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Biochemical Study to Evaluate the Role of Heavy Metals and Oxidative Stress in the Incidence of Kidney Disease in Iraqi Patients

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Abstract

The study was conducted on kidney patients (60 male and female, and healthy subjects control n=60, Serum was used to assay antioxidant enzymes activities and to measure MDA to prove such lipid peroxidation occurring as MDA considered an indirect measurement for lipid peroxidation, GSH in control group (11.65±0.01) while the increase in patient male and woman (18.55±0.01, 30±0.01), which MDA in control group (0.227±0. 1) that increase in patient male and woman (0.384±0.02, 0.138±0.01) and GST in control group (3.3±0.01) while the increase in both groups (3.8±0.01, 3.6±0.01). The control group's TAC (4.00±0.32), were calculated. Additionally, the TAC values for renal patients were (2.38±0.55), while in women (2.16±0.34). The mean TAC in patients is significantly decreasing from the control group (P<0.001). The control group's serum chromium (Cr) concentrations were (0.94±0.11). Additionally, the serum chromium (1.08±0.14) levels of the children with kidney disease, then in women with (1.5±0.13). Compared to the control group in the current study, the mean blood Chromium level in patients with Kidney is considerably greater (P<value 0.001). The serum Lead (1.62±0.22) of readings for the control group. Additionally, the blood Lead (Pb) levels (0.94±0.13) of readings for the kidney patients'.

Keywords: Kidney, Oxidative Stress, Cr, Pb, Hg, MDA, Glutathione Peroxidase

1. Introduction

In particular, the kidneys play a critical role in maintaining the body's internal mineral and water balance (sulphate, magnesium, phosphorus, calcium, chloride, potassium, sodium). Producing erythropoietin and other hormones, the kidneys are a component of the endocrine system. 1, 25-dihydroxycholecalciferol is a kind of vitamin D. (calcitriol). Erythropoietin is a protein that aids in the formation of red blood cells. The development of bones is aided by red blood cells and calcitriol. Aside from hypertension, there are a number of other conditions that might cause renal function might deteriorate as a result of medical disorders that damage the kidneys. Intensely or chronically for a long period of time in such circumstances, it's critical to figure out what the problem. Renal failure is caused by a variety of factors (Arman and Aggarwal 1993) [1]. As a result, the primary emphasis of inquiry should be to rule out post-renal causes. Anomalies of the renal pelvis, ureters, bladder, and urethra are among them. Urologists generally deal with disturbances in these locations. Severe septic shock, hemorrhage, significant surgery, including aortic aneurysm, and occlusion of renal arteries are all prerenal causes of kidney injury. Collaboration with experts from a variety of fields is crucial in this case. Primary renal illness, such as glomerulonephritis or interstitial nephritis caused by local side effects of pharmacological medications or toxins, is a cause of kidney injury. If such a cause is suspected, it is critical to make contact with a nephrologist as soon as possible to avoid delaying treatment options. Acute renal failure is classified as a) prerenal: caused by hypoperfusion, b) renal: caused by infections, tumors, primary and secondary nephritis, acute tubular necrosis, cardiovascular illness, drug/intoxications, tubular blockage, and c) postrenal: caused by various types of congestion. Haemoglobin levels are generally normal in acute renal failure, and the kidneys may be normal, big, or enlarged. Urinary output can be lowered to oliguria (500 milliliters per day) or anuria (100 milliliters per day). Urine output may be higher than normal despite significant kidney impairment (i.e., non-oliguric acute renal failure). A measurement or estimate of the glomerular filtration rate and the extent of urine output are used to stage acute kidney damage.

As acute kidney injury proceeds, numerous uremic metabolites and water are retained. Furthermore, medications that are ordinarily eliminated by the kidneys will build up in the body and cause more or less serious adverse effects. Potassium is an important metabolite to consider since it can cause abrupt cardiac arrhythmia. Another issue is fluid retention, which can lead

to acute pulmonary oedema. These patients should be sent to hospitals as soon as possible by their general practitioner, where they can be treated by multidisciplinary teams. The focus of this thesis will be solely on chronic kidney damage and related processes.

1.1 Aim of This Study

1. Studying the relationship of oxidative stress associated with kideny patients
2. Evaluation the antioxidant enzymes (TAC,GSH-PX), and studying the relationship between antioxidant with Cr, Pb, Hg levels in sera of control and kideny patients.
3. Studying the the relationship between antioxidant with MDA levels in sera of control and kideny patients.

2. Materıals and Methods

2.1 Subjects

A. Group 1: 60 apparently healthy subjects were chosen as healthy group, don't have any history of kideny diseases.
B. Group 2: 30 male kideny patients were included in this study.
C. Group 3: 30 female kideny patients were included in this study. The study was carried out from the first of January 2022 to march 2022. The Kidney Center in Heat City provided the patient samples. The research was carried out in the College of Medicine, University of AL-Biochemistry Ramadia's Department lab.

A questionnaire was submitted to the patients and controls as explained in Table 1.

Table 1: Questionnaire

Number:		
Name:		
Sex:	Male	Female
Age:		
Drug used:		
Dose:		
Duration of treatment:		
Duration of disease:		
Other disease:		
Other drug used:		
Weight:		
Height:		
Family history:		
Smoking:		
Passive smoking:		
Residency:		
Recent hospitalization:		

2.2 Collection of Blood Samples

Venous After a time of fasting, blood samples were taken using disposable syringes while the subjects were seated from asthmatic patients and healthy controls. Without applying a tourniquet, five milliliters of blood were gently drawn from each patient and placed into non-anticoagulant, plain disposable tubes. The serum was obtained by centrifuging the blood at 1500 g for 10 to 15 minutes after the blood had been allowed to clot. Serum samples were then put into fresh, disposable, simple tubes.

2.3 Methods

2.3.1 Determination of plasma total antioxidantcapacity

Principle: The TAC Assay Kit was created by Bio Vision and can assess proteins alone in the presence of our unique Protein Mask or proteins in conjunction with both small molecules antioxidants. Both tiny molecules and proteins can change a Cu^{2+} ion into Cu^{2+}. By preventing protein from reducing Cu^{2+}, the Protein Mask allows for the study of just small-molecule antioxidants. A colorimetric probe is used to chelate the reduced Cu2+ ion, and the result is a wide absorbance peak that is proportional to the overall antioxidant capacity at roughly 570 nm.

A. Determine sample antioxidant trolox equivalent concentrations:

Sample antioxidant capacity = [(sample absorbance – blank absorbance) x (µl of the sample)] / slope of the standard curve, or Sa / Sv = nmol/µl or mMTrolox equivalent

2.3.2 Determination of malondialdehyde

The following approach was built on the spectrophotometric analysis of the color produced by the reaction of thiobarbituric acid (TBA) and MDA, (Figure 3.2) (Lunec 1990) [3].

2.3.3 Determination of Cr

The samples were digested by adding (4 ml) of (1:1) [con. HNO3 and con. HClO4] to (0.5 ml) of serum in a spyrex test tube, and then immersing the test tube in an oil bath at 160° C for an hour. then the tubes were taken out of the bath and allowed to cool at room temperature. 0.5 M HCl was then added to bring the volume up to 10 ml (Spycher *et al.* 2010) [7].

2.3.4 Determination of Pb

Transferring (0.5 ml) of serum into a spyrex test tube, adding (4 ml) of (1:1), and putting in an oil bath at 130 °C for an hour were the steps taken to digest the samples. In order to dilute the tubes to a final volume of 10 ml with 0.5 M HCl, the tubes were removed from the bath and allowed to cool at room temperature.

2.4 Flame Atomic Absorption Spectrophotometric Analysis

Khalid *et al.* described a procedure for determining lead, chromium, and cadmium. Samples from the same brands will be carefully mixed before analysis in order to provide homogenous and representative samples. The digested samples underwent Pb, Cr, and Cd analysis by AAS. Each element's concentration was calculated by contrasting the measured absorbance with the appropriate standard (calibration curve). The median concentration was used to contrast with the tentative weekly intake for food additives that the Food and Agriculture Organization and the World Health Organization had established (JECFA).

2.5 Statistical Analysis
All findings were provided as the mean standard deviation value following data analysis using the statistical application SPSS 18.0. Data put into a database were examined using SPSS. The differences between the groups that were studied were compared using the t test, and a value of P<0.05 was deemed statistically significant.

3. Results and Dıscussıon
3.1 Level of Related Oxidative: Antioxidant Parameters
It was advised to measure MDA to demonstrate that this lipid peroxidation was happening because MDA is regarded as an indirect marker for lipid peroxidation, as stated in Table 2. The amount of MDA increased to a point where it was producing as much peroxynitrate as was expected. This finding suggests that the increase in antioxidant enzyme activities coincided with a decline in free radical levels, and that the opposite is also true. However, additionally, it implies that crucial balances between the production of oxygen free radicals and antioxidant defense enzymes throughout development have already taken place (Kohen and Nyska 2002) [4]. In order to deactivate free radicals and defend themselves against their toxicity, organisms employ this crucial balance between their antioxidant defense systems and those that are created (Sies 1991) [8].

Table 2: Levels of MDA in patient and control group

Parameters	Group (1) Control	Group (2) Pateint (mean)	Group (3) Pateint (woman)
GSH(µM)	11.65 ±0.01	18.55±0.01*	30±0.01*
MDA(M)	0.227±0. 1	0.384±0.02*	0.138±0.01*
GST(µU/L)	3.3±0.01	3.8±0.01*	3.6±0.01*

* Significant difference (P < 0.05).

This defense mechanism could be brought on by an increase in oxidative stress since there is increased endogenous production of free radicals. This study proposes that the production of antioxidant enzymes throughout development is related to the change in free radical levels because an increase in oxidative stress exhibits a substantial relationship with immune system activation. The gene expression of key antioxidant enzymes is thought to directly quench reactive oxygen species, mediating the antioxidant effects **(**Grundmann *et al.* 2010) [12]. In contrast, it has been suggested to look at the levels of two significant components, GST and GSH, as antioxidant factors opposing the impact of the MDA (oxidative stress marker), in order to confirm the effects of the oxidative-antioxidant axis. The results show that the control group differs from the others in a number of ways, one of which is that reduced glutathione levels seem to be higher in the patient (men) in conjunction with an increase in Glutathione-S-transferase activity to about 3.3 µU/L, which reveals information about the body's defense against free radicals. Malondialdehyde (MDA), a sign of lipid peroxidation, was determined to be 0.138 M, but was reduced to 50 pM by peroxynitrate. It has been demonstrated that this new biological balancing role exists.
As compared to their level in control, the results show an increase in pathogenic species for all three parameters. The activity's essential radicals are reduced by GSH. However, GST accelerated the reaction between GSH and either hydrophilic or electrophilic molecules, which uses more GSH (Van-Konynenburg 2004) [15].

In order to keep oxidation and anti-oxidation in a healthy equilibrium, GSH is crucial. In order to control important functions including the production and repair of DNA, the synthesis of proteins, and the activation and regulation of enzymes, the cell uses GSH, an internal antioxidant.
According to the Fig 1, both axis, the antioxidant and oxidative stress have aerobically role in the cell, thus a suggestion have been made by the author that as the cell try to protect itself. It was obviously shown that an increasing in the three parameters were occurred, on opposite the free radicals liberation can resulting tissue damage.

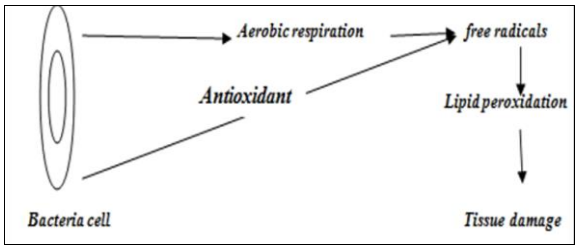

Fig 1: Antioxidant defense systems and free radical generation must live peacefully

The production of free radicals and their elimination by antioxidants make up the oxidative state **(**Christman *et al.* 1985) [2].
Unpaired electrons in the other orbital of a molecule or molecular fragment are known as free radicals. During metabolism, a number of potent oxidants are formed, including superoxide (O2), hydrogen peroxide (H2O2), peroxy radicals (ROO-), peroxynitrate, and hydrogen radicals (OH-), and others (ONOO-). Antioxidant defense mechanisms of both the enzymatic and non-enzymatic types are used to combat free radicals. In cellular detoxification, GST are essential **(**Baynes and Dominiczak 2004). Additionally, in bacteria, GST participates in a range of different processes, including the biotransformation of hazardous substances, the generation of tolerance to various stresses, and the development of antibacterial drug resistance (Oakley 2011) [14].

3.2 Age and Sex Distribution
Age and sex distribution is elucidated in Table 3.

Table 3: Age and sex distribution

Age (yrs)	Sex		Total	Percentage
	Female	Male		
20–29	15	30	50	50.22
30–39	28	30	50	52.99
Total	58	60	100	100.00

3.3 Levels of Total Antioxidant Capacity in the Patient and Control Groups
The serum of the three groups had their total antioxidant capacity (TAC) tested; the findings are shown in Table 4.

Table 4: Mean of the study groups' average total antioxidant capacity

Groups	Number	TAC Mean ±SD nmol	P-value
G1	60	4.00 ± 0.32	
G2	30	2.38 ± 0.55	P<0.001
G3	30	2.16±0.34	P<0.001

The control group's TAC values, with a mean and standard deviation of (4.00±0.32), were calculated. Also, the values of TAC of the kideny patients with the mean ± SD (2.38 ± 0.55) in mean while in woman (2.16±0.34).The mean TAC in patients is significantly decrease from the control group (P<0.001). Also, the mean TAC in patients is significantly decrease from the control group (P<0.001), According to the report (Shikotra *et al*. 2012) [6].

3.4 Levels of Serum Chromium Concentrations

Chromium (Cr) was measured in the sera of the three groups; the results are given in Table 5.

Table 5: Mean levels of serum chromium in the studied groups

Groups	Number	Chromium (Cr) Mean ±SD (mg/dL)	P-value
G1	60	0.94±0.11	
G2	30	1.08 ±0.14	P<0.001
G3	30	1.5±0.13	P<0.001

The control group's serum chromium (Cr) concentrations were mean standard deviation (0.94±0.11). Additionally, the mean±SD values for serum chromium (Cr) in pediatric patients were 1.08±0.14 mg/dl and 1.53±0.13 in female patients. When compared to the control group in the current study, the mean blood Chromium (Cr) level in patients with Kideny is considerably greater (P<value 0.001). It is possible that some of the side effects of long term therapy may, in part, be due to the increse of Chromium (Cr) stores in the body (Sigurs *et al*. 2000) [9].

In this study, patients' mean blood chromium (Cr) levels are considerably higher than those of the control group, a minor but not statistically significant rise that may be attributed to therapy. The decrease in serum Chromium (Cr) concentration may be an important risk factor in oxidant release and the development of DNA damage and cancer, Chromium (Cr) is co-factor in proteins involved in antioxidant defense system, electron transport, DNA repair and protein expression. The metal-regulatory transcription factor (MTF)-1's Chromium (Cr) finger structures may bind to increased quantities of free Chromium (Cr) ions, which causes thionein to express itself. Additionally, the production of the oxidized protein thionin (Tox) and the simultaneous release of chromium occur when (ROS) or nitrogen species (RNS) oxidize thiols (Cr) (Stern *et al.* 2007).

3.5 Levels of Serum Lead (Pb) Concentrations

Lead (Pb) was measured in the sera of the three groups; the results are given in Table 6.

Table 6: Mean levels of serum lead (Pb) in the studied groups

Number	Groups	Lead (Pb) Mean±SD (mg/L)	P-value
60	G1	1.62 ±0.22	
30	G2	0.94 ± 0.13	P<0.001
30	G3	0.82±0.12	P<0.001

The mean±SD of the serum Lead of (Pb) (1.62±0.22 mg/dl) readings for the control group. Additionally, the mean SD (0.94±0.13) mg/dl readings for the kideny patients' blood Lead (Pb) levels. The mean Lead (Pb), in patients is significantly different from the control group. Also, the mean Lead (Pb), in the patients is significantly different from the control group. Besides, the mean Lead (Pb),level is significantly different between control group and pateint group. Our findings confirmed previous research by demonstrating that the BA group's lead (Pb) content is much lower than that of the healthy group (Salam *et al.* 2005) [11] A reduction in lead (Pb) levels may result in the removal of free radicals from the body. The need for Lead (Pb) for various iron transport and utilization-related enzymes is the basis for the process through which lead (Pb) deficiency causes anemia (Stein *et al.*1997) [10].

3.6 Levels of Mercury (Hg) in the Patient and Control Groups

Mercury (Hg), was measured in the sera of the three groups; the results are elucidated in Table 7.

Table 7: Mean mercury (Hg), in the studied groups

Number	Groups	Mercury (Hg) Mean±SD	P-value
60	G1	1.74 ± 0.32	
30	G2	0.87±0.14	P<0.001
30	G3	0.78±0.12	P<0.001

The Mercury (Hg), (1.74 ± 0.32) for the group controls with the mean ± SD. Also, the Mercury (Hg), of the kideny patients with the mean ± SD (0.87±0.14). Mercury (Hg) levels were much lower in G2, G3 than they were in G1, which may be related to a drop in Cr levels. Despite the fact that certain research have linked increased levels of serum Mercury (Hg) with increased childhood illnesses including failing (Roger and Clive 2003) [5]. Our findings are in line with recent research that demonstrated that individuals with chronic kidney disease had increased serum Mercury (Hg) levels (Thorstensen 2014) [13]. As a result, there are conflicting findings about the blood levels of Cr and Mercury (Hg) in individuals with childhood disorders. As a result, it is probable that changes in Mercury (Hg) or Cr levels are less significant than changes in the ratio of Cr to Hg.

4. Conclusions

The organisms exploit this important equilibrium between the production of free radicals and the antioxidant defense system to deactivate and defend themselves against the toxicity of free radicals., Free radical levels are falling, and this is accompanied by rising antioxidant enzyme activity. The opposite is also true. It is found from this study that the oxido index for such biological samples is very good indication for detection severity of free radicals and the ability of antioxidant to overcome such effects. According to the study's hypothesis, changes in the levels of free radicals, which exhibit a significant association with immune system activation owing to the elevated levels of antioxidant markers, are connected to the synthesis of antioxidant enzymes throughout development. According to the study, the level of Cr is much lower in the KD group than in the healthy group. This suggests that a decline in enzyme activity, which is what removes free radicals from the respiratory system, may be linked to the low level of Cr. Decrease in level in Cr, Pb, Hg compare with control group in kideny disorder.

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