Article

Determination of Lead in Serum of Urolithiasis Patients in Basra Governorate

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Abstract

Kidney stones are a significant public health issue globally, linked to high environmental levels of metallic elements, including lead, which contaminates both natural and industrial environments. The primary aim of this research was to highlight the biochemical relationship between blood levels of Pb and the incidence of urolithiasis. The present study compared 44 healthy individuals from Iraq's Basra Governorate with 44 kidney stone patients. We use (ICP-MS Agilent 7500/USA) the Agilent Inductively Coupled Plasma Mass Spectrometry was used to measure lead amounts in participants' blood. The urolithiasis individuals exhibited a highly significant variation in their lead levels. The lead level in the healthy group were 49.38 ppb, whereas in the kidney stones group it was 64 ppb. There are notable variations between the healthy and patient groups regarding the antioxidant enzymes (catalase, glutathione peroxidase, malondialdehyde, xanthine oxidase, and superoxidase dismutase), and the elevated lead levels may impact these enzymes. Antioxidant enzymes are tested using the enzyme-linked immune sorbent assay method. The study also evaluates kidney and liver function using markers such as ALP, GOT, GPT, urea, uric acid, serum creatinine, total serum bilirubin, direct and indirect bilirubin. The biochemical parameters related to liver and kidney functions substantial differences between the patient and healthy group. Lead was one of the contributing causes for the development of renal calculi production in the study. Urolithiasis may be prone to high serum lead levels, and also there is evidence that reporting increasing exposure to lead in the blood is linked to a higher likelihood of urolithiasis in adults.

Keywords: **antioxidant enzymes, Basra, Heavy metals, Kidney stones, lead.**

Introduction

Lead is found in nature; however, it has been shown that human activity is the primary risk factor of the elevated lead level in the environment [1]. Lead is released into the atmosphere through the mining of lead, the use of lead compounds and alloys in manufacturing, vehicle emissions, and the combustion of fossil fuels [2]. Although lead is deposited in teeth and bones and is transmitted to the skeleton, organs, and tissues, it is not an alien substance to the human body; in adults, lead levels are only 10 μg/dL, and in children, they are only 1.4 μg/dL [3,4]. Lead has a World Health Organization guideline threshold of 0.01 mg/L [5]. With about thirty days is the half-life in the bloodstream, lead spreads to several systems, such as the renal. Eventually, it accumulates as lead phosphate in the bones, teeth, and hair [6]. Lead poisoning produces reactive oxygen species (ROS), and the human body experiences oxidative stress when the system is out of balance [2]. Lead also inhibits the function of catalase and superoxide dismutase, which are key antioxidant enzyme [2]. Lead inactivates glutathione by binding to its sulfhydryl group, increasing oxidative stress and rendering GSH replenishment inefficient [8]. The kidney's function in eliminating toxic and metabolic wastes through the urine may be connected to its status as a major location for lead buildup [9]. Remarkably, it has been observed that there are several consequences associated with chronic and low levels of environmental lead exposure, including kidney damage, cancer, malfunction, and neurotoxicity. Additionally, the function of the lead-binding protein in the process of renal carcinoma or how lead harms the kidney is worth considering [10]. It has been indicated that the buildup of lead in the cells lining the proximal renal tubules leads to malfunction in these tubules. This accumulation also resulted in the formation of inclusion bodies, specifically lead-protein complexes, responsible for causing lead nephrotoxicity [11]. Lead poisoning in mitochondria results in an ATP shortage, which lowers salt reabsorption, a crucial kidney function, and increases sodium excretion in the kidneys [2]. Moreover, renal tubular dysfunction due to lead and oxalate results in a calcium imbalance or denudation of epithelial cells, which may promote the pathological conditions that trigger the formation of crystal nuclei. According to epidemiological research, subjects who have blood lead levels (BLL) ranging from 0.48 to 3.85μM have a greater prevalence of chronic kidney disease, possibly related to lead-induced nephrotoxicity at lower concentrations [7,13]. Remarkably, it has been reported that the rising in lead level content with an average of twice was linked to a 35% higher chance of kidney stone production and that elevated blood lead level (BLL) among humans has been demonstrated to be an important trigger for the development of nephrolithiasis. The creation of CaOx crystals and epithelial nucleation would be significantly aided by lead-induced calcium release, offering a mechanistic explanation for the association of lead exposure with the development of CaOx renal stones [14]. Exposure to lead has been demonstrated to result in interstitial edema, the disintegration of cell nuclei, and the detachment of kidney tubular epithelial

cells [15]. Furthermore, mice exposed to lead acetate showed signs of vascular congestion, perivascular fibrosis, tubular degeneration, glomerular atrophy, and congestion. [16]

Materials and Methods

Participants' criteria

Forty-four volunteers, aged between 23 and 75 years, were diagnosed with kidney stone(s) by a specialist urologist and participated in this research. The samples were collected from Al-Mwanei Hospital after the diagnosis of patients as kidney stones. The social history of each subject was taken according to a questionnaire (age, gender, weight, height, protein food, UTI recurrence, renal stones recurrence, smoking, chronic disease, and chronic drug intake).

Control group

Forty-four individuals, aged between 24 and 69, were selected as the control group from the general community. These healthy individuals were chosen from both hospital attendees and patient attendants.

Ethical committee approval

The study protocol was reviewed and approved by the University of Basra. All clinical trials were conducted according to the dependent ethical principles regarding the permission from the patients to participate in the current study and collect blood samples for research purposes.

Blood samples collection and biochemical determination

Both the patients and healthy control subjects had venous blood collection by vein puncture with 5mL sterile syringes, with 5 milliliters of blood were obtained from each individual participated in the present study. To get the serum, a blood sample was centrifuged at 2450xg for 10 minutes after being left to clot on the bench for 20 minutes. Subsequently, it was divided into three Eppendorf tubes. The initial Eppendorf tube contains serum which was utilized to examine the biochemical parameters: uric acid, urea, and creatinine, liver enzymes, and function including alanine aminotransferase, aspartate aminotransferase, total serum bilirubin, direct bilirubin, and alkaline phosphatase. Those parameters were analyzed using the laboratory chemical monitor Architect Chemistry System (Abbott, USA). The following Eppendorf tube was kept at -20°C until they were used for metal assay (using Agilent Inductively Coupled Plasma Mass Spectrometry (ICP-MS Agilent 7500/USA). The final Eppendorf tubes group was kept at a temperature of -20°C until the ELISA technique was used for assays of antioxidant enzymes.

Body mass index

BMI is now utilized to define adult anthropometric height/weight characteristics. It was computed using the square-meter formula BMI=Weight (kg)/Height.

Statistical Analysis

In this work, the results of all experiments were offered as Mean ± S.D For statistical analysis, one-way ANOVA was used, followed by Dunnett's t-test. When probability (P) value ≤ 0.05 the difference is known as significant, when p value ≤ 0.001, the difference is known as highly significant, when p value > 0.05 the difference is known as non- significant.

Results and Discussion

Fundamental characteristics of study participants: This study included 88 participants who completed health checks, tested different biochemical markers and filled out questionnaires. Among them, 61 (69.31%) were men and 27 (30.68%) were women. Patients with renal stones varied in age (23 – 75 years old, mean is 42.6 years). For healthy control individuals aged 24 to 69 years, the mean is 40.7 years. Serum creatinine (CR), blood urea and uric acid (UA), serum lead (Pb), total serum bilirubin, direct and indirect bilirubin, liver enzymes (GOT, GPT, ALP), and oxidative enzymes (catalase, glutathione peroxidase, malondialdehyde, xanthine oxidase, and superoxidase dismutase) were all statistically associated between the kidney stone patients with healthy. The observed variances showed statistical importance (P<0.05). No statistically significant difference was reported for age or BMI, table 1, figure 1.

Table 1: BMI, age, weight, and lead (Pb in ppb) for patients and control groups.

Parameter	Patients	Healthy	*P* value
BMI (Kg/m^2)	28.9±4.6	27.1±6.1	0.22
Age (year)	42.6±12.6	40.7±10.7	0.074
Pb (ppb)	64±14.3	49.38±17.4	0.0014

Table 1 indicates the level of lead as a heavy metal in sera of patients and control groups. It was highly significance increased (P = 0.0014) in patient group (64 ppb) compared to the healthy group (49.38 ppb). Figure 2

Regarding hepatic: The glutamic oxaloacetate transaminase (GOT) level in the patient group (27.81 U/L) was significantly increased (P = 0.0019) compared to the healthy group (14.84 U/L). The alanine aminotransferase (ALT or GPT) level in the patient group (23.31 U/L) was also significantly increased (P = 0.0067) compared to the healthy group (16.15 U/L). The alkaline phosphatase (ALP) level in the patients group

(58.95 U/L) were elevated in high significance (P = 0.0003) compared to the healthy group (26.47 U/L), as mentioned in table 2 and figure 5.

Table 2: Biochemical parameters related to the liver and kidney functions in sera of patients and control groups.

Parameter	Patients	Healthy	*P* value
GOT (U/L)	27.81±8.1	14.84±7.9	0.0019
GPT (U/L)	23.31±9.4	16.15±7.7	0.0067
ALP (U/L)	58.95±16.2	26.47±13.5	0.0003
B. Urea (mg/dL)	37.97±13.5	26.52±7.5	0.002
S. UA (mg/dL)	6.23±1.52	4.55±1.12	0.0001
S. Cr (mg/dL)	0.85±0.27	0.55±0.14	0.0003
TSB (mg/dL)	0.82±0.22	0.63±0.15	0.0009
Dir. Bilirubin (mg/dL)	0.36±0.17	0.22±0.09	0.0017
Ind. Bilirubin (mg/dL)	0.46±0.1	0.41±0.12	0.013

Biochemical parameters show differences in significance as follows: Blood urea level in the patients group (37.97 mg/dl) was highly significant increased (P=0.002) in comparison to the healthy group (26.52 mg/dl). Uric acid level in sera of patients (6.23 mg/dl) was highly significant increased (P=0.0001) in comparison to the healthy group (4.55 mg/dl). Serum creatinine levels in the patients group (0.85 mg/dl) was highly significant increased (P=0.0003) in comparison to the healthy group (0.55 mg/dl). The total serum bilirubin level in the patients group (0.82 mg/dl) was highly significant increased (P=0.0009) in comparison to the healthy group (0.63 mg/dl). Direct bilirubin levels in the patients group (0.36 mg/dl) was significantly increased (P=0.0017) in comparison to the healthy group (0.22 mg/dl). Indirect bilirubin level in the patients group (0.46 mg/dl) was highly significant increased (P=0.013) in comparison to the healthy group (0.41 mg/dl). As mentioned in table 2, figure 3, figure 4.

Table 3: The activities of antioxidant enzymes in sera of patients and control groups.

Parameter	Patients	Healthy	*P* value
Catalase (U/L)	129.45 ± 44.5	116.62 ± 26.7	0.0019
Glutathione peroxidase (ng/ml)	5.95 ± 2.1	3.41 ± 1.6	0.049
Malondialdehyde (nmol/ml)	11.83 ± 8.6	9.02 ± 7.6	0.039
Xanthine oxidase (ng/ml)	19.54 ± 9.2	16.42 ± 8.4	0.032
Superoxidase dismutase (ng/ml)	21.35 ± 8.9	14.11 ± 4.6	0.0063

Catalase activity in sera of patients group (129.45 KU/L) was significantly increased (P=0.0019) in comparison to the healthy subjects (116.62 KU/L). Glutathione peroxidase activity in sera of patients group (5.95 ng/ml) was significantly increased (P = 0.049) compared to the healthy group (3.41 ng/ml). Malondialdehyde level in patients group (11.83 nmol/ml) was significantly increased (P = 0.039) compared to the healthy group (9.02 nmol/ml). Xanthine oxidase activity in sera of patients group (19.54 ng/ml) was highly significant increased (P = 0.032) compared to the healthy group (16.42 ng/ml). Superoxidase dismutase activity in the patient group (21.35 ng/ml) was highly significant increased (P = 0.0063) compared to the healthy group (14.11 ng/ml). Table 3 have shown the results of antioxidant enzymes. Figure 6

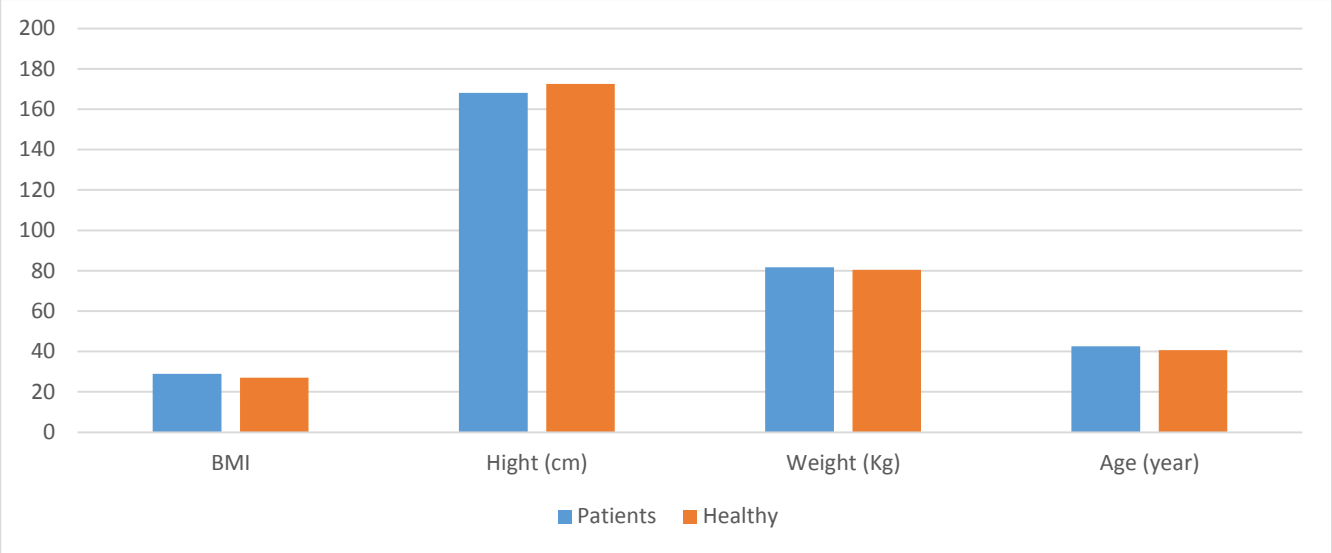

Figure 1: BMI (kg/m2), height(cm), weight(kg), and age(year) for the patients and control groups.

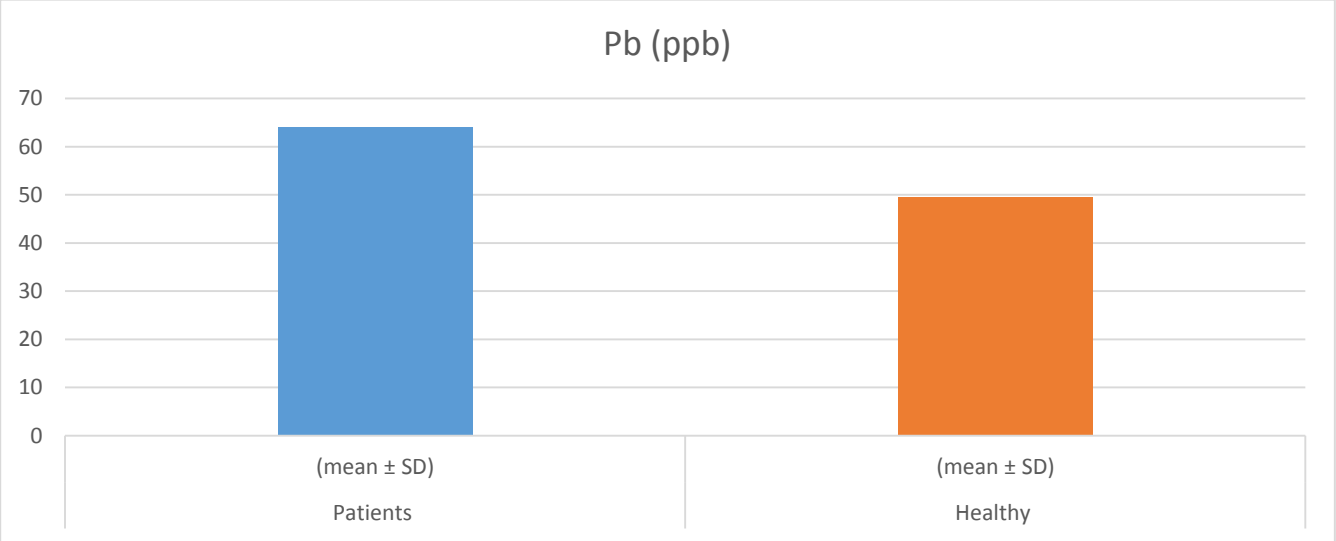

Figure 2: Lead level (ppb) in the serum of the patient and control group.

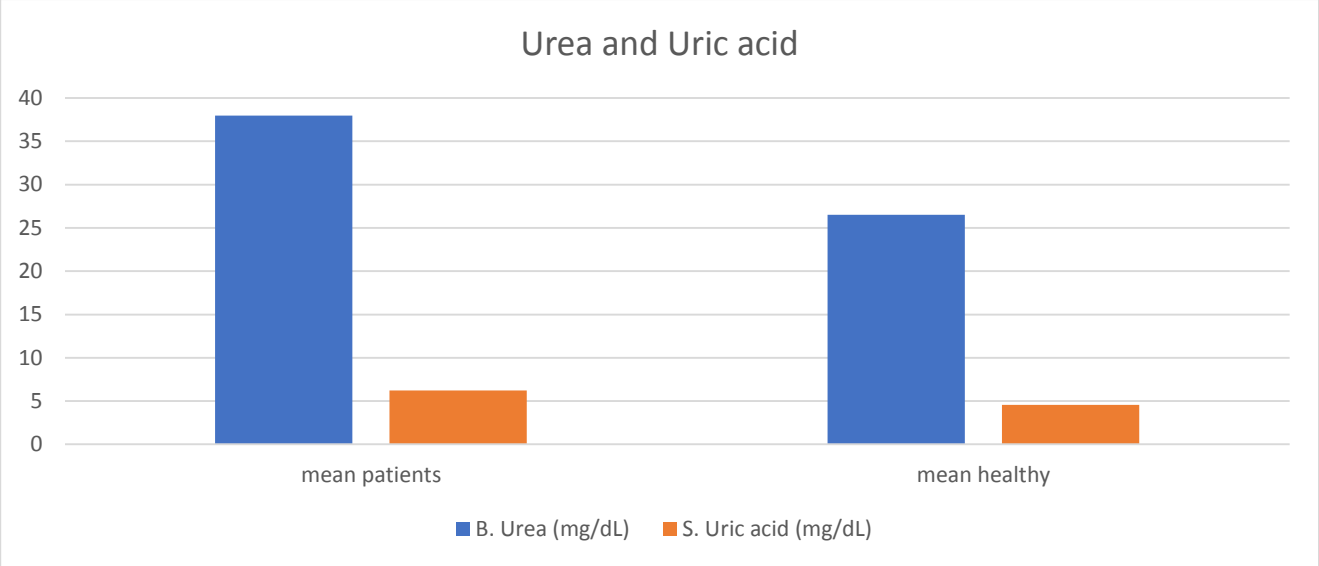

Figure 3: The levels of urea and uric acid in sera of patients and control groups.

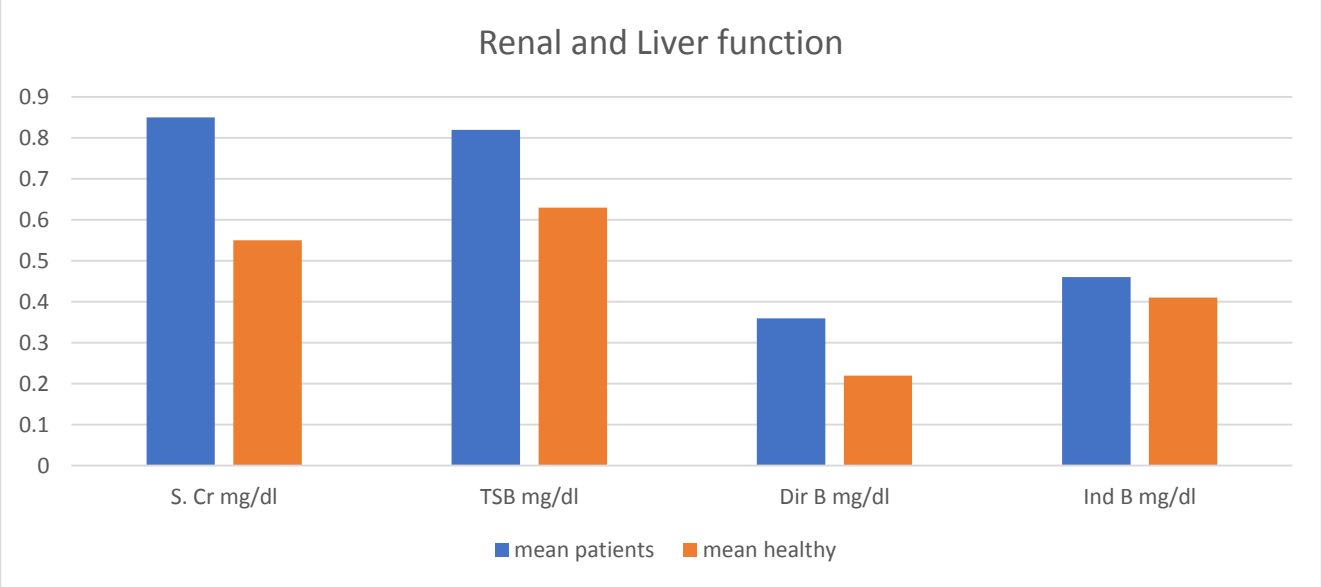

Figure 4: The levels of creatinine, total serum bilirubin, direct bilirubin, and indirect bilirubin in sera of patients and control groups.

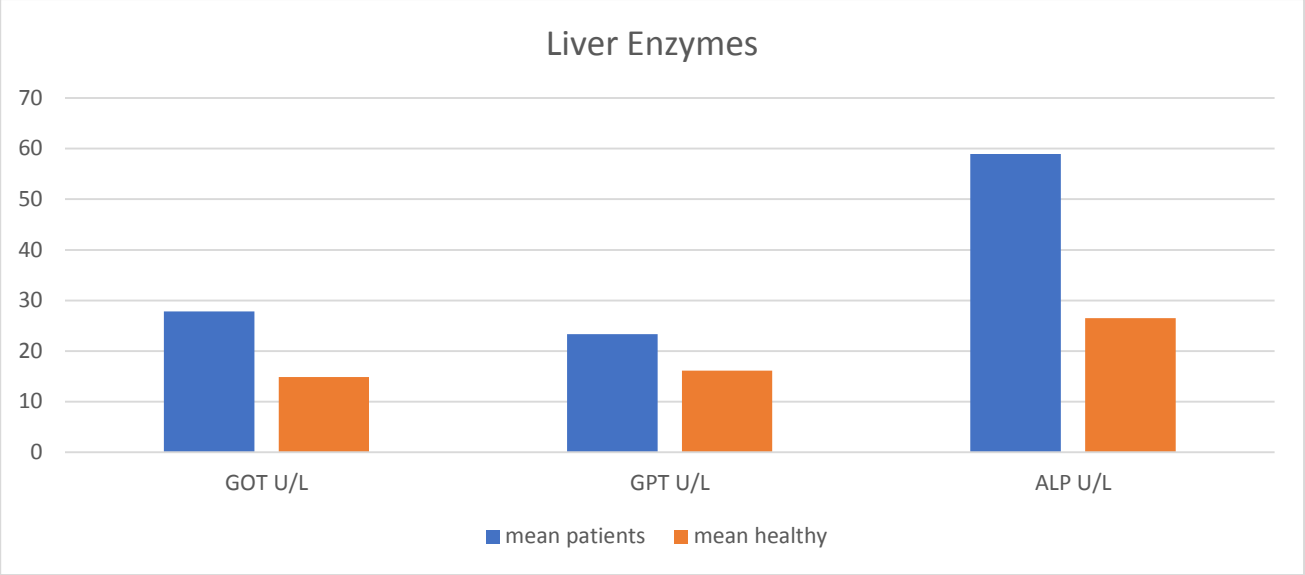

Figure 5: The activities of Liver enzymes (GOT, GPT, and ALP) in sera of patients and control groups.

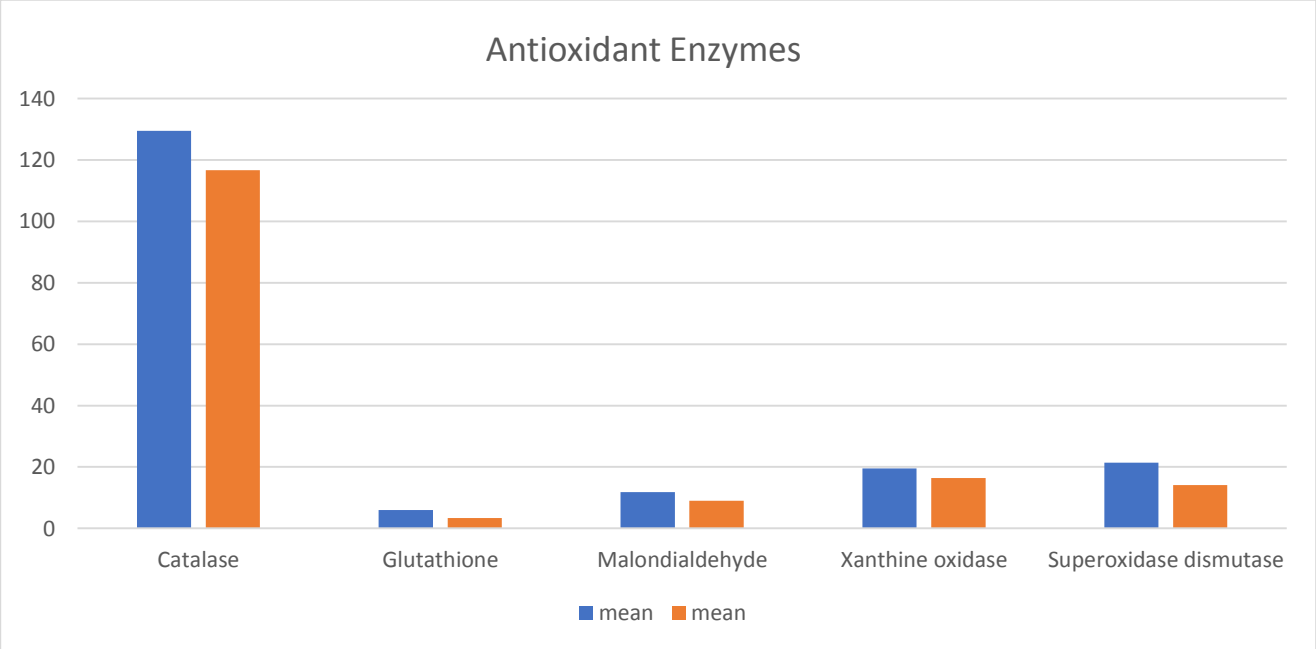

Figure 6: The activities of antioxidant enzymes (catalase (KU/L), glutathione peroxidase(ng/ml), malondialdehyde(nmol/ml), xanthine oxidase(ng/ml), and superoxidase dismutase(ng/ml)) in sera of patients and control groups.

The kidney's function in expulsing toxic and metabolic wastes through the urine may be connected to its status as one of the main locations where lead accumulates [9].

Exposure to lead has been demonstrated to cause interstitial edema, disintegration of cell nuclei, and kidney tubular epithelial cell detachment [15]. Furthermore, mice exposed to lead acetate showed signs of vascular congestion, perivascular fibrosis, tubular degeneration, glomerular atrophy, and congestion [16]. The kidney tissues of individuals subjected to minimal levels of mercury, cadmium, and lead also showed pathological symptoms such as renal arteriosclerosis, glomerulosclerosis, tubular atrophy, and interstitial fibrosis [17].

A previous study has reported that exposure to lead was linked to a higher potential for renal calculi among the Flemish population [14]. Also, a recent study has revealed a substantially increased susceptibility to kidney stones in men when lead levels exceeded 100 ug/L [25]. On the other hand, when lead concentration was within 5 ug/dL, Sun et al. found that kidney stone risk had an inverse relationship related to lead level [26].

The precise correlation of lead exposure with kidney stones still needs to be fully understood. One possible mechanism could be linked with the occurrence of hypercalciuria as a result of lead exposure. Animals subjected to lead in experiments were observed to result in increased protein and calcium in urine in mice, accompanied by notable tissue death and inflammation [19]. Urinary stone formation is more likely when the calcium levels in the urine exceed 200 mg/d [20]. Hypercalciuria is the primary underlying cause of calcium-based kidney stones. These stones form when calcium phosphate crystals accumulate in the kidney's interstitial tissues due to excessive calcium levels in the urine [21,22]. Most kidney stones, over 80%, consist of the phosphate and oxalate forms of calcium [27].

The Ca SR is an extracellular calcium-sensitive (G protein-coupled) receptor in the renal tubular cell plasma membrane. It detects the amount of unbound blood calcium and is crucial in maintaining calcium balance [28]. Lead ions may attach to the CaSR, causing hypercalciuria by impairing the kidneys' calcium handling [23].

This evidence agrees with our results regarding the levels of urea, creatinine, and uric acid (37.97, 0.85, 6.23 mg/dl, respectively) which were higher in the kidney stone patients than in the healthy ones (26.52 mg/dl, 0.55 mg/dl, 4.55 mg/dl, respectively, Table 2 and Figure 3.

Medical professionals often use the traditional UREA, CR, and UA markers when evaluating renal function. The creatinine level in the blood is a better indicator of glomerular filtration function [29]. An increasing body of epidemiological evidence gives a hint to a link between elevated blood uric acid levels and urolithiasis, raising the prospect that it can negatively impact glomerular and tubular function [30,31]. There was a high correlation between lead exposure, the presence of albuminuria, and a decrease in estimated GFR [18].

A recent study conducted on a group of workers in a lead mine reported a reasonable increase in the blood levels of bilirubin, AST, ALK, ALT, and LDH

(P<0.001) compared to individuals not exposed to lead. Additionally, the blood levels of protein in subjected people were less than those in the not subjected. Furthermore, a significant inverse association was noticed between blood lead levels with total protein (P<0.001)[32].

Lead (Pb) forms chemical bonds with sulfhydryl groups in structural and cytosolic proteins like glutathione. This interaction reduces the ability of these proteins to act as antioxidants, making them less effective in protecting cells from damage, Pb also increases its toxicity by causing peroxidation of lipids in cell membranes, including those of mitochondria and endoplasmic reticulum. The strong attraction between Pb with amino acids sulfhydryl groups led to reduced functionality of several enzymes, including superoxide dismutase, glutathione peroxidase, and catalase. A recent study has highlighted the higher activity of these enzymes but the mechanism remains partially understood. [33].

The elevation of antioxidant enzymes may reflected by the oxidative stress from high amounts of reactive nitrogen and oxygen species resulting from heavy metal toxication. Oxidative stress disrupts the equilibrium between free radicals and antioxidants, producing reactive nitrogen and oxygen species. Mitochondrial dysregulation increases the oxidation of DNA, proteins, and lipids, increasing ROS generation and lowering antioxidant levels. Inducing pro-inflammatory mediators worsens cellular damage and accelerates the course of CKD [34].

Exposure to lead can also cause the activation of genes that promote inflammation, such as interleukins and necrosis factor. Additionally, antioxidant enzymes like superoxide dismutase are produced [35]. Oxidative stress, caused by an excess of ROS causes structural and functional alterations in cells by damaging cell membranes and rendering essential enzymes inactive [12]. Oxidative stress-induced harm to kidney tubular epithelial cells is a significant factor in forming calcium oxalate crystals. The injured cells can act as sites for the attachment of these crystals [24]. Pb increases cellular susceptibility to oxidative damage via altering cell membrane stability and fatty acid composition. This alteration affects the integrity of cell walls, and the concentration of fatty acids is associated with an increase in the amount of malondialdehyde in the liver [36,37]. Pb causes DNA damage by its direct contact with DNA replication. A rise in ROS production is one possible cause of this [38]. The present study submits a good finding to the field of research in the term of pollution regarding heavy elements in humans, animals, plants, foodstuffs, soil and water and the extent of their impact on human health [39-42].

Conclusion

The present study submits new evidence to the field of research by reporting that the lead as a heavy metal is a key biochemical marker for Iraqi patients with renal stones in Basra Governate. Also, the present study highlighted a biochemical relationship

between prolonged exposure to lead and the activities of antioxidant enzymes. To successfully address this public health risk, the study's findings emphasize the importance of monitoring the degree of exposure to environmental heavy metals. According to the study results, individuals are more likely to develop kidney stones if their blood lead levels are high.

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Conflicts of Interest

The authors declare no conflict of interest.

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Author Contribution

All authors contributed equally to the research and preparation of this manuscript: data collection and analysis, writing and preparation of the manuscript for publication, and final editing.

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