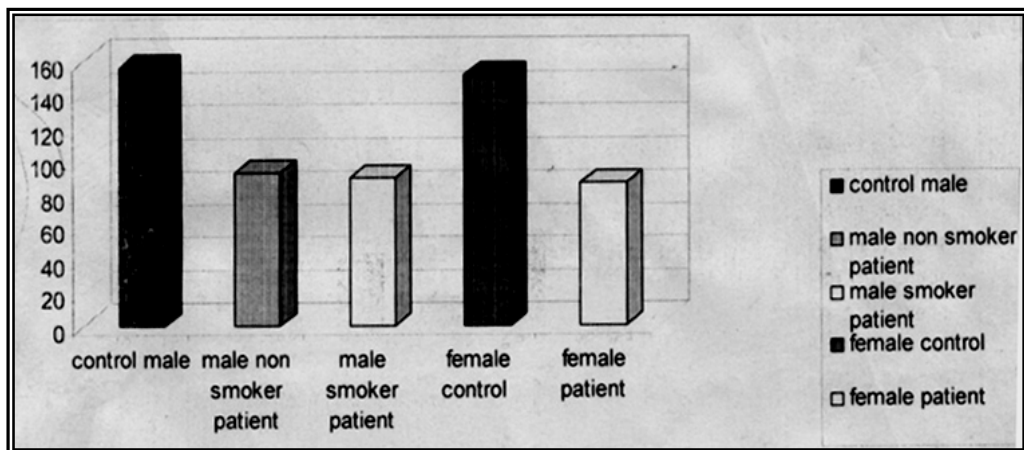


Figure (2): Distribution of the individual and coruloplasmin activity in plasma of bladder cancer patients and normal individuals.

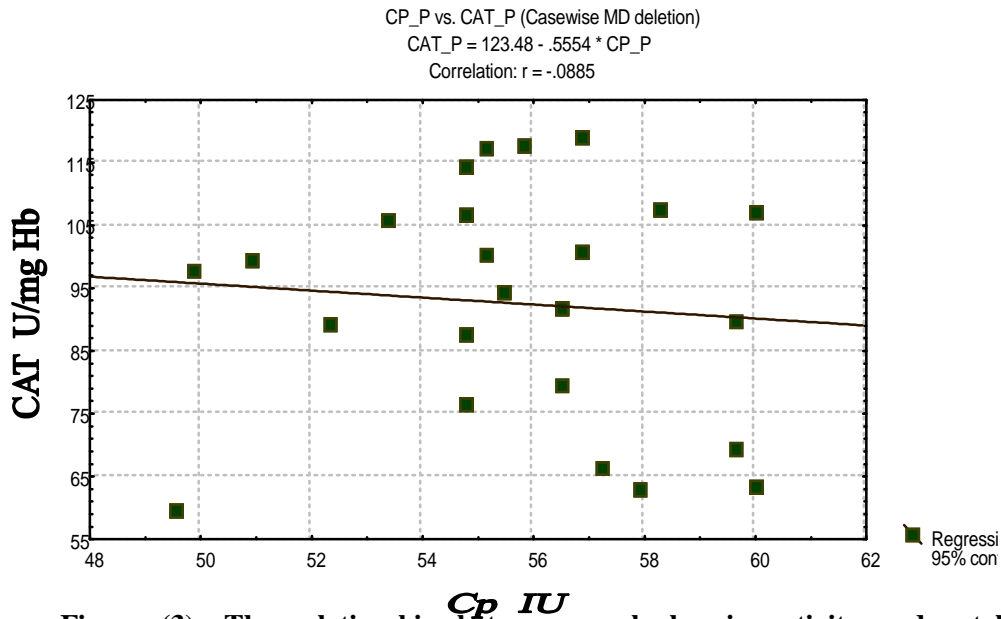


Figure (3): The relationship between ceruloplasmin activity and catalase activity in patient groups.

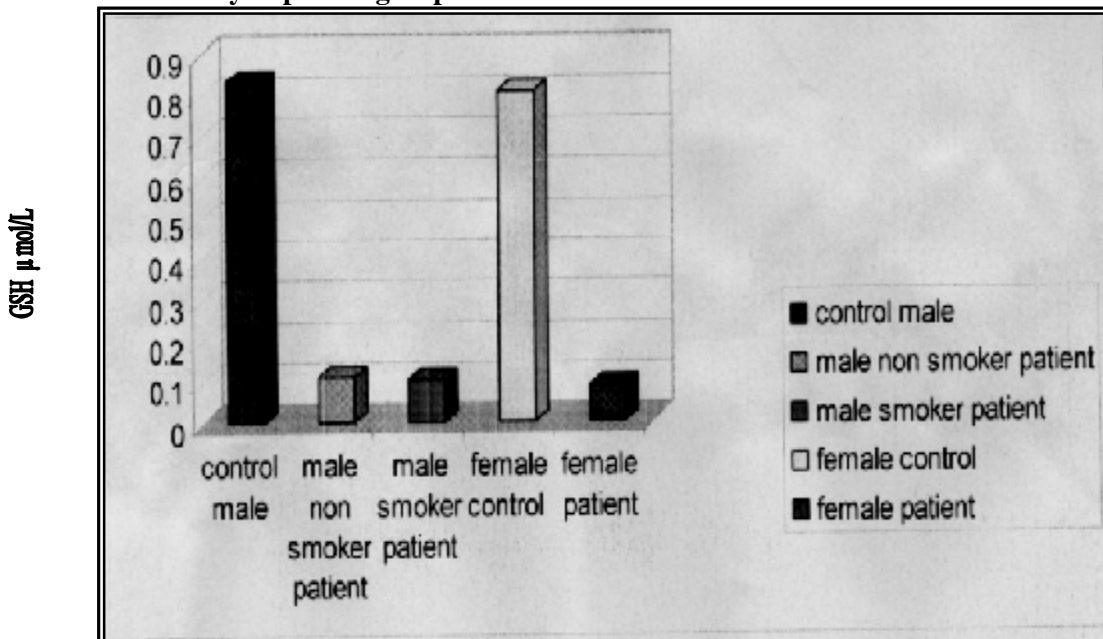


Figure (4) : Distribution of the individuals and glutathione level In plasma of patients with bladder cancer and normal Individuals.

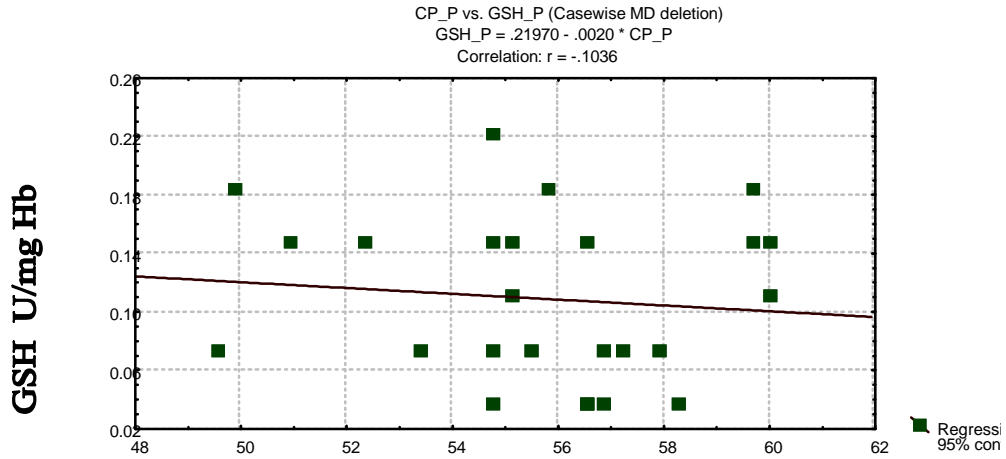


Figure (5): Relationship between glutathione level and ceruloplasmin activity in patient groups.

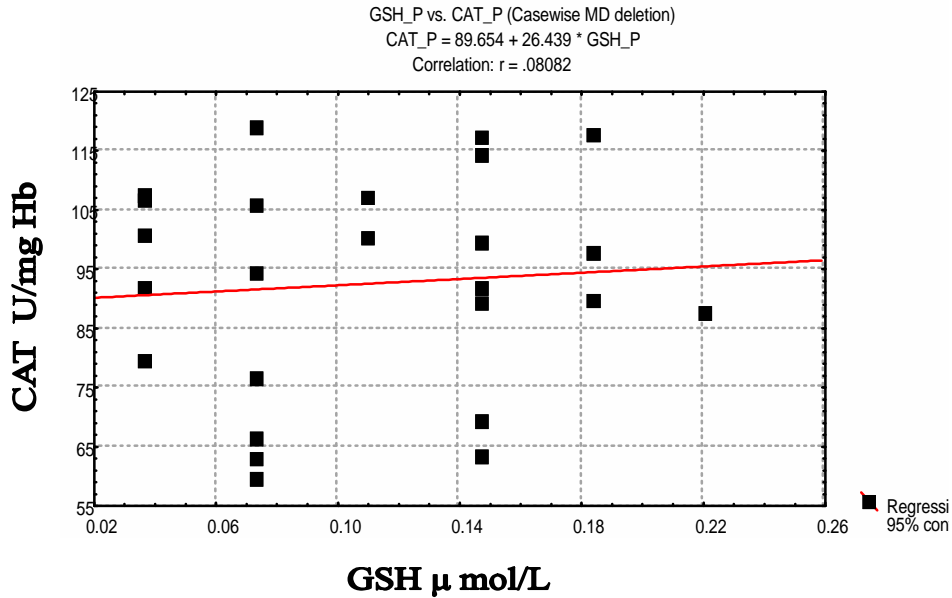


Figure (6): Relationship between catalase activity and glutathione levels in patient groups.