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Association between Lead Poisoning and Chronic Kidney Disease in a Sample of Iraqi Population

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Researcher

Dedication

To the one who led mankind’s hearts and minds to the harbor of safety, the first teacher of mankind, Muhammad, may God’s prayers and peace be upon him and his family,

For the sake of his name with pride... To whom I miss since childhood... To whom I comfort my heart to remember... To my father's soul,

To the one through whom I saw the path of my life... and drew from her my strength and pride in myself... to the fountain that never tires of giving... to the one who woven my happiness with threads woven from her heart to my dear mother, may God prolong her life,

To whom was my shadow when fatigue struck me, my faithful husband,

To the seed of the heart and the hope of tomorrow, my sons "Mustafa, Zahraa, Fatima"

To my brothers and sisters, a source of pride and honor.

To all of them I dedicate this study, hoping that God will make it a window of knowledge and a knowledge card... and that it will benefit us and benefit us,,

Researcher

Abstract

Study included to estimate blood lead levels (BLL) in sera of patients with chronic kidney disease and its comparison with those in the control group, estimation of lead in the blood as a toxic element and its association with the development of chronic kidney disease among a sample of Iraqi population and to investigate the relationship between antioxidants, oxidative stress, and blood lead levels and their effect on the kidneys function tests that can be seen in CKD patients.

A case-control study included 77 subjects, their ages ranged from (17-72) years, 45 of whom were diagnosed with chronic kidney disease, while the other 32 were diagnosed as healthy controls. Samples were collected from December 2021 until May 2022.

The results of this study shows the mean of BLL (mg/dl) were highly significant increase in patients with CKD group as compared to controls group ($p<0.001$), and found that highly significant elevation in biomarkers SCr, B Ur, TC, HDL, Non-HDL-C, LDL and MDA in patients group as compared to healthy groups in $p<0.001$, and found that highly significant diminution in e GFR, TAC and Conc. Se in patients group as compared to healthy groups in $p<0.001$, and found that non- significant in Age, BMI, TG, and VLDL in patients group as compared to healthy groups in $p\geq0.05$. Also there is positive significant correlation between BLL, B Ur, S Cr, and MDA $p\leq0.001$, also there is negative significant correlation between BLL, e GFR, TAC and Conc. Se $p<0.001$.

From the results of this study it is concluded that the rise in lead levels in the blood leads to a decrease in antioxidants and an increase in oxidants in patients, and associated with low eGFR in patients with CKD, and so it is

involved in the development of chronic kidney disease, and increased BLL levels were a major risk factor for CKD and may be clinically meaningful, and the high level of lead and low level of selenium in the blood of patients with chronic kidney disease has a relationship with increase the risks of oxidative stress, kidney failure and through a decrease in the level of antioxidants (TAC) and an increase in the final product of MDA.

List of Tables

List of Abbreviations	
Abbreviations	**Description**
ATP	Adenosine triphosphate
apoB	Apolipoprotein B
BLL	Blood Lead Level
BSA	Is the investigated subject's body surface area (in square meters)
BUN	Blood urea nitrogen
BMI	Body Mass Index
CKD	Chronic kidney disease
Da	Daltons or atomic mass unit
DNA	Deoxyribonucleic acid
DDW	Deionized water
e GFR	Estimated glomerular filtration rate
ESRD	End-stage renal disease
GFR	Glomerular filtration rate
GPX	Glutathione peroxidase
HD	Hemodialysis

HDL	High-density lipoprotein
LDL	Low-density lipoprotein
LPL	Lipoprotein lipase
LDLR	Low-density lipoprotein receptor
LPO	Lipid peroxidation
MDRD	Modification of diet in renal disease
MDA	Malondialdehyde
NADPH	Nicotinamide adenine dinucleotide phosphate
NO	Nitric oxide
Non-HDL-C	Non-high-density lipoprotein
OS	Oxidative stress
pH	Potential of hydrogen or power of hydrogen
ppb	Parts per billion
ROS	Reactive oxygen species
RNS	Reactive nitrogen species
S Cr	Serum creatinine
SOD	Superoxide dismutase

SePP	Selenoprotein
T.Ch	Total cholesterol
TG	Triglyceride
TAC	Total antioxidant capacity
VLDL	Very-low-density lipoprotein

CHAPTER ONE:

INTRODUCTION

1.Introdaction

Characterized by a slow decline in renal function over months to years, chronic kidney disease (CKD) is an indicator of premature cardiovascular disease and risk of mortality. Chronic kidney disease (CKD) has become a global public health issue as a result of its rising prevalence, high management costs, and function as a risk factor for cardiovascular disease. The main causes of CKD include diabetes and hypertension. Many other cardiovascular disease risk factors, such as age, obesity, smoking, and hypercholesterolemia, have been related to CKD[1]. Heavy metals levels in the environment, such as lead (Pb) have been established nephron toxins at high levels of exposure[2] . Long-term exposure to low levels are common in today's environment, particularly in urban areas. These metals gradually accumulate in the body in industrializing countries [3]. Lead (Pb) in the environment, in particular, exposure can be found in the air, food, cigarettes, gasoline, and other products. crops that have been polluted, and seafood that has been infected[4] . However, their significance is debatable.At low-level environmental exposures [5]. Poisoning has been demonstrated to produce mitochondrial enlargement and impair energy generation in renal tubular cells.[6] The loss of nephrons is irreversible in CKD, and the glomerular filtration rate (GFR) eventually decreases [2].In an attempt to compensate for the loss of sick nephrons, these changes are accompanied by structural, functional, and molecular changes in the remaining functional nephrons [7]. Numerous experimental investigations conducted over the past ten years have indicated that oxidative stress, which is a major factor in the development of chronic kidney disease complications, is prevalent in CKD patients[8]. The imbalance of free radicals and antioxidants is what is referred to as oxidative stress. Increased radical generation and/or low antioxidant levels have the ability to modify biomolecules chemically,

resulting in a variety of structural and functional changes in the plasma of CKD patients. Changes in the quantities of some important trace elements, like selenium(Se), can affect how much oxidative stress is present[9] . These minor elements work with antioxidant enzymes to catalyze the destruction of free radicals as cofactors or structural elements[10]. Free radicals are challenging to measure because of their short half-lives and reactive nature.

1.2.Chronic kidney disease

Chronic kidney disease (CKD) is a major public health issue that affects people all over the world. It affects ten to thirteen percent of the population. The incidence and prevalence of this condition have increased in the recent decade, with a large increase in the number of patients requiring dialysis therapy[11,12], Nearly 20 million people are thought to have impaired kidney function[13]. Cardiovascular disease is the leading cause of death in patients with chronic renal disease, and it is directly linked to decreasing kidney function[14]. Kidney failure is commonly caused by glomerulonephritis, diabetic nephropathy, and polycystic kidney disease[15].

1.2.1.Kidney failure and disease

CKD is characterized by a gradual loss of nephrons and a reduction in renal function. This will result in an increase in uremic symptoms, such as elevated urea and creatinine levels in the blood, as well as electrolyte and water balance problems. Furthermore, anemia can be caused by a decrease in erythropoietin production, while hyperparathyroidism can be caused by a decrease in vitamin D synthesis[16].In addition CKD refers to a range of renal impairments marked by a decrease in glomerular filtration rate (GFR). The GFR is the most accurate way to assess renal function. In the process of urine production, GFR refers to the

volume of plasma ultra-filtrate supplied to the nephrons per unit time[17]. The idea of clearance, which is defined as the equivalent volume of plasma from which a chemical would have to be completely eliminated to account for its rate of excretion in urine per unit of time, is used to calculate GFR indirectly[18]. The clearance of a substance is estimated by dividing its excretion rate by its plasma concentration (Cx=UxV/ Px), where Ux and Px are the urine and plasma concentrations of substance x, respectively, and V is the urine flow rate. Cx=GFR when a substance is freely filtered, not protein-bound, and not reabsorbed, secreted, or metabolized by the kidney. Cx is measured in milliliters per minute and is commonly converted to a standard 1.73 m^2 idealized adult body surface area (Cx in milliliters per minute per 1.73 m^2) using the factor 1.73/BSA, where BSA is the investigated subject's body surface area (in square meters)[19,20].

The condition is usually diagnosed by measuring (GFR), and it is divided into stages (table 1.1)[21,22].

Table 1.1: Stages of Chronic Kidney Disease.

Stage of CKD	GFR (ml/min/1.73m^2)	Description
1	>90	Normal kidney function , signs of kidney disease
2	60-89	Mildly reduced kidney function, signs of kidney disease
3	30-59	Moderately reduced kidney function
4	15-29	Severely reduced kidney function
5	<15,dialysis	Very severe kidney failure

Kidney function is normal in stage one, and it minimally diminished in stage two. These stages of disease may go unnoticed by the patient, and they are not always associated with uremic symptoms. However, symptoms of kidney illness such as proteinuria, hematuria, structural abnormalities of the kidney, or genetic diagnosis can be detected at both stages, and diagnosis disease progression and cardiovascular disease risk. Patients in stage 3 have moderately reduced kidney function, necessitating more dietary restrictions, medical care, and disease monitoring. At stage 4, kidney function is severely impaired, and at stage 5, the disease is life-threatening, necessitating renal replacement therapy[23].

1.2.2.Chronic kidney disease and Serum Creatinine

The final result of creatine and phosphate metabolism is creatinine. Creatinine is a nitrogenous organic acid made up of three amino acids: glycine, arginine, and methionine[24]. It is produced primarily in the kidney and liver, but also to some extent in the pancreas. This process consumes up to 10 percent of the daily intake of glycine, 22 percent of arginine, and 42 percent of methionine[25].

Serum creatinine (molecular weight 113 Da) is produced in muscle cells by a non-enzymatic, spontaneous hydration of creatine. The non-enzymatic cyclization of creatine to creatinine is pH and temperature dependent, with higher temperatures and acidic environments increasing creatinine synthesis[26]. In-vivo, this process is irreversible and occurs at a constant rate (about 1.1 percent of the body's creatine and 2.6 percent of creatine phosphate are converted to creatinine per day)[27,28] (Fig. 1.1). The most prevalent biomarker of renal function is serum creatinine (SCr). Using the modification of diet in renal disease (MDRD) or chronic kidney disease epidemiology collaboration (CKD-EPI) equations, serum creatinine can be used to determine glomerular filtration rate in the steady-

Stata[29]. It has been established that a decrease in urine creatinine excretion is linked to a higher risk of renal failure and mortality in patients with CKD[30].

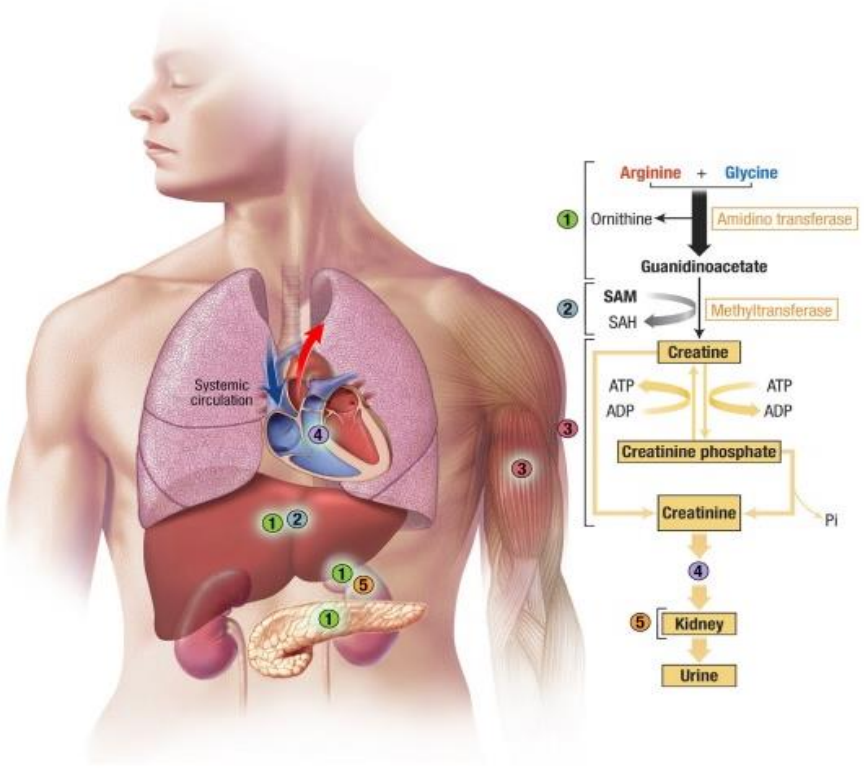


Figure1.1:Metabolism of creatinine[31]

Although it is usually assumed that serum creatinine is primarily eliminated via the kidney, creatinine appears to have an altered rate of generation and alternate routes for breakdown and removal in the setting of renal insufficiency[32]. Patients with advanced CKD have different rates of creatinine production and different

plasma amounts of guanidinoacetate, creatine, or creatine phosphate[33]. As a result, a rise in serum creatinine throughout the progression of CKD may be disproportionate to the loss in GFR[34]. The fundamental cause for this divergence could be attributable, at least in part, to an incremental shift in the activity of saturable proximal tubular creatinine secretion as GFR declines. As GFR declines, the amount of creatinine cleared by tubular secretion rises, and creatinine clearance consequently overestimates GFR to a greater extent in more advanced stages of CKD[35].

1.2.3.Chronic kidney disease and blood urea

Urea is the major metabolite produced by the breakdown of food protein and tissue protein. It is reabsorbed by the renal tubules after being freely filtered at the glomerulus but not secreted[36,37]. Furthermore, as the rate of urine flow decreases, more urea is reabsorbed. The nitrogen component of serum urea is measured by blood urea nitrogen (BUN)[38]. The deterioration of renal function is inversely related to BUN levels. Experiments have shown that increased blood osmolality causes the production of vasopressin and the activation of the aldose reductase-fructokinase pathway, which may be linked to kidney injury. BUN is one of the chemicals that impacts cSosm levels (calculated serum osmolality)[39]. As a result, there is a rise in cSosm and BUN levels in patients with advanced CKD[40]. Furthermore, a recent study found that higher BUN and cSosm levels were independently linked to the development of CKD, which was defined as a drop in eGFR to less than 60 mL/min/1.73 m^2 in patients with preserved kidney function (average eGFR of 86.7 mL/min/1.73 m^2)[39,41].

1.2.4.Chronic kidney disease and lipid profile

Despite the fact that atherosclerosis and cardiovascular disease (CVD) are the leading causes of increased morbidity and mortality in patients with chronic kidney disease (CKD), epidemiological studies and clinical trials have raised questions about the impact of dyslipidemia on CVD risk, particularly in patients with end-stage renal disease (ESRD)[42,43]. Figures 1.2 and 1.3 show the reported CKD changes in lipid and lipoprotein concentrations[44].

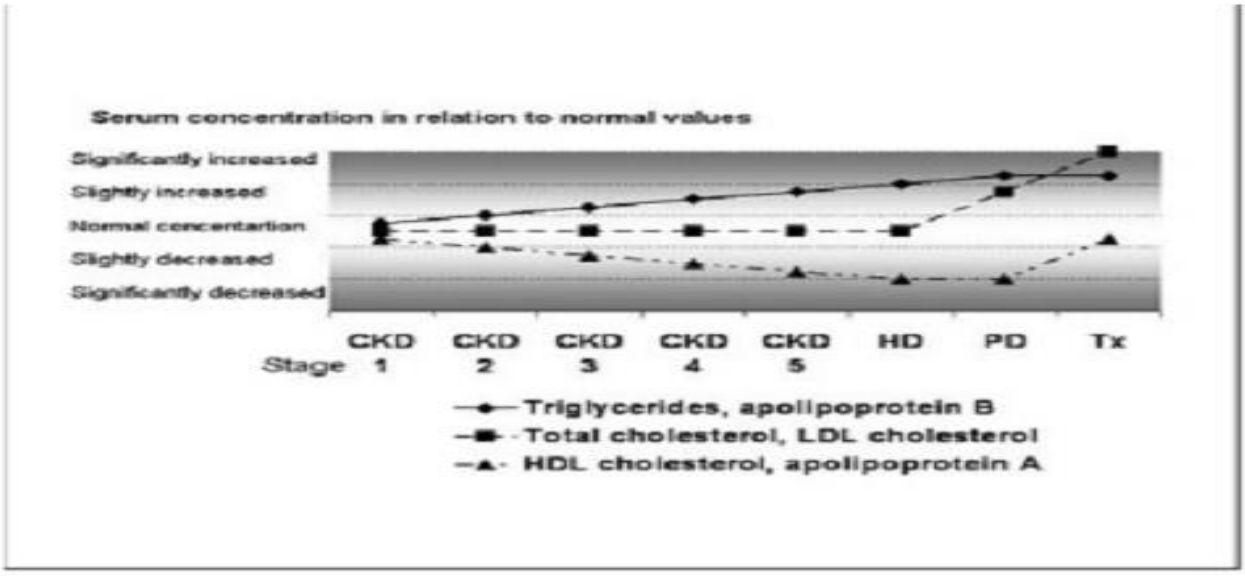


Figure 1.2[35]: A simplified plot depicting variations in lipid and Apo lipoprotein concentrations as chronic kidney disease (CKD) progresse. HD stands for hemodialysis, HDL stands for high-density lipoprotein, LDL stands for low-density lipoprotein, PD stands for peritoneal dialysis, and TX stand for kidney transplantation.

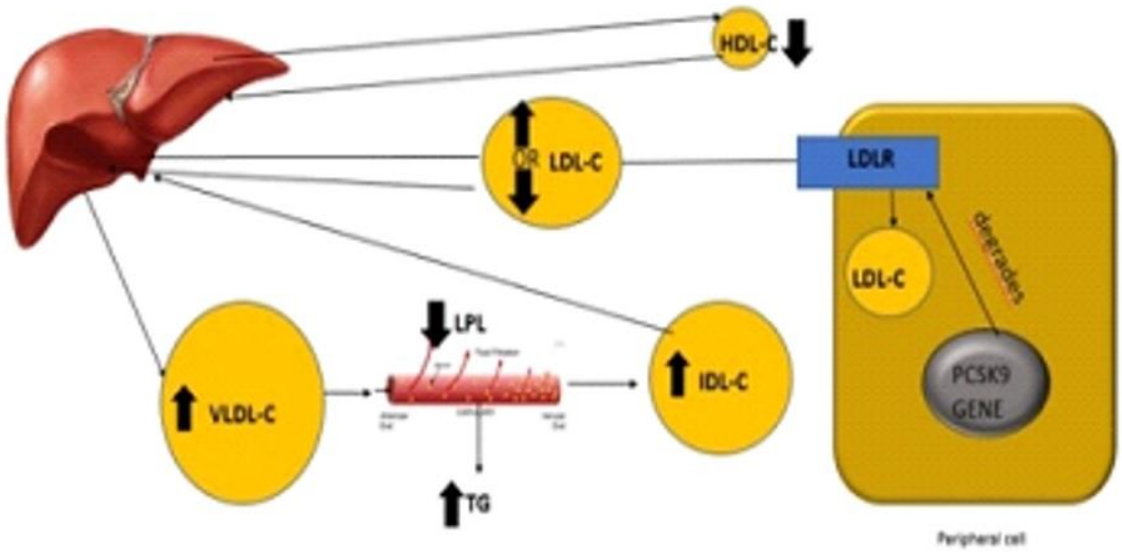


Figure 1.3[35]: Aerial view of lipoprotein alterations in chronic kidney disease. VLDL very-low-density lipoprotein, LPL lipoprotein lipase, TG triglyceride, IDL intermediate-density lipoprotein, LDL low-density lipoprotein, LDLR low-density lipoprotein receptor, HDL high-density lipoprotein, or PCSK9 proprotein converses subtilisin-Kexin like-9. Changes in the number of particles in CKD are indicated by arrows.

Hypertriglyceridemia, which is already evident in early stages of CKD and found in up to 70% of ESRD patients, is the most common dyslipidemia CKD disturbance[45]. Although hemodialysis has the ability to enhance triglyceride profile, renal replacement therapy does not result in a reduction in this proportion. High levels of apoB and triglyceride-rich lipoproteins, such as very-low-density lipoproteins (VLDL) and intermediate-density lipoproteins (IDL), as well as higher postprandial chylomicron concentrations, are associated with high triglycerides[46,47]. Hypertriglyceridemia in CKD can be caused by two different mechanisms: (a) increased triglyceride production and (b) impaired triglyceride clearance[48,49]. The latter appears to be the primary offender. Low expression and activity of lipoprotein lipase (LPL) and hepatic lipase(HL) cause decreased lipolysis. Furthermore, in experimental CKD, lower expression of LDL-related protein (LRP) and VLDL-receptor has been linked to reduced clearance of triglyceride-rich lipoproteins[50,51]. Total and LDL cholesterol levels in CKD are

normally within normal ranges, with only 20% -30% of cases surpassing them, similar to what is seen in the general population[52,53]. However, even when LDL cholesterol levels are normal, LDL particles in ESRD patients tend to be smaller, denser, and thus more atherogenic. Furthermore, when they undergo more oxidative changes, they lose their ability to be recognized by LDL receptors. Another common CKD symptom is low HDL cholestero[54].

Low activity of lecithin cholesterol acyltransferase (LCAT), which esterifies cholesterol taken up by HDL and allows HDL to acquire future cholesterol particles, is one of the pathophysiological pathways leading to lower HDL cholesterol levels[55]. Increased activities of cholesterol ester transfer protein (CETP) and acyl CoA: cholesterol acyltransferase (ACAT), as well as decreased activity of LPL and HL, all contribute to lower HDL cholesterol . Importantly, HDL paraoxonase, an enzyme that mediates HDL's antiatherogenic actions, is also reduced in CKD patients[56] . This most likely leads to a reduction in HDL's ability to protect against oxidative stress[57,58].

1.2.5.Kidney failure and lead exposure

Because of its tendency to reabsorb and concentrate divalent metals, the kidney is a target organ in heavy metal toxicity. The severity of kidney damage is determined by the nature, dose, and duration of exposure[59]. In general, the toxicity mechanism of acute damage varies from that of chronic damage. As a result, clinical characteristics and therapy approaches differ[60].

Non-diffusible (protein-bound) and diffusible (complexed and ionized) heavy metals exist in plasma. The bound form and the free form can both be found in the luminal fluid of the early proximal tubule[61]. The ionized form is poisonous

and causes direct cellular toxicity; the mechanism involves membrane rupture and mitochondrial respiration uncoupling, as well as the production of various death signals such reactive oxygen species and cytokines[62].

Metals are swiftly removed from the circulation and sequestered in a variety of tissues[63]. The apical membrane of the first zone of the proximal tubule is the predominant site of reabsorption in acute poisoning, but the loop of Henle and the terminal segments can also contribute in heavy metal reabsorption. The bound, inactive form, on the other hand, is conjugated with metallothionein and glutathione during chronic poisoning, and subsequently released into the bloodstream via the liver and kidney[64]. These chemicals are then reabsorbed in the proximal tubule's segment S1 via an endocytotic mechanism[65].

Lead is one of the oldest occupational poisons, with evidence of lead poisoning dating back to the time of the Romans. Lead is the most common nephrotoxic metal, and people are exposed to it through the air, food, and water[66].

Figure 1.4 shows how humans are exposed to lead contamination and how it affects them[67].

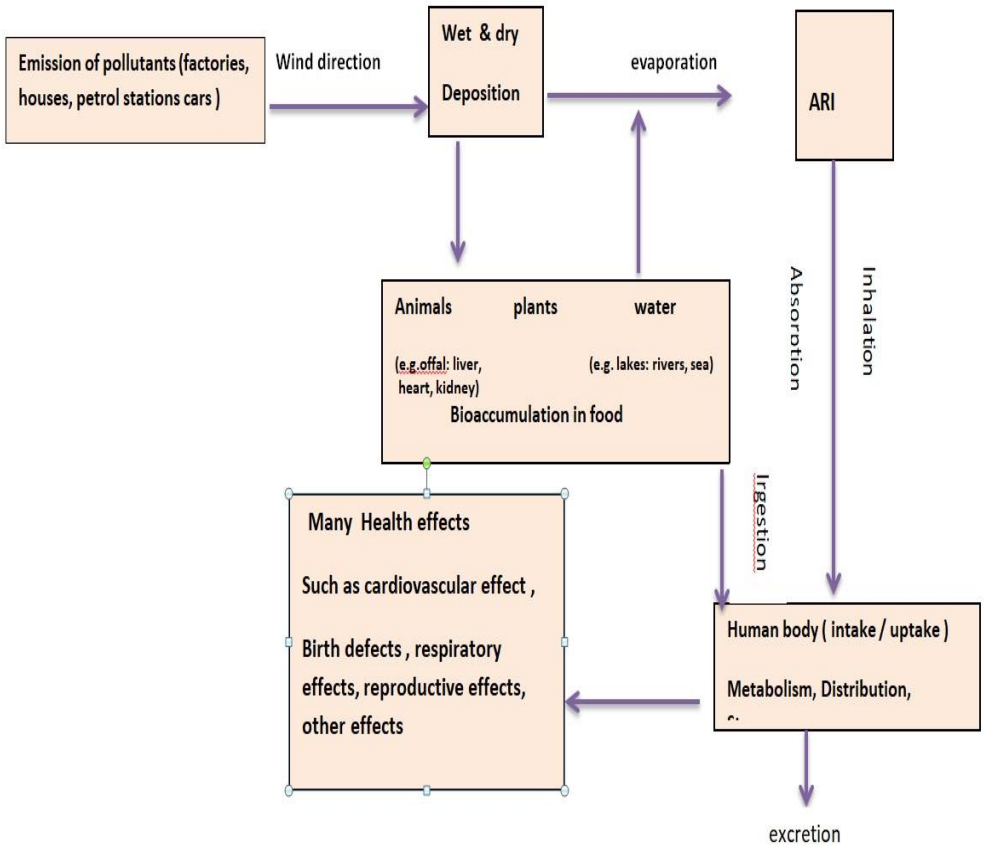


Figure 1.4: Stepsare exposed to lead contamination and how it affects them[67]**.**

lead's origin Metallic lead, inorganic lead (water-soluble lead salts), and organic lead, such as tetramethyl lead, which is more hazardous than the inorganic form, are all sources of exposure[68]. Acute exposure is a term that refers to a situation in which

Accidental or intentional intake of water-soluble inorganic lead salts or inhalation of tetramethyl lead causes acute intoxication, which is extremely unusual. a long-term exposure[69].

Lead poisoning can occur via lead paint, drinking water, lead-glazed pottery, and Asian traditional treatments[70].

Certain occupations, such as the fabrication of ammunition, batteries, sheet lead, bronze piping, radiation shields, and intravenous pumps, expose workers to high quantities of lead. Antiknock chemicals in automobiles also pollute exhaust with lead (tetramethyl lead)[71].

The proximal tubular architecture is disrupted, with histologic alterations such as eosinophilic intranuclear inclusions in tubular cells containing lead-protein complexes and mitochondrial edema. where there is a long-term exposure Increased urate production, vasoconstriction, and glomerulosclerosis with hypertension and interstitial fibrosis cause injury to both the proximal and distal tubules[72,73].

1.3.Oxidative stress

Oxidative stress is defined as an imbalance between free radicals and antioxidant enzymes in the body's stable agent[74]. When free radicals are present in low concentrations, they play a crucial function in cellular signaling and physiological control, but when they are present in large concentrations, they can cause cell damage[75]. Furthermore, oxidative stress has been shown to be advantageous in some circumstances. It can, for example, inhibit apoptosis in order to prepare the birth canal for delivery. Furthermore, oxidative stress strengthens cellular defense systems with appropriate physical activity and ischemia[76]. As a result, a better description of oxidative stress may be a state in which oxidation overcomes antioxidant systems due to a breakdown of equilibrium between them[77].

Cells respond to oxidative stress by activating or silencing genes that encode protective enzymes, transcription factors, and structural proteins in order to diminish oxidant effect and restore balance[78]. The ratio of oxidized to reduced

glutathione (2GSH/GSSG) is one of the most important markers of oxidative stress in the body[79]. Increased levels of reactive oxygen species (ROS) in the body can affect DNA structure, causing changes in proteins and lipids, as well as activate various stress-induced transcription factors and release inflammatory and anti-inflammatory cytokines[80]. Although low oxidative stress can trigger apoptosis, excessive oxidative stress causes mitochondrial activity to be compromised, culminating in ATP depletion and necrotic cell death[81]. Oxidative stress and nitrosative stress are terms for the damage caused by free ROS and RNS radicals, which can cause potential biological harm. If there is an excess of ROS / RNS formation as well as a lack of enzymatic and non-enzymatic antioxidants, this is seen in biological systems[82]. The redox state/oxidative stress technique is challenging. The effect on the body varies depending on the type of oxidant, where it is produced, and how powerful it is, as well as the structure and function of different antioxidants and the capability of repair mechanisms[83]. Oxidative stress can result in tissue damage or even cell death, which occurs primarily through two pathways: necrosis and apoptosis[84]. Oxidative stress causes a wide range of diseases, including many of the diseases that cause death, such as cancer (Figure 1.5)[85].

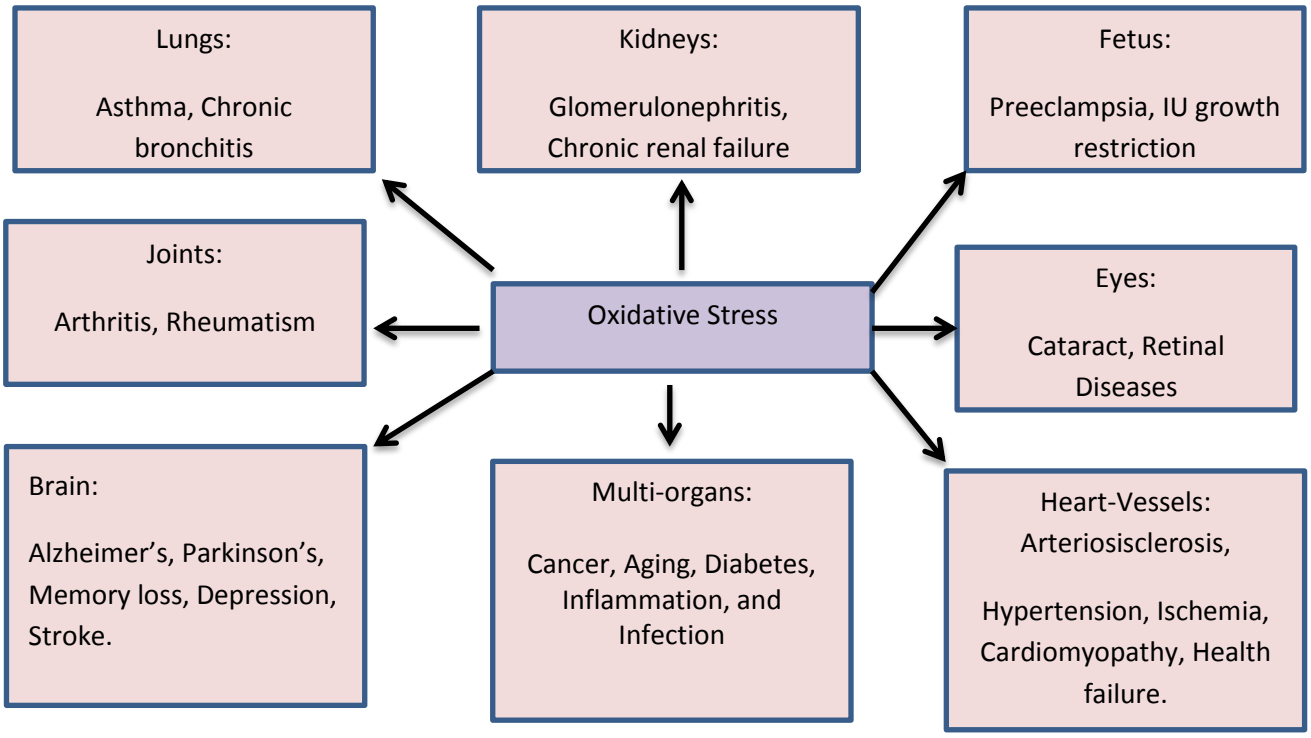


Figure 1.5: Human diseases caused by oxidative stress[85]

1.3. 1. Free radical's biological effects

Because the harm of processes overlaps extensively, oxidative stress may cause the destruction of all molecular targets, including DNA, lipids, and proteins (Figure1.6)[86]. The first line of attack is not usually visible. The first cellular target of OS can change; DNA is a significant first target of damage[87]. When the lipid peroxidation process begins, it will generate a series of chain reactions that will continue until completed products are formed. As a result, lipid peroxidation end products such as malondialdehyde (MDA), F2-isoprostanes, and 4-hydroxy2-nonenol (4-HNE) are produced in the biological systems[88].

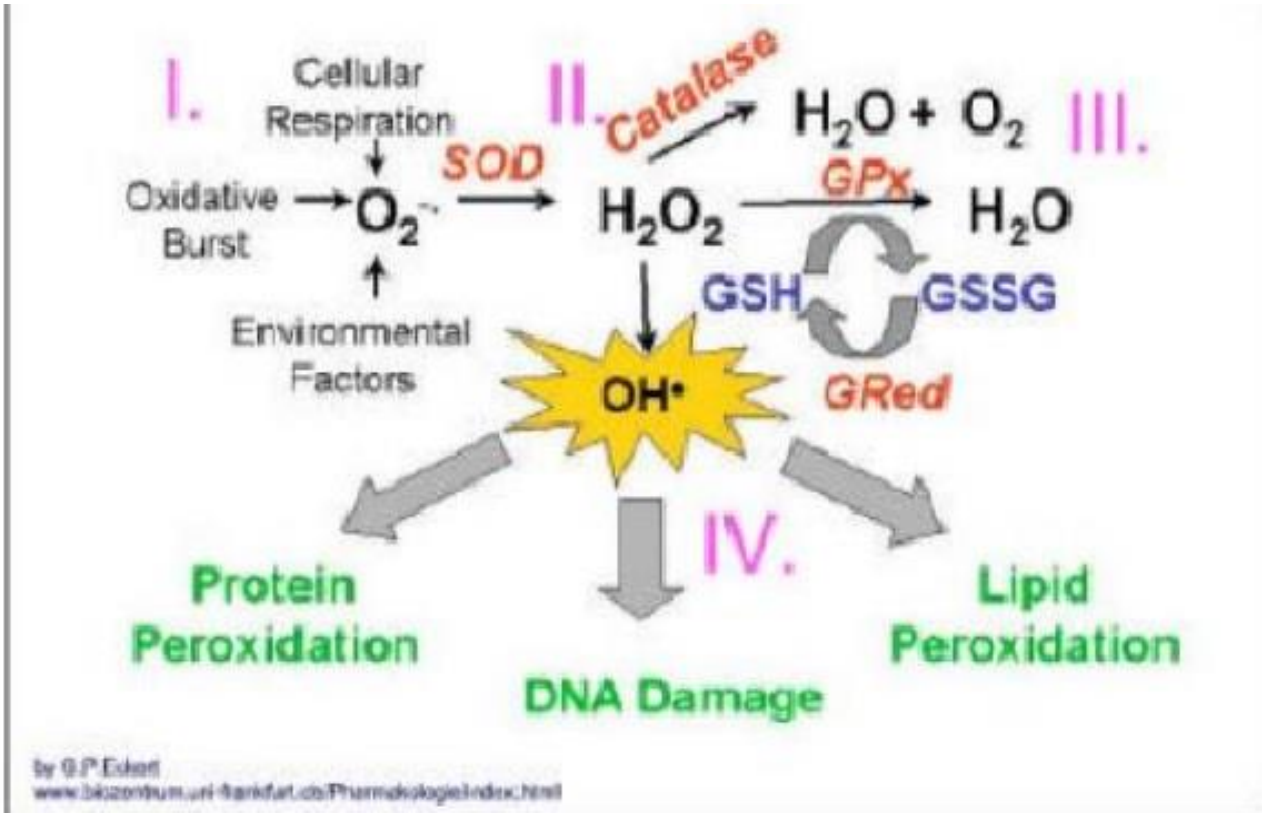


Figure 1.6: Free radical's biological effects[86].

1.3. 2.Malondialdehyde(MDA) and Lipid peroxidation

At first, lipid peroxidation appears to be the oxidative degradation of polyunsaturated lipids since it is a free radical mediated chain reaction. Pieces of biological membranes are the most popular uses[89]. When produced in biological membranes, many numbers of this harmful substance, such as end peroxides and aldehydes, can be used to start or improve processes. Malondialdehyde (MDA) is a three-carbon compound generated by peroxidized polyunsaturated fatty acids, the majority of which are arachidonic acids[90]. This is also one among the membrane lipid peroxidation's end products. The various correlations with free radical damage were discovered because the level of MDA is enhanced in several diseases among the abundance of oxygen free radicals[91]. In the 1980s, Esterbauer

and his colleagues conducted extensive study on the numerous different aldehydes that can be formed as secondary products by lipid peroxidation, such as malondialdehyde (MDA), hexanal, and propanol[92]. MDA is thought to be the most common mutagenic result of lipid peroxidation[93]. The membrane's lipids, notably polyunsaturated fatty acids, are the remaining phospholipids, which are highly susceptible to oxidation by free radicals[94]. Because of its association with a variety of clinical conditions, lipid peroxidation is critical for Vivo[95]. The effects of lipid peroxidation in the membrane include reduced fluidity, deactivation of membrane-bound enzymes, and receptor inactivation[96].

1.3. 3.Antioxidant system's control over oxidative stress

Various compounds known as antioxidants can either neutralize oxidants or stop the oxidation of downstream macromolecules[97]. Antioxidants can be categorized according to how they scavenge ROS—either generally or specifically depending on the substrate[98]. They can also be divided into groups based on their solubility in lipids or water; the former is crucial for limiting the oxidation of cell membrane components, whereas the latter controls the redox status of plasma and cytoplasm[99]. Similar to ROS generation, different antioxidants predominate in different cell types and intracellular sites[100].

1.3.3.1.Enzymatic antioxidants

Superoxide dismutase (SOD), catalase, peroxiredoxin, and glutathione peroxidase are the principal enzymatic antioxidants[101]. The three SOD isoforms, which are abundant near cells where ROS are produced, such as the

mitochondria, catalyze the breakdown of O_2^- into H_2O_2 and oxygen[102]. Extracellular SOD, which is generated and secreted by vascular smooth muscle cells and binds to elements of the extracellular matrix to regulate interstitial redox state, is one of the additional SOD isoforms in addition to mitochondrial SOD[103].

By lowering the potential for numerous cellular processes, reduced glutathione plays a crucial part in intracellular redox control[104]. The cytoplasm contains a large number of isoforms of the enzyme glutathione peroxidase (GPx). GPx-1 lowers H_2O_2, whereas GPx-4 prefers oxidized lipids as a substrate[105]. During this process, glutathione (GSH) is oxidized to glutathione disulfide (GSSG), which can then be recycled back to GSH by the enzyme glutathione reductase with nicotinamide adenine dinucleotide phosphate (NADPH)serving as the electron donor[106].

The small, ubiquitous proteins known as thioredoxins play a vital role as intracellular antioxidants[107]. By lowering the thiol disulphide linkages that form as a result of oxidative stress, thioredoxins, which have decreased cysteine residues, enable enzymatic repair of oxidative damage to cellular proteins[108]. Thioredoxins are reduced by the enzyme thioredoxin reductase so that they can be recycled for use in these processes[109].

1.3. 3.2.Antioxidant non-enzymes

Low molecular weight proteins that remove ROS by going through their own oxidation are the majority of non-enzymatic antioxidants[110]. These include urate, ascorbic acid, vitamin E, glutathione and ascorbic acid (vitamin C)[111]. A number of enzymes require ascorbic acid, a six-carbon ketolactone, as an essential cofactor[112]. Ascorbic acid must be consumed through diet in order to prevent vitamin C shortage in humans because we, like all primates, are unable to

synthesis it. the prototypical vitamin C deficiency syndrome[113]. Ascorbic acid acts as a reducing agent to scavenge ROS, and also acts as an antioxidant by stabilising tetrahydrobiobterin(BH_4), facilitating its action as a co-substrate for nitric oxide NO formation[87,114]. Ascorbic acid, on the other hand, has pro-oxidant qualities as well, generating ROS through an interaction with iron. Especially present in cell membranes, vitamin E is a lipophilic antioxidant[115].

The antioxidant activity of glutathione, a cysteine-containing peptide, is usually exerted intracellularly, where it is present in large concentrations[116]. The cysteine residues' active thiol group contributes electrons to neutralize ROS and keep NADPH in its reduced form[117]. In order to assist ascorbic acid's antioxidant activity, reduced glutathione also keeps ascorbic acid in its reduced state[117,118]. The ratio of glutathione to glutathione disulphide is frequently evaluated as a marker of the redox status. Glutathione reductase and NADPH reduce oxidized glutathione to glutathione disulphide[119].

1.4.Selenium's impact on kidney disease.

When Schwarz and Foltz revealed that vitamin E and selenium deprivation caused nutritional necrotic liver degeneration in rats, they proved the necessity of selenium for mammals in 1957[120],. Se was added to stop this kind of harm. Twelve years later, the Se activity's mechanism was clarified[121].

Se needs in humans were unknown for a very long period. The American National Academy of Sciences advised people over the age of 7 to ingest 50-200 µg Se per day based on animal studies conducted in the 1980s[122]. According to human research, the daily dietary need for selenium is 55 µg for females and 70 µg for males, or 1 µg/kg body weight[123]. Byard was the first to recognize trimethylselenium ion(TMSe) as a urine Se metabolite in 1969. Palmer ***et al***.

(1969; 1970) determined TMSe to be the primary Se metabolite in rat urine almost simultaneously[124]. These authors have demonstrated that TMSe makes up 20–50% of the urine Se and is a common urinary metabolite from all examined Se sources[125]. It has been widely accepted that when Se exposure exceeds the nutritional requirement, the TMSe level in urine rises. Later, a human urine sample contained this Se metabolite[126]. Figure(1.7)[127]. depicts the metabolism of se in animals and how it becomes methylated[128].

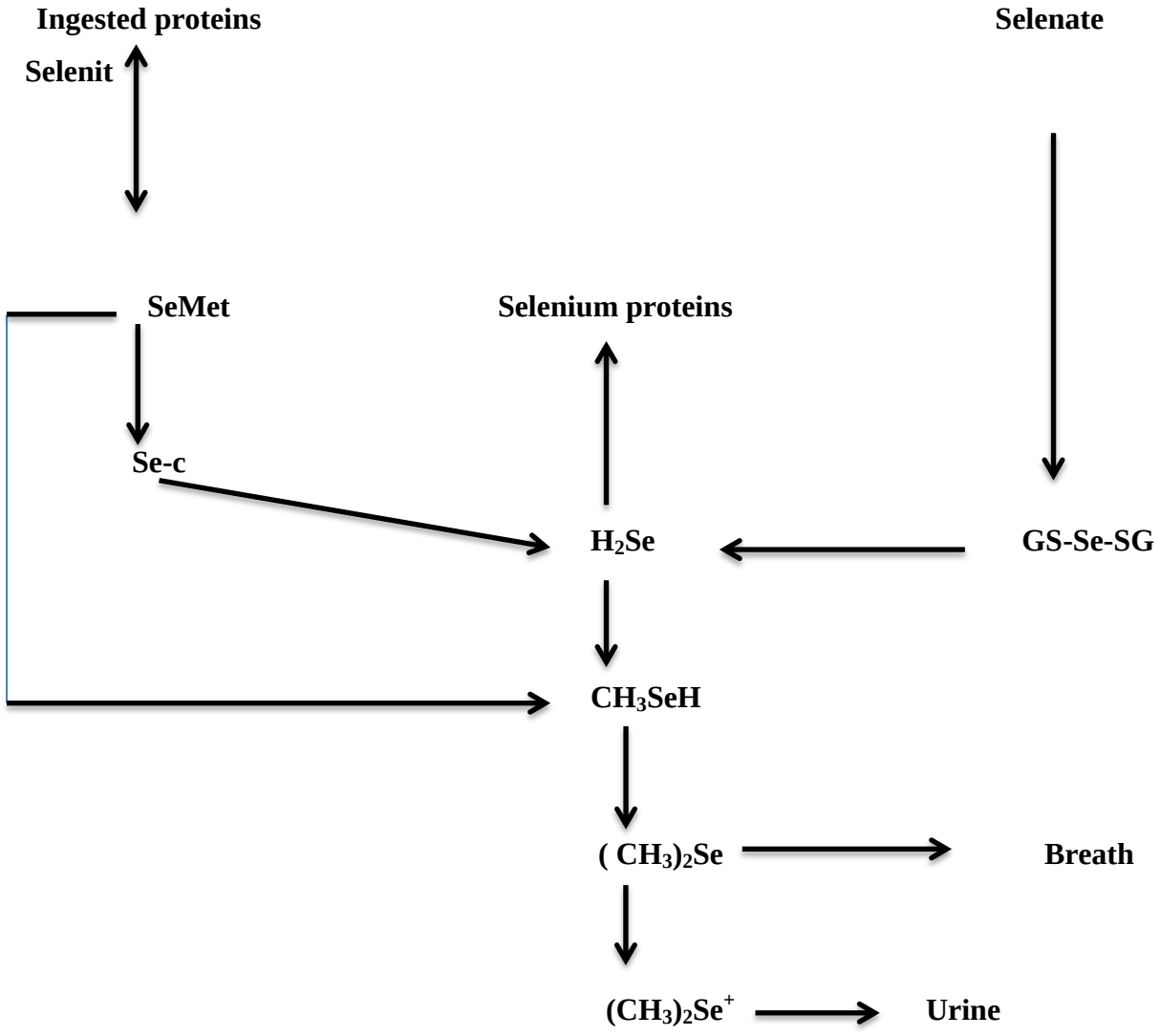


Figure 1.7: Mammal metabolism of selenium. Sec; seleno- cysteine[127].

Se has a significant part in many illnesses, including cancer, cardiovascular, and kidney disease, as a component of several proteins. Many tiny molecules and elements, including Se, have a central place in the homeostasis of the kidneys[129]. In actuality, among all human organs, the thyroid and kidneys contain the highest Se concentrations. Se is transported by SePP, which has at least 10 Se atoms, from the liver to the kidneys[130].

Se was considerably lower in CKD than healthy subjects in whole blood, plasma, serum, and red blood cells[131]. In fact, numerous studies have demonstrated that Se levels decline according to the severity of the disease. In whole blood and plasma, Se has been found to be lower but statistically insignificant at the early stages of illness[132]. Whole blood and plasma Se, however, were considerably decreased (by 50%) in ESRD patients compared to healthy controls ($p \leq 0.0001$). The Se concentration was even lower in hemodialysis patients. Uncertainty exists regarding the importance of low blood Se levels[133]. Cardiomyopathy in dialysis patients has been linked to decreased serum Se without severe insufficiency. For HD patients, in whom oxidative stress is considerably enhanced, mild Se deficiency appears to increase vulnerability to oxidative stress[134]. Does the issue of the reason of low Se in CKD come up? blood component Diet, or the primary source of this component, has a clear impact on se concentration[135]. Reduced protein intake and increased urine protein excretion are both associated with decreased whole blood and plasma Se in uremia[136]. Patients are recommended to limit their protein intake in these situations to ease uremic symptoms and stop the progression[137]. Se is primarily found in proteins, hence dietary consumption is drastically decreased[138]. This phenomena may also be brought on by other elements like poor intestinal absorption, strange binding proteins that transport Se, or medication therapy[139]. Different kinds of se are excreted in the urine. Trimethylselenium was once believed to be the primary urinary form (TMSe) It

was interestingly shown that TMSe accounted for 20–50% of the Se excretion in rat pee. TMSe was also found in human urine in 2004[140]. Se-containing carbohydrates were found to be the main urine metabolites in later research. Se is transferred to the kidney after hepatic metabolism, with extra Se eliminated as

selenosugar[141]. Currently, TMSe is viewed as a less important metabolite. Plasma creatinine concentration, creatinine clearance, or glomerular filtration rate (GFR) measurements are frequently used to monitor the progression of CKD[142]. Plasma creatinine concentration is several times greater in CKD patients compared to healthy controls, and it is maximum in ESRD patients[143]. HD by itself has little effect on the concentration of creatinine, even though it may even be higher in HD and peritoneal dialysis[144]. Other investigations showed a strong negative relationship between Se and creatinine in CKD . Others have made similar discoveries[144],[145],[146].

1.5. Designs of the several instruments (device types) used in absorption spectrometry

The following categories can be used to categorize the many device designs employed in the measurement of absorption spectrometry in the visible and ultraviolet light spectrums:

1. A variety of devices using a single beam appear from it:

a) Non-scanning instruments (there are three kinds of them).

i. Photometers, which are gadgets that use filters.

ii. Spectrophotometers, which are gadgets that make use of a monochromator.

iii. Multichannel instruments are gadgets that make use of a monochromator and a multi-channel detector.

b) Scanning apparatus (which are also of three types):

i. Instruments without a reference beam that use a monochromator.

ii. Monochromator-based instruments having a reference beam and a second detector, It is known as beam dual.

2.Devices that use a double beam and fall into one of two categories:

i. i. Devices that use filters

ii. ii. Devices that use monochromators

It is worth noting that the double beam devices can be with two detectors, and they are called space in, or with a single detector, called time in.

Spectrophotometers that measure UV-Vis absorption were used to calculate the levels of urea, creatine,total antioxidant, and lipids. It was determined using a JENWAY 7315 Spectrophotometer that this type of equipment uses a monochromator with a dispersion element to select the appropriate wavelength for measurement with high accuracy and without the need to change any filters, and that it is obvious to move the dispersion element (grating, prism), either manually or with the aid of a motor. The detector is a silicon diode or a photomultiplier tube, depending on the application.The components of the device are shown in Figure 1.9. Because the device operates in the UV and Vis regions, a deuterium lamp is used to cover the UV region before switching to a tungsten-halogen lamp (as modern technology has forced the use of semiconductors because they can be manufactured at a lower cost, smaller size, and with the same performance, if not better)to cover the Vis area. The process of measurement starts with selecting the proper wavelength to analyze at and the location of the

reference solution in the cell where the detector first reads P_o. Next, the reference solution is switched out for the sample solution so that the device reads P, and finally, the device processor provides us with the value of the absorbance or percent of transmittance[147,148].

$\%T=100-10^{-A}$;Where T: Transmittance; A: Absorption.

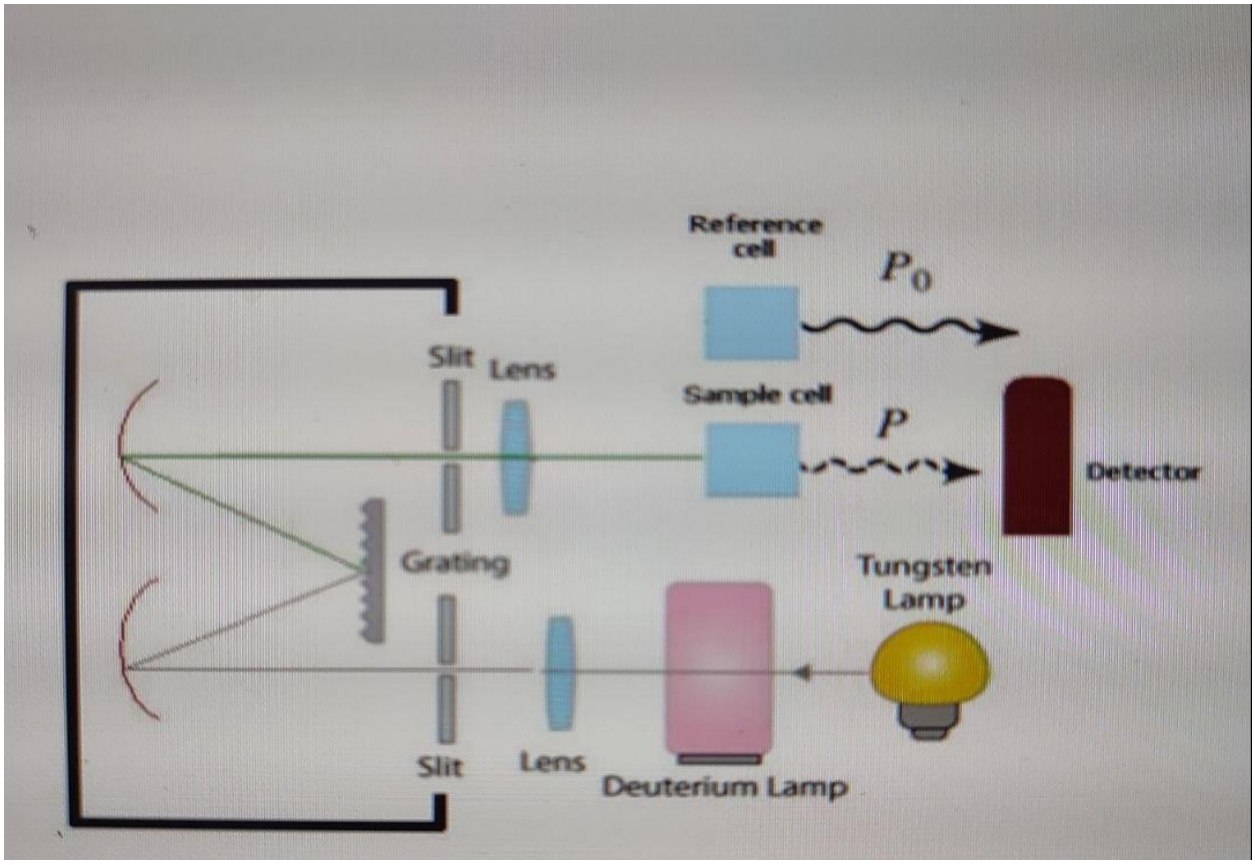


Figure 1.8: The components of the UV-Vis absorption spectrophotometer[147]

1.6. ELISA technology (Enzyme Linked Immunosorbent Assay)

It may be claimed that it is a frequently used laboratory test to identify antibodies in the blood since it is an enzymatic immunoassay that permits the detection of the interaction between an antigen and an antibody, owing to a colorimetric reaction. Antigens are substances that cause the body's immune system to be activated, specifically the stimulation of antibodies, whereas

antibodies are substances that are already existing inside the body's immune system and are specifically created when the body is exposed to foreign bodies. The four primary[149].

ELISA technique types are as follows:

1- Direct ELISA
2- Indirect ELISA
3- Competition ELISA
4- ELISA Sandwich

Competition ELISA technology was used to estimate Malondialdehyde in blood serum. Biotek EL.x50 ELISA device was used, characterized by high sensitivity, where the typical detection range of ELISA technology is 0.01-0.1 ng.

Where the antigen to be measured in the sample competes with the labeled antigen to bind to the antibody. In this case, there is an inverse relationship between the amount of required antigen measured by the labeled antigen. The key event in competitive ELISA (also known as fixation ELISA) is the process of competitive interaction between the antigen sample and the microtiter-bound antigen with the primary antibody[150].

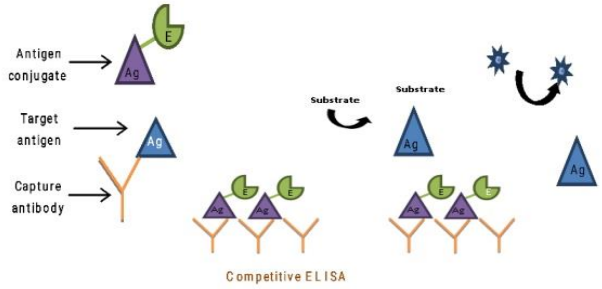
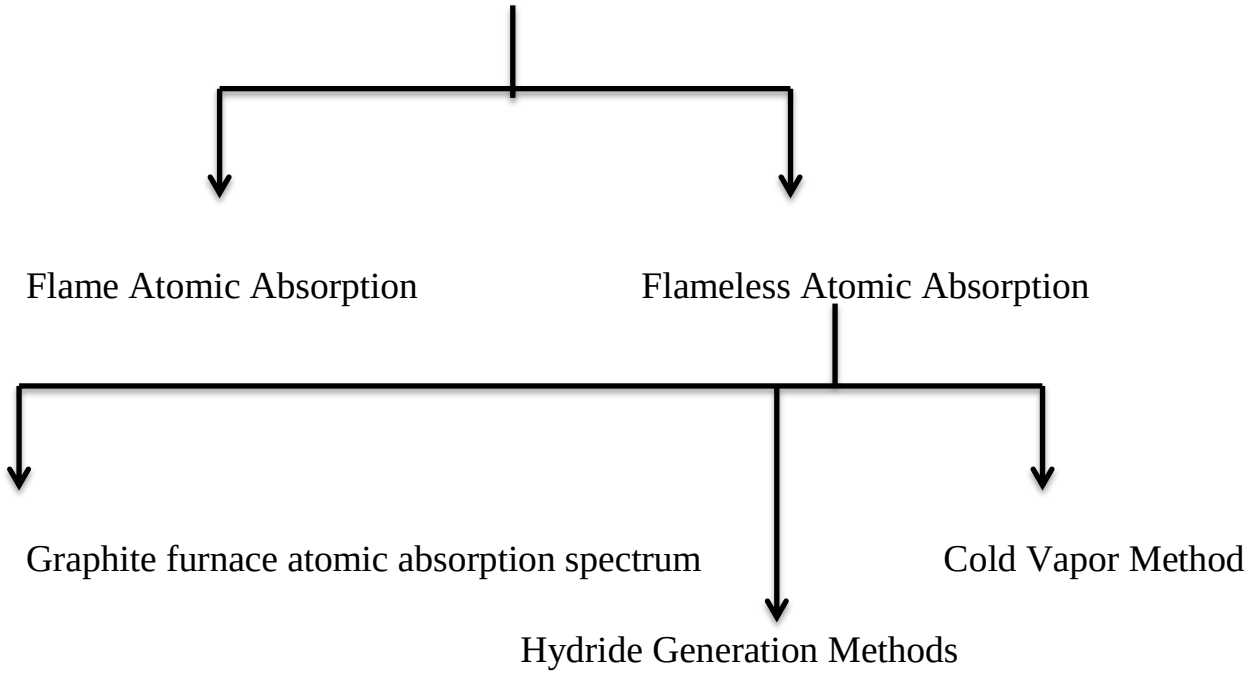


Figure 1.9: Stages in competitive ELISA[150].

1.7. Atomic absorption spectroscopy

Atomic absorption spectrum: This is the spectrum formed when free atoms of an element absorb light energy at a particular wavelength; the wavelength varies on the element. The investigation of how atoms absorb radiant radiation is known as atomic absorption spectroscopy (AAS). It involves dissolving the mixed elements into free atoms with the capacity to absorb radiant radiation as an analytical technique.

Atomic absorption methods are classified into:



The method of flameless atomic absorption spectrometry using graphite furnace technology (GFAAS) was used to estimate the elements lead and selenium in the blood serum, where a SHIMADZU AA 6300 atomic absorption spectrometer was used for the purpose of determination, characterized by high sensitivity, which can reach low detection limits (in ppb units).

A graphite furnace tube is used in this method, which is also known as electrothermal atomic absorption spectrometry (ETAAS), to vaporize the graphite sample in three stages: drying, atomization, and ablation. The effectiveness of this method depends on the element's free atoms' ability to absorb light with particular wavelengths that are typical of that element from the cathode lamp. Within specific bounds, the concentration of the element present in the amount of light absorbed reflects that concentration and can be linearly dealt with that concentration. When high temperatures and high currents are applied to

the graphite furnace, the majority of elements can produce free atoms from samples.

In this method, very small amounts of samples (10–20 microliters) are injected into a tiny graphite tube or a graphite tube coated with carbon, which may then be heated to a wide range of temperatures to evaporate and then atomize the sample. Atoms absorb electromagnetic radiation in the visible or violet range, which causes electrons to move to higher electronic energy levels to the excited state, where they eventually return to the ground state by releasing their own characteristic light that may be used to quantify the concentrations of samples. Depending on the element being studied, the temperature of the graphite tube can climb within seconds and exceed 3000°C[147,151].

1.8.Literature review

A review of the literature reveals an association between blood lead levels and CKD, and the following is a summary of some of the studies.

Many researchers have been interested in chronic kidney disease associated with heavy metals such as lead, cadmium and others, as in some of the following studies:

According to several studies there was a statistically significant effect on kidney function. It revealed that the levels of lead in the blood had a negative effect to some extent on the glomerular filtration rate, as the correlation between the levels of lead in the blood and the glomerular filtration rate was inversely related. It was also noted that there was a positive correlation between the levels of lead, creatinine and urea[152,153,154,155,156,157,158].

While other studies have found the development of end-stage renal disease with high levels of lead in the blood[159].

While another study discovered an inverse relationship between lead levels and kidney size in pregnant women, but not at the beginning of early pregnancy[160,161].

Another study in which lead levels were linked to creatinine-based glomerular resting rates, although the association was not statistically significant and was weaker than cysteine-dependent glomerular resting rates[162].

A study also observed a statistically significant correlation between cadmium and lead levels with glomerular filtration rate and albuminuria, where the effect of cadmium was higher than that of lead[163].

While other studies showed that there is an improvement for chronic kidney patients who have high levels of lead in the blood and who receive chelation therapy, where it was observed that their glomerular filtration rates improved significantly during a period of 4 months[164,165].

While the current study showed that there is a statistically significant inverse correlation between lead levels and levels of selenium and antioxidants in the blood of chronic kidney patients, and a positive, statistically significant correlation between lead levels and the final product of oxidation (MDA) in the blood of chronic kidney patients.

2.Aims of the Study

* Estimation of blood lead levels in chronic kidney disease and its comparison with those in the control group

* Evaluation of the relationship between blood lead levels and oxidative stress in people with chronic kidney disease

* Evaluate the role of selenium as an antioxidant by evaluating selenium levels in the blood of chronic kidney patients and comparing them with those in the control group.

* Study the correlations between blood lead levels, creatinine, urea, glomerular filtration rate and oxidants - antioxidants.

CHAPTER TWO:

MATERIALS

&

METHODS

2. Materials and Methods

2.1. Materials

2.1.1. Chemicals and Kits

The chemicals and kits in the present study are used as supplied from purchases without additional purification . Kits and chemical used in the present study are shown in the Table (2-1).

Table (2-1) The Chemicals and Kits

Chemicals and Kits	**Formula or Symbol**	**Concentration**	**Company and/or Country**
Ammonium dihydrogen orthophosphate	$NH_4H_2PO_4$	20 %	India
Triton X-100	$C_{34}H_{62}O_{11}$	10%	India
Nitric acid	HNO_3	69%	India
Standard lead solution	$Pb(NO_3)_2$	1000ppm	
Standard selenium solution	SeO_2	1000ppm	HORIBA
Urea Kit	CH_4N_2O		LiNEAR (Spain)
Creatinina Kit	$C_4H_7N_3O$		BIOLABO (France)
Triglyceridi (TG) Kit	TG		SANYMED (Italia)
Total Cholesterol (TC) Kit	T.C		SANYMED (Italia)
HDL-Cholesterol Kit	HDL		SANYMED (Italia)
Malondialdehyde ELISA Kit	MDA		Elabscience (USA)
Total Antioxidant Capacity Colorimetric Assay Kit	T-AOC		Elabscience (USA)

2.1.2. Instrument and Equipment's

All Instrument and tools which have been used in this study are listed in table (2-2).

Table (2-2) : Instrument and Equipment .

Instruments	Company and/or Country	Workplace
Sensitive balance	Germany	Kerbala University College of Education for Pure Sciences
Vortex mixer	China	Kerbala University College of Medicine Biochemistry Branch
Water bath	Germany	Kerbala University College of Medicine Biochemistry Branch
Centrifuge	Germany	Kerbala University College of Medicine Biochemistry Branch
Incubate	Japan	Kerbala University College of Medicine Biochemistry Branch
Magnetic drive		

Volumetric bottles		
Conical flask		
Baker		
Inserted cylinder		
Micropipette 100-1000 µL	China	
Micropipette 10-100 µL	China	
UV-visible Spectrophotometer	Jenway 7315	Kerbala University College of Education for Pure Sciences
Elisa device ELx50	Bio Tek (USA)	Kerbala University College of Medicine Biochemistry Branch
Micro plate reader	Bio Tek (USA)	Kerbala University College of Medicine Biochemistry Branch
Graphite Furnace Atomic Absorption Spectrophotometry	Shimadzu AA-6300 Japan	Kerbala University College of Medicine Biochemistry Branch

2.2. Subjects

2.2.1. Study design

The study is case-control study at the Adel Al-Sabbah Dialysis Center of Al-Hussein Teaching Hospital ,Holy Karbala governorate, Iraq. Included in the following criteria is both patients and healthy people: The age (17 to 72) years of CKD who visited Adel Al-Sabbah Dialysis centers during the study period from November (2021) to October (2022).

2.2.2. Patient Group

Patient group involved (45) patients they were diagnosed with CKD , With a mean age of (17-72) years. All patients were diagnosed by physicians.

2.2.3.Control Group

The Control group involves (32) approximately healthy subjects. All subjects do not show any signs and symptoms of diseases. Controls subjects were matched with patients in sex and age to increase the accuracy of the results. Any patient with the following problems was excluded from the current study:

1- heart diseases and hypertension 2- Smokers 3-diabetic.

2.3. Methods

2.3.1 Collection of Samples

Disposable syringes and needles are used for blood collection. Blood samples (5 ml) were obtained from Patients and the control group. Blood samples were allowed to clot, and then centrifuged at 4500xg for 10 minutes. Sera are separated and divided into fraction in Epperdrof tubes then frozen until use.

2.3.2. Body Mass Index (BMI)

Body mass index was calculated in all subjects according to ratio depend on weight and height obtained by applying a mathematical equation, in which the weight in kilogram is divided by the square height in meter, and the results were considered as follows :

BMI (kg/m2) = weight (kg) / height (m^2)

Underweight ≤ 18.5(kg/m^2)

Normal weight between 18.5 - 24.9(kg/m^2)

Overweight between 25-29.9(kg/m^2).

Obese ≥30(kg/m^2)[166] .

2.4. Biochemical measurement

2.4.1.Determination of Creatinine

The colorimetric method for quantitative in vitro diagnostic measurement using the Creatinine kit was being used to assess the serum Creatinine . using a device JENWAY 7315 Spectrophotometer .

2.4.1. 1. Principle

Colorimetric reaction (Jaffe reaction) of creatinine with alkaline picrate measured kinetically at 490 nm (490-510) , without any pre-treatment step. This reaction has been improved (specificity , speed and adaptability) by the development of an initial-rate method .

2.4.1.2. Reagents :

1- Reagent 1 :- Consist of 6.4 mmol/L of disodium phosphate and 150 mmol/L of sodium hydroxide .

2- Reagent 2 :- Consist of 0.75 mmol/L of sodium dodecyl sulfate and of 4.0mmol/L picric acid pH 4.0 .

3- Reagent 3 :- Consist of 177 µmol/L (2mg/L) of standard of creatinine .

2.4.1.3. Procedure :

The addition was made to a set of three tubes represented sample , reagent , and blank.

Pipette in a 1 cm path **Length cuvette**	**Blank**	**Standard**	**Sample**
Working Reagent (R1 + R2)	**1mL**	**1mL**	**1mL**
Deionized Water	**100µL**		
Standard		**100µL**	
Sample			**100µL**

All reagent was mixed well and after 30 second the absorbance A1 was measured at 490 nm against reagent plank . The absorbance A2 was measured after 2 min .

Calculations :

Creatinine mg/d L = $\frac{(A2-A1)SAMPLE}{(A2-A1)STANDARD} * standard\ concentration$

2.4.2. Determination of Urea :

The colorimetric method for quantitative in vitro diagnostic measurement using the Urea kit was being used to assess the serum Urea . using a device (JENWAY 7315) Spectrophotometer .

2.4.2.1. Reagent :

1- Reagent 1 (Enzyme reagent) :- Consist of urease > 500 U/m L and of 0.05 g/L sodium azide .
2- Reagent 2 (Buffered chromogen 0 :- Consist of 20 mmol/ L of phosphate buffer pH 6.9 , 2mmol/L of EDTA , 60 mmol/L of sodium salyciliate and 3.4 mmol/L of sodium nitroprusside .
3- Reagent 3 (Urea standard) :- Consist of 50 mg/d L (8.3 mmol /L) .

2.4.2.2 Principle

Urea is hydrolyzed by urease , into ammonia and carbon dioxide . the ammonia generated react with alkaline hypochlorite and sodium salicylate in presence of sodium nitroprusside as coupling agent to yield a green chromophore . The intensity of colour formed is proportional to the concentration of urea in the sample .

$$\text{Urea} + H_2O \longrightarrow 2\,NH_3 + CO_2$$

NITROPROUSSIDE

$$NH_4^+ + \text{Salycilate} + NaClO \longrightarrow \text{Indophenol}$$

OH^-

2.4.2.3. Procedure :

The addition was made to a set of three tubes represented sample , reagent , and blank.

TUBES	Blank	Sample	Standard
Working reagent	**1.0 m L**	**1.0 m L**	**1.0 m L**
Sample		**10 µL**	
Standard			**10 µL**
All reagent was mixed well and incubate for 5 minutes at 37 ^{0}C or for 10 minutes at room temperature (16-25 ^{0}C) . Pipette .			
R3	**1.0 mL**	**1.0 mL**	**1.0 Ml**

All reagent was mixed well and incubate for 5 minutes at 37 ^{0}C or for 10 minutes at room temperature (16-25 ^{0}C) . The absorbance (A) of the sample and the standard were measured at 600 nm against the blank .

2.4.3.Determination of e GFR

GFR was estimated using the CKD-EPI equation

GFR = 141 × min(Scr/κ, 1)α × max(Scr/κ, 1)-1.209 × 0.993Age × 1.018 [if female] _ 1.159 [if Black ethnicity]

where: Scr is serum creatinine (mg/dL)

κ is 0.7 for females and 0.9 for males,

α is -0.329 for females and -0.411 for males,

min indicates the minimum of (Scr/κ) or 1, and

max indicates the maximum of (Scr/κ) or 1 [30].

2.4.4. Determination of Lipid Profile Levels

2.4.4.1. Determination of Serum Total Cholesterol

The total Serum cholesterol is determined by the colorimetric method for the quantitative in the laboratory diagnostic measurement using a kit [167] .

2.4.4.2. Principle

This method is for the measurement of the total serum cholesterol, which involves the use of three enzymes: cholesterol esterase (CE), cholesterol oxidase (CO) and peroxidase (POD). In the presence of the former, the mixture of phenol and 4-aminoantipyrine (4AA) is condensed by hydrogen peroxide to form Quinoneimine dye proportional to the concentration of cholesterol in the sample.

The Serum cholesterol is measured using the enzymatic method based on the following reactions:

$$\text{Cholesterol ester} + H_2O \xrightarrow{CE} \text{Cholesterol} + \text{Free Fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{CO} \text{Cholestene-3-one} + H_2O_2$$

$$2H_2O_2 + \text{phenol} + \text{4-amino-antipyrine} \xrightarrow{POD} \text{Quinoneimine (pink)} + 4H_2O$$

Where.

CE = Cholesterol esterase

CO = Cholesterol oxidase

POD = Peroxidase

2.4.4.3. Reagents

1- Reagent 1 (Buffer) : consist of 100.0 mmol/L of goods buffer pH 6.8 ; 20.0 mmol/L of phenol ; ≥ 500 U/L of cholesterol esterase ; ≥ 800 U/L of cholesterol oxidase ; ≥ 2500 U/L peroxidase ; 1.6 mmol/L of 4-aminophenazone ; conservatives and preservatives .

2- Standard : consist of 200 mg/d L (5.17 mmol/L) of cholesterol .

2.4.4.4.Procedure

Three sets of tubes (Sample , standard and blank) are prepared as the following :

Reagent	Blank	Standard	Sample
Working reagent	1000 µL	1000 µL	1000 µL
Standard	…….	10 µL	…….
Distilled water	10 µL	…….	…….
Sample	…….	…….	10 µL
All reagent was mixed well , then incubate for 5 minutes at 37 ^{o}c . The absorbance of sample (EC) and standard (ESTD)were measured at wavelength 510 nm against the reagent blank .			

Calculation

The total cholesterol (mg /d L) = Abs. of sample / Abs. of standard × Conc. STD (200 mg/ d L)

. Reference Values

The total cholesterol concentration in serum is < 200 mg/d L .

2.4.4.2.Determination of serum Triglycerides (TG)

Serum triglycerides were determined by the colorimetric method for quantitative in vitro diagnostic measurement using a Kit .

2.4.4.2.1. Principle

The method is based on the enzymatic hydrolysis of serum or plasma triglyceride to glycerol and fatty acids by lipoprotein lipase (LPL) , which is transformed by glycerolchinnase (GK) , ATP and glycerol-3-p-oxidase (GPO) , into diidroxiaceton –phopsphate and H_2O_2. The hydrogen peroxid catalyzed from peroxidase (POD) reacts with 4- aminophenazone and 4-phenol-chloride giving a coloured compound whose intensity is proportional to the concentration of triglycerides in the sample .

2.4.4.2.2. Reagents

1- Reagent 1 (buffer) : consist of 100.0 mmol/L of pipes buffer ; 16.0 mmol/L of 4-clorophenol ; ≥ 4000 U/L of lipoproteinlipase ; ≥ 2000 U/L of glicerolkinase ; ≥ 2500 U/L of peroxidase ; 0.8 mmol/L of adenosine triphosphate (ATP) ; 1.4 mmol/L of 4-aminophenazone and ≥ 2000 U/L of glycerol-3-p-oxidase .

2-Reagent 2 (standard) : consist of 200 mg/dl (2.26 mmol/l) of triglycerides .

2.4.4.2.3. Procedure

Three sets of tubes (Sample , standard and blank) are prepared as the following :

Reagent	Blank	Standard	Sample
Working reagent	1000 µL	1000 µL	1000 µL
Standard	…….	10 µL	…….
Distilled water	10 µL	…….	…….
Sample	…….	…….	10 µL
All reagent was mixed well , then incubate for 5 minutes at 37 ^{o}c . The absorbance of sample (EC) and standard (ESTD) were measured at wavelength 510 nm against the reagent blank .			

Calculation

TG in (mg/dl) = Abs.(sample) / Abs. (standard) × con. of standard (200 mg/dl)

Reference Values

The triglycerides concentration in serum is between 40 – 165 mg/d L .

Where:

Men	60 – 165 mg/d L
Women	40 – 140 mg/d L

2.4.4.3. Determination of High Density Lipoprotein-Cholesterol (HDL-Ch) concentration

Serum or plasma (HDL-Ch) was determined by the elimination method in the laboratory direct enzymatic measurement using a kit.

2.4.4.3.1. Principle

The assay is based on a modified polyvinyl sulfonic acid (PVS) and polyethylene-glycol-methyl ether (PEGME) coupled classic precipitation method with the improvement in using optimized quantities of PVS/PEGME and selected detergents .Low density lipoprotein –cholesterol (LDL-C) , very low density lipoprotein –cholesterol (VLDL-C) and chylomicron (CM) react with PVS and PEME and the reaction results in inaccessibility of LDL, VLDL and CM by cholesterol oxidase (CHOD) and cholesterol esterase (CHER) . The enzymes selectively react with HDL to produce H_2O_2 which is detected through a Trinder reaction .

2.4.4.3.2. Reagents

1- Reagent 1 : consist of MES buffer 6.5 pH ; TODB N,N-Bis(4-sulfobutyl)-3methylaniline ; Polyvinyl sulfonic acid ; Polyethylene-glycol-methl ester ; $MgCl_2$; Detergent ; EDTA .

2-Reagent 2 : consist of MES buffer 6.5 pH ; Cholesterol esterase ; Cholesterol oxidase ; Peroxidase ; 4-aminoantipyrine ; Detergent .

3-Reagent 3 : consist of 50 mg/dl of standard HDL Cholesterol .

2.4.4.3.3. Procedure

Three sets of tubes (Sample , Standard and Blank) are arranged as the following :

Reagent	Blank	Standard	Sample
Reagent 1	225 µl	225 µl	225 µl
Distilled water	3 µl	…….	……
Standard	……..	3 µl	……
Sample	…….	…….	3 µl
All reagent was mixed well , incubate at 37 °C for 5 min and then was added			
Reagent 2	75 µl	75 µl	75 µl
All reagent was mixed well , then incubate for 5 min at 37 °C and the absorbance of sample (A1C) and standard (A1STD) were measured at 600 nm . The second absorbance of sample (A2C) and standard (A2STD)were measured after 5 min against the reagent blank .			

Calculation

HDL-C (mg/dl) = (A2C – A1C)/ (A2STD – A1STD) × Standard con. (50 mg/dl) .

Reference values

The HDL-C concentration in serum is between 35 – 65 mg/d L .

Where :

Men	35 – 55 mg/d L
Women	45 – 65 mg/d L

2.4.4.4. Assessment of Very Low Density Lipoprotein-Cholesterol

Very low density lipoprotein- cholesterol (VLDL-C) is calculated by dividing the triglycerides concentration by 5 and it characterizes the concentration in milligram per deciliter [168].

2.4.4.5. Assessment of Low Density Lipoprotein-Cholesterol

Low density lipoprotein- cholesterol(LDL-C) is calculated by the indirect method [169] .

LDL-C= total cholesterol – (HDL-cholesterol + VLDL cholesterol).

LDL-C = total cholesterol - (HDL-cholesterol + TG/5)

- **Reference Values**

Normal value of LDL cholesterol less than 100 mg/dl

2.4.5. Total Antioxidant Capacity (T-AOC) Assay : The Colorimetric method .

2.4.5.1. Principle

A variety of antioxidant macromolecules, antioxidant molecules and enzymes in a system can eliminate all kinds of reactive oxygen species and prevent oxidative stress induced by reactive oxygen species . The total level reflect the total antioxidant capacity in the system . Many antioxidants in the body can reduce Fe^{3+} to Fe^{2+} and Fe^{2+} can form stable complexes with phenanthroline substance . The antioxidant capacity (T-AOC) can be calculated by measuring the absorbance at 520 nm .

2.4.5.2. Reagents

1- Reagent 1 : consist of 60 ml of buffer solution .

2- Reagent 2 : consist of chromogenic agent which preparation by dissolve with 120 ml distilled water (It can be dissolved by incubating in 80-90 ^{o}C water bath) It can be used after cooling to room temperature .

3- Reagent 3 : consist of 1.5 ml of ferric salt stock solution dilute with a ferric salt diluent (60 ml) at the ratio of 1: 19 .

4- Reagent 4 : consist of 24ml of stop solution .

2.4.5.3. Procedure

Two sets of tubes (Sample and Control) are arranged as the following

Reagent	Sample	Control
Reagent 1 (ml)	1.0	1.0
Sample (ml)	0.1	
Reagent 2 (ml)	2.0	2.0
Reagent 3 (ml)	0.5	0.5
All reagent was mixed well and incubate the tubes at 37 ^{O}C for 30 min .		
Reagent 4 (ml)	0.1	0.1
Sample (ml)	0.1	0.1
All reagent was mixed well and stand for 10 min at room temperature . Set to zero with distilled water and measured the absorbance of each tube at 520 nm with 1 cm optical path quartz cuvette .		

Calculations :

T – AOC activity (U/m L) = $\frac{\Delta A}{0.01} \div 30 \times \frac{V1}{V2} \times F$

Note :

ΔA : abs. sample _ abs. control .

30 : the reaction time (minute) .

V1 : the total volume of reaction , ml .

V2 : the volume of sample added to the reaction , ml .

F : Dilution factor of sample before tested .

2.4.6. Determination of Malondialdehyde

2.4.6.1. Principle

This ELISA Kit uses the competitive-ELISA principle . The micro ELISA plate provided in this Kit has been pre-coated with MDA . During the reaction , MDA in samples or Standard competes with a fixed amount of MDA on the solid phase supporter for sites on the Biotinylated Detection Ab specific to MDA. Excess conjugate and unbound sample or standard are washed from the plate , and Avidin conjugated to Horseradish Peroxidase (HRP) are added to each microplate well and incubated . Tgen a TMB substrate solution is added to each well . the enzyme-substrate reaction is terminated by the addition of stop solution and the color change is measured spectrophotometrically at a wavelength of 450 ± 2 nm . The concentration of MDA in the sample is then determined by comparing the absorbance of the samples to the standard curve .

2.4.6.2.Reagents Preparation

1- All reagents were brought to room temperature (18 – 25 ^{o}C) before use .

2- Wash Buffer : Dilute 30 ml of concentrated Wash Buffer with 720 ml of distilled water to prepare 750 ml of Wash Buffer .

3- Standard working solution : Centrifuge the standard at 10,000 × g for 1 min . 1 ml of Reference Standard & Sample Diluent was added , let it for 10 min and invert it gently several times . After it dissolves fully , was mixed it thoroughly with a pipette . this reconstitution produces a working solution of 2000 ng / ml . Then make serial dilutions as needed . The recommended dilution gradient is as follows : 2000, 1000 , 500 , 250 , 125, 62.5 , 31.25 ng /ml . Dilution method 7 EP tubes were taken , Added 500 µL of Reference Standard & Sample Diluent to each tube . Pipette 500 µL of the 2000 ng /ml working solution to the first tube and mixed up to produce a 1000 ng /ml working solution . Pipette 500 µL of the solution from the former tube into the latter one according to this step . The illustration below is for reference . Note : the last tube was regarded as a blank . Do not pipette solution into it from the former tube .

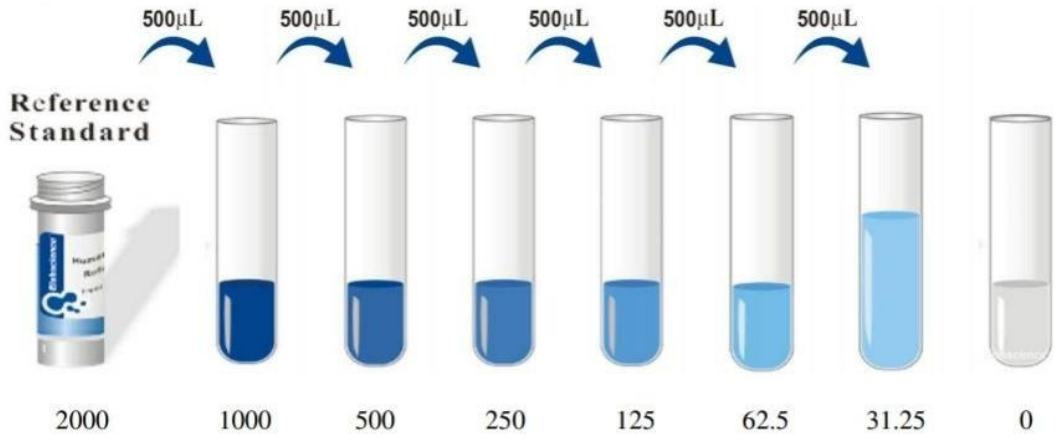


Figure 2.1: Standard MDA dilution

4- Biotinylated Detection Ab working solution : Centrifuge the Concentrated Biotinylated Detection Ab at 800×g for 1 min , then dilute the 100× Concentrated Biotinylated Detection Ab to 1× working solution with Biotinylated Detection Ab

Diluent (Concentrated Biotinylated Detection Ab: Biotinylated Detection Ab Diluent = 1:99) .

5- HRP Conjugate working solution : Centrifuge the Concentrated HRP Conjugate at 800×g for 1 min , then dilute the 100× Concentrated HRP Conjugate to 1× working solution with HRP Conjugate Diluent (Concentrated HRP Conjugate: HRP Conjugate Diluent= 1:99) .

2.4.6.3. Assay procedure

1- Determine wells for diluted standard, blank and sample. Add 50 µL each dilution of standard, blank and sample into the appropriate wells (It is recommended that all samples and standards be assayedin duplicate). Immediately add 50 µL of Biotinylated Detection Ab working solution to each well. Cover the plate with the sealer provided in the kit. Incubate for 45 min at 37℃. Note: solutions should be added to the bottom of the micro ELISA plate well, avoid touching the inside wall and causing foaming as much as possible.
2- Decant the solution from each well, add 350 µL of wash buffer to each well. Soak for 1 min and aspirate or decant the solution from each well and pat it dry against clean absorbent paper. Repeat this wash step 3 times. Note: a microplate washer can be used in this step and other wash steps. Make the tested strips in use immediately after the wash step. Do not allow wells to be dry.
3- Add 100 µL of HRP Conjugate working solution to each well. Cover the plate with a new sealer. Incubate for 30 min at 37°C.
4- Decant the solution from each well, repeat the wash process for 5 times as conducted in step 2.
5- Add 90 µL of Substrate Reagent to each well. Cover the plate with a new sealer. Incubate for about 15 min at 37°C. Protect the plate from light. Note: the reaction

time can be shortened or extended according to the actual color change, but not more than 30 min. Preheat the Microplate Reader for about 15 min before OD measurement.

6- Add 50 μL of Stop Solution to each well. Note: adding the stop solution should be done in the same order as the substrate solution.

7- Determine the optical density (OD value) of each well at once with a micro-plate reader set to 450 nm.

Assay Procedure Summary

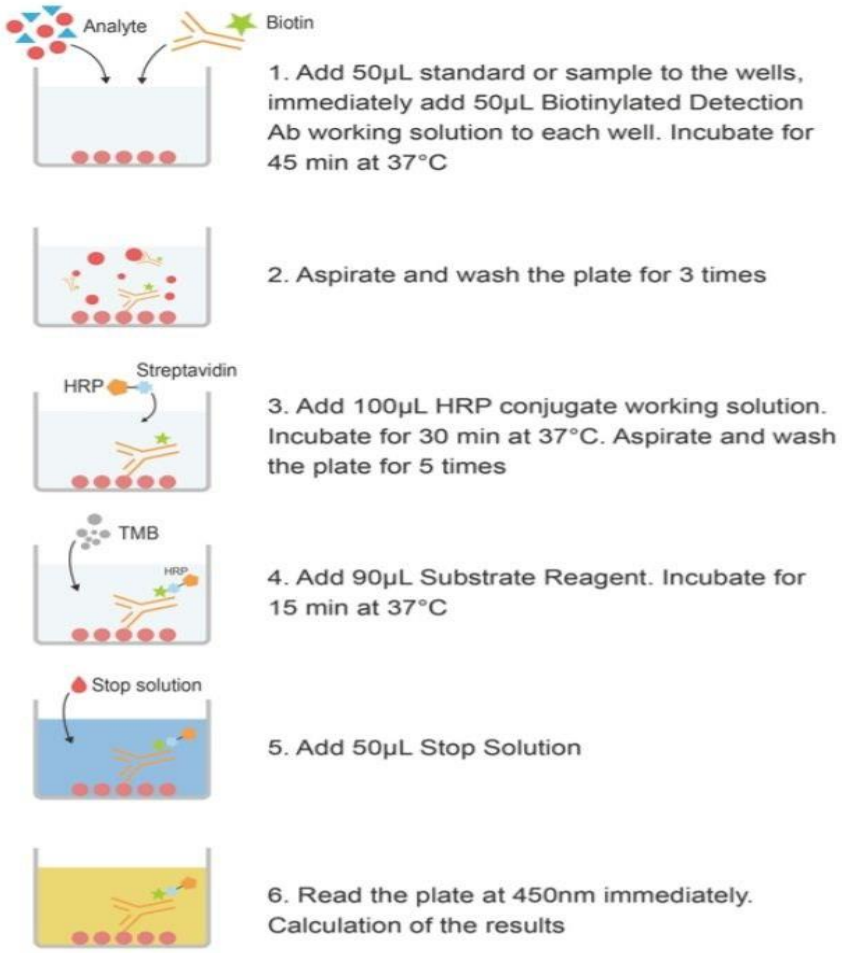


Figure 2.2: Assay Procedure Summary

Calculation of results

Average the duplicate readings for each standard and samples. Plot a four parameter logistic curve on log-log axis, with standard concentration on the x-axis and OD values on the y-axis. If the OD of the sample under the lowest limit of the standard curve, you should re-test it with an appropriate dilution. The

actual concentration is the calculated concentration multiplied by the dilution factor.

Table 2.3: Calibration Curve of MDA

Logcon. (std)	logAbs. (std)
2.69897	-0.07007
2.39794	-0.22768
2.09691	-0.47496
1.79588	-0.64397
1.49485	-0.8041

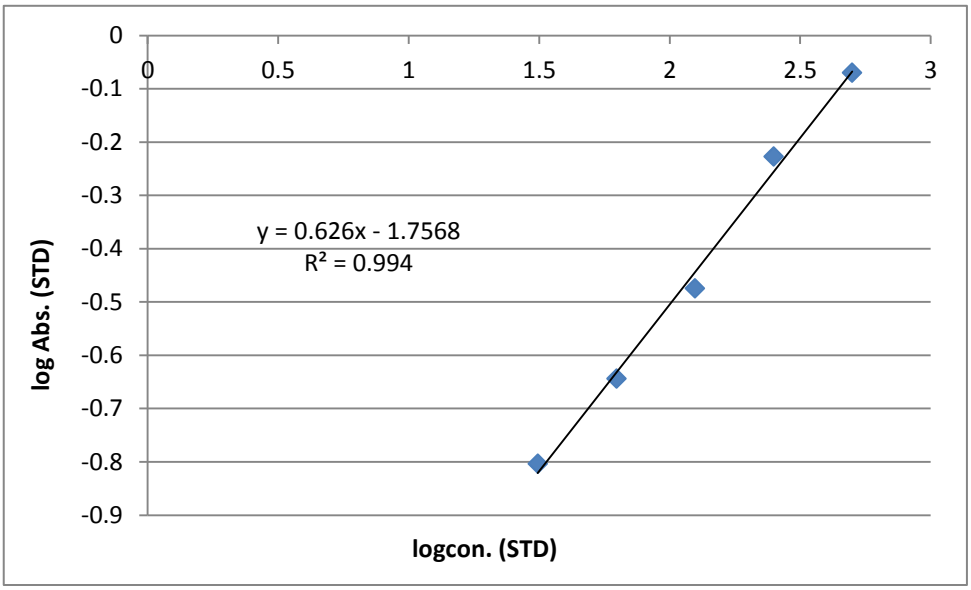


Figure 2.3: Standard curve of Malondialdehyde

2.4.7.Determination of Lead and Selenium Elements :

2.4.7.1. Preparation of Reagents , Standards , and Specimens for Analysis

2.4.7.2. Modifier Preparation (0.2% (v/v) HNO_3 , 0.5% (v/v) Triton X-100 , 0.2% (w/v) $NH_4H_2PO_4$

1- Stock solution 10% (v/v) Triton X-100 : Using a volumetric pipette, transfer 10 mL of Triton X-100 to approximately 80 mL of deionized water, warmed slightly in a glass beaker. Mix thoroughly using a magnetic stir bar for at least one hour. When cool, transfer to a 100-mL volumetric flask, and dilute to the mark. Prepare monthly and store at ambient temperature, appropriately labeled with date and analyst's initials.

2- Stock solution 20% (w/v) $NH_4H_2PO_4$: Dissolve 20 g $NH_4H_2PO_4$ in approximately 75 mL of deionized water in a glass beaker, dilute to 100 mL in a volumetric flask .

3- Modifier diluent: Add approximately 300 mL of deionized water to a 500-mL flask. Using a micropipette, carefully add 1.00 mL of concentrated HNO_3 and swirl to mix. Add 25 mL of 10% stock Triton X-100, 5 mL of 20% stock $NH_4H_2PO_4$, and dilute to 500 mL with deionized water. Mix thoroughly and transfer modifier diluent to a 500-mL opaque plastic bottle labeled with contents, concentrations, and date of preparation[170] .

2.4.7.3. Preparation of Aqueous Pb and Se Calibration Standards

Three working Pb and Se standards are prepared by serial dilution of a 1,000 mg/L primary Pb and Se standards, traceable to an appropriate reference material. The intermediate stock solutions and working standards are all maintained at 1%

(v/v) nitric acid relative to concentrated HNO_3, i.e., 1 mL concentrated HNO_3 per 100 mL standard[170].

2.4.7.4.Determination of Lead Element :

To prepare a multi-point calibration curve 100, 300, and 600 ppb Pb working standard solutions were prepared in 1% HNO_3 . The final standard solutions were prepared by mixing 100 µL each of the working standard solution and 900 µL of matrix modifier in the autosampler vessels to produce 10, 30, and 60 ppb. These standard solutions were set aside until the bubbles were dissipated. Where the calibration curve was as shown in the figure below [171].

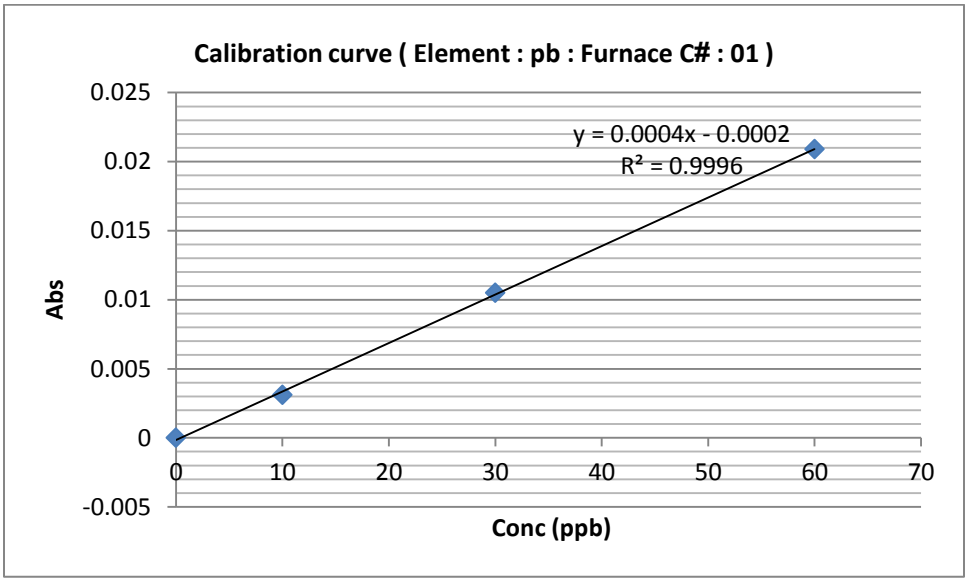


Figure 2.4: Calibration curve (Element : pb: C£ : 01)

The samples were prepared by mixing 100 µL of whole blood (with anti-coagulant) with 900 µL matrix modifier. Where the concentration of Lead in the samples was measured after measuring the standard solution based on the calibration curve[171] .

Table (2.4): Instrument and analytical conditions for Lead

Variable	Ideal condition
Lamp current	10 mA
Wavelenght	283.3 nm
Slit width	0.7 nm
Lighting mode	BGC-D2
Sample volume	20 µL
Replicates	3

2.4.7.5.Determination of Selenium Element

To prepare a multi-point calibration curve 50, 100, 200, and 400 ppb Se working standard solutions were prepared in 1% HNO_3 . The final standard solutions were prepared by mixing 100 µL each of the working standard solution and 900 µL of matrix modifier in the autosampler vessels to produce 0, 5, 10, 20, and 40 ppb. These standard solutions were set aside until the bubbles were dissipated. Where the calibration curve was as shown in the figure below [172].

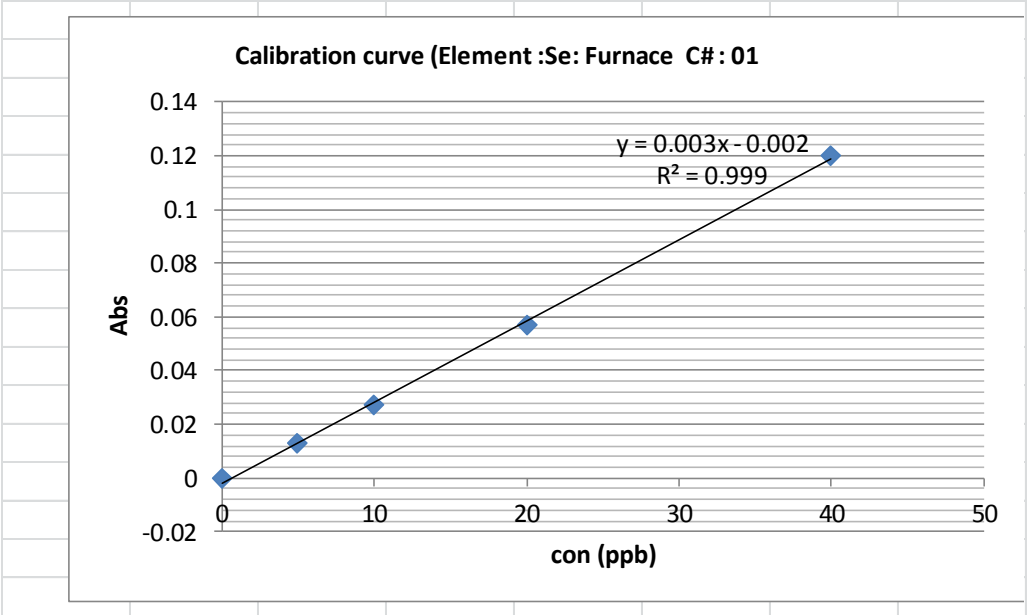


Figure 2.5: Calibration curve (Element: Se: Furnace C£: 01)

The samples were prepared by mixing 100 µL of whole blood (with anti-coagulant) with 900 µL matrix modifier. Where the concentration of Lead in the samples was measured after measuring the standard solution based on the calibration curve [171].

Table (2.5): Instrument and analytical conditions for Selenium

Variable	Ideal condition
Lamp current	23 m A
Wavelength	196.0 nm
Slit width	0.5 nm
Lighting mode	BGC-D2
Sample Size	20 µl
Replicates	3

2.4.8.Statistical analysis :

In this study, the statistical package for the social sciences (SPSS) version 21 was used to compute the data using Microsoft Excel 10. Mean plus standard deviation (SD) was employed to represent descriptive statistics, and the T-test was utilized to determine if the patient and control groups differed. The relationships between each variable were assessed using Pearson's correlation coefficient[173]. . Differences that were judged significant were those with $p \leq 0.05$ and more was non-significant.

CHAPTER THREE:

RESULTS

&

DISCUSSION

3-Results and discussion

The study included 77 person age from (17-72) years old , They were classified into two groups 45 as a patient group and 32 apparently healthy as a control group patients gave written informed .

The patient group can be investigated by some criteria including serum creatinine , blood urea and e GFR .

3.1.Comparison in biochemical parameters between groups

For the comparison between studied groups used independent T-test, and the results were expressed as mean ± standard deviation (SD) and extraction P-value to show the difference variation , considered significant if P-value are $P \leq 0.05$, whereas $P > 0.05$ is non-significant while $P \leq 0.01$ is highly significant .

Table 3.1 comparison of S Cr , B Ur , e GFR , BMI , TCh , TG , HDL , MDA , TAC , BLL , Se conc.in patients and control groups.

Table 3.1: Clinical characteristics of CKD with control subject.

Parameters	Patients group N=45 Mean±SD	Control group N=32 Mean±SD	P-value
Age (years)	47.40±12.63	44.59±13.41	NS
BMI (kg/m^2)	26.95±6.98	27.39±5.05	NS
S Cr (mg/dl)	8.86±3.61	0.95±0.26	HS
B Ur (mg/dl)	111.10±39.32	26.06±8.32	HS
e GFR (ml/min/1.73m^2)	7.09±8.10	82.78±21.87	HS
T C (mg/dl)	317.40±89.22	185.44±48.48	HS
T.G (mg/dl)	117.42±29.30	132.34±47.14	NS
HDL (mg/dl)	44.89±10.18	37.50±4.91	HS
Non-HDL-C	271.52±81.33	147.94±45.96	HS
VLDL (mg/dl)	23.48±5.86	26.46±9.42	NS
LDL (mg/dl)	249.02±78.78	121.46±42.82	HS
TAC (U /ml)	8.75±2.26	15.36±2.93	HS
MDA (ng/ml)	13.97±1.95	6.55±4.08	HS
Conc. Se (µg/dl)	5.00±0.92	14.19±1.23	HS
BLL (µg/dl)	24.38±9.06	14.14±4.04	HS

NS: t-test P-value ≥0.05 ; S: P-value ≤0.05 ; HS: P-value ≤0.01 .

According to the presented data the mean of BLL (24.38±9.06) (14.14±4.04) patients and control groups respectively which is significantly increased in patients group of CKD (P<0.01). and this result agreed with the results of a study that found the kidneys excrete roughly 75% of all lead in a person with normal renal function, while the rest is removed through the gastrointestinal tract. Lead poisoning, on the other hand, can arise when a person's ability to remove the metal is exceeded[174] while , another study it showed that Anemia, renal failure, and brain impairment are all clinical symptoms of lead toxicity that can be assessed clinically and followed up with lab tests [175] . Results of current study was found e GFR (7.09±8.10);(82.78±21.87) for patients and control groups

respectively were significantly decreased in CKD than in control group ($p<0.001$) while, that found the mean of serum creatinine (8.86±3.61);(0.95±0.26) and blood urea (111.10±39.32);(26.06±8.32)for patients and control groups respectively were significantly increased in patients group of CKD than in control group ($p<0.001$) this result agrees with study of Gerhardsson *et al* that found when Chronic exposure to high lead concentrations damages the glomerulus of the kidney, causing it to lose its capacity to selectively filter high molecular weight proteins. High molecular weight proteins like albumin and macroglobulin are excreted more often in the urine as a result of this[176].

According to results in table 3.1 There was statistically non-significant changes in (age , BMI, T.G and VLDL) means values between the normal control group and the patients group of CKD .This results agrees with studies that found drugs, the uremic state, the dialysis procedure, and these patients' underlying medical conditions can all result in alterations in trace element levels. dialysis water quality, as well as anemia-related to lessen underlying illness and erythropoietin levels mechanisms, and metabolic changes related to renal disease failure[177,178].

In the presented data explained by figure 3.1 showed the mean of e GFR (ml/min/1.73m^2) decreased in CKD patient than control group , while figure 3.2 showed the mean levels of BLL (mg/dl) increased in CKD patients group than control group , and so is the case for the means levels of S Cr (mg/dl) and B Ur (mg/dl) increased in CKD patients than control group as shown in the figures 3.3 ; 3.4 respectively .

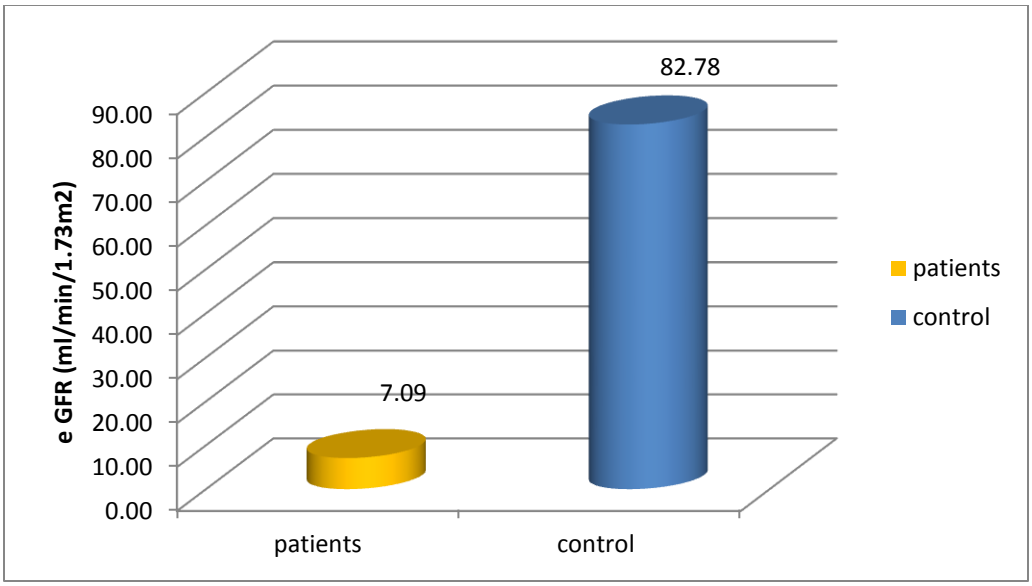


Fig 3.1: The levels of e GFR in patients group of CKD versus controls subject

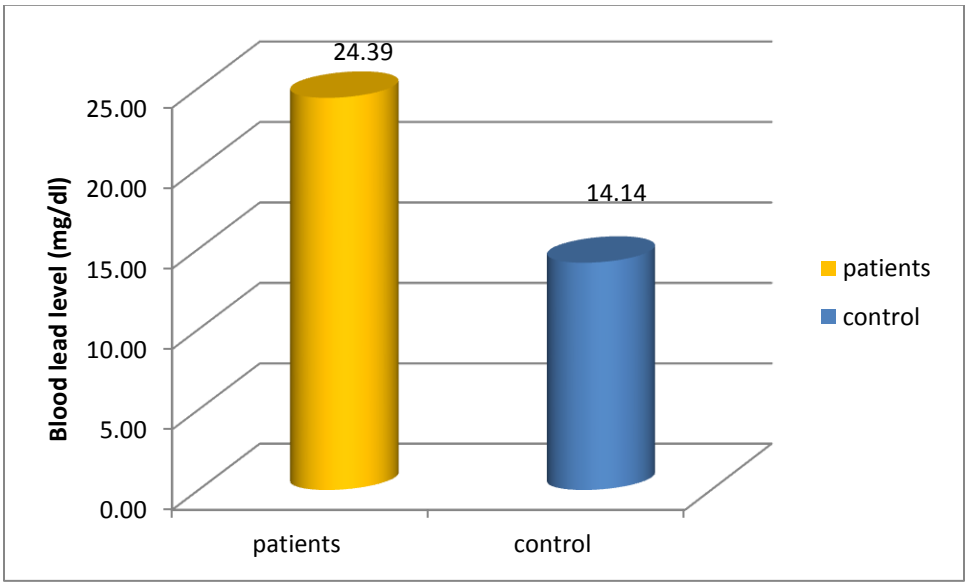


Fig 3.2 : The levels of BLL in patients group of CKD versus controls subject

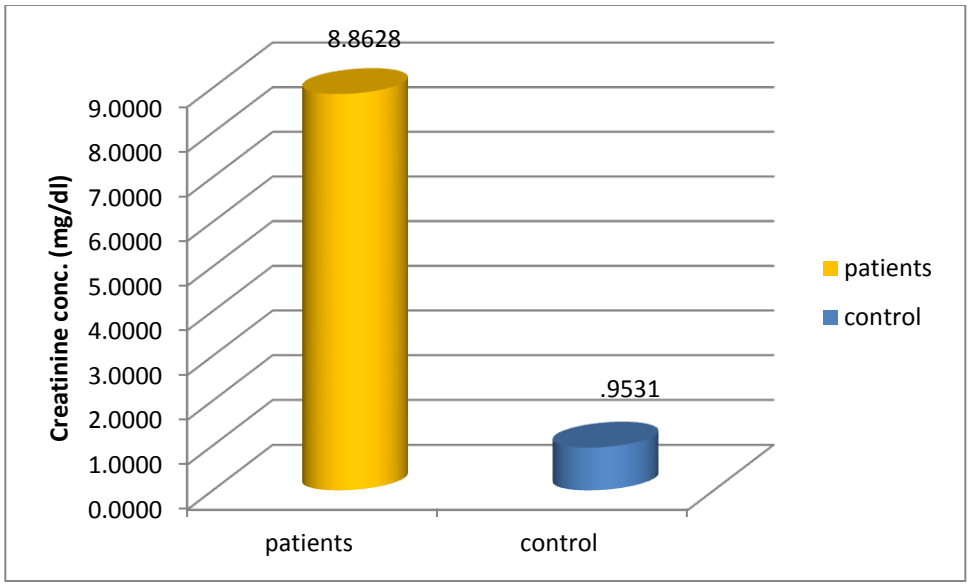


Fig 3.3 : The levels of S Cr in patients group of CKD versus controls subject

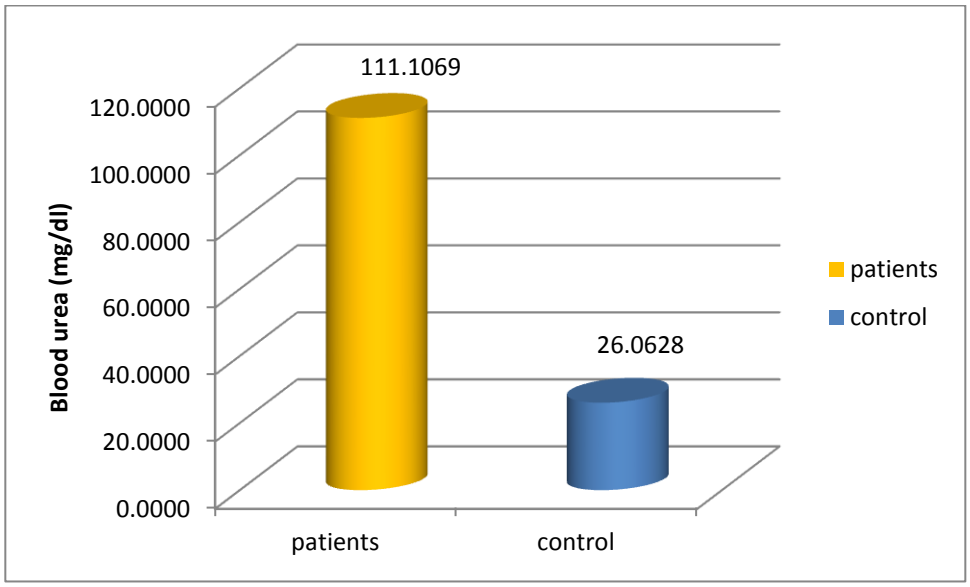


Fig 3.4 : The levels of B Ur in patients group of CKD versus controls subject

According to the presented data the means of TC, Non-HDL-C, and LDL (317.40±89.22) (185.44±48.48);(271.52±81.33) (147.94±45.96);(249.02±78.78) (121.46±42.82) patients and control groups respectively which are significantly increased in patients group of CKD ($P<0.001$), while the means of T.G and

VLDL (117.42±29.30) (132.34±47.14); (23.48±5.86) (26.46±9.42) patients and control groups respectively which are non-significantly decreased in patients group of CKD (P>0.01), And the mean of HDL (44.89±10.18) (37.50±4.91)patients and control groups respectively which is significantly increased in patients group of CKD (P<0.001), And the rationale for the increase in average HDL in patients is that they are taking a statin medication. This agrees with other result of studies that found, a 17 (mg/dl) rise in HDL-C concentration was linked to a 0.8 percent increase in e GFR (P=0.004) and a decreased probability of e GFR 60 (ml/min.1.73m^2) ((P<0.001) in chronic kidney disease patients. There was no evidence of a link between LDL-C, T.G levels, and any measure of renal disease[179,42]. After controlling for confounding variables, researchers discovered that HDL-C is still linked to renal dysfunction, but that LDL-C, TG, TC, apoA1 and apoB have no link to renal dysfunction[180]. Previous research has shown that HDL-C has ant-inflammatory, antithrombotic properties, and antioxidant, which could help to prevent atherosclerosis in renal and other arteries. It could also protect renal functions[181,43].

Malondialdehyde (MDA) is a lipid peroxidation (LPO) product that can occur when lead is present. Many studies have found that there has been an upsurge in MDA levels were linked to greater BLLs, implying an increase in LPO as a result of ROS[182].

According to results in table 3.1 The mean of MDA (13.97±1.95);(6.55±4.08) in patients and control groups respectively which is significantly increased in patients group of CKD when compared with healthy control (P<0.001) and this result agreed with the results of a study by Patil and *et al* who found the serum MDA content was significantly increased (P<0.001) and the activities of

antioxidant enzymes were significantly reduced ($P<0.001$) in battery manufacturing workers[183]. Other studies shows that MDA levels were frequently used biomarker for measuring lipid peroxidation LPO which found MDA levels to be significantly higher in lead exposed groups than in the control groups[184,185].

The results of TAC and conc. Se highly significant decreased in patients with CKD when compared with healthy control, this agreed with the result of other studies that found Pb-induced abnormalities in the antioxidant defense system are to blame for the increased amounts of ROS. Pb decreases the activity of antioxidant enzymes TAC[186,187]. And its agreed with the result of a study by Wu and ***et. al*** who found the red blood cell lead and cadmium were significantly correlated with the increased ROS for CKD . In contrast, the levels of plasma selenium were negatively related to the ROS of CKD[188]. Many studies have shown the levels of dietary lead are significantly associated diabetes or prediabetes and hypertension, However they found an interesting result that regardless of whether CKD had comorbidity (i.e., diabetes or hypertension) blood lead level was significantly increased and plasma selenium levels was significantly decreased as compared to the control group[8,190] .

Many epidemiological and animal research have revealed that Pb can generate oxidative stress and alter numerous enzymatic and non-enzymatic components of antioxidant defense. Pb's potential to generate oxidative stress in the blood could be due to a number of ways. Pb, like many other hazardous metals, binds to enzymes' functional –SH groups, rendering them nonfunctional and suppressing their activity by generates the production of H2O2, O2, OH and ROS[191,192] . The ability of Pb to replace elements that serve as important co-factors of these enzymes can cause increases in their activity as a result of increased ROS production, while their suppression can be explained by the

ability of Pb to replace elements that serve as important co-factors of these enzymes[193].

In the presented data explained by figure 3.5 showed the mean of TAC (U/ml) decreased in CKD patient than control group , while figure 3.6 showed the mean of MDA conc. (ng/ml) increased in CKD patient than control group , and so in the case for the mean levels of Se (mg/dl) decreased in CKD patient than control group asshown in the figure (3.7) .

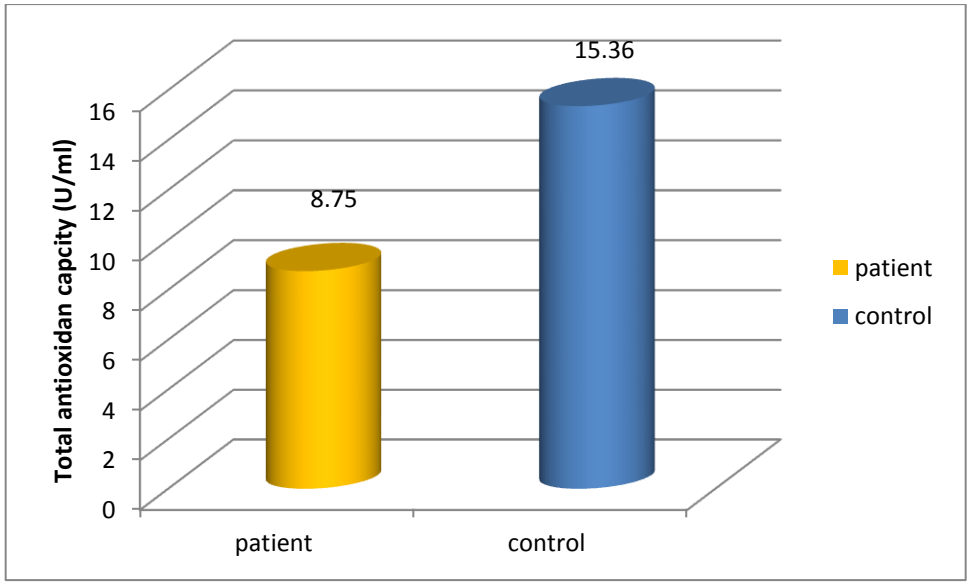


Fig 3.5 : The levels of TAC in patients of CKD versus control subject

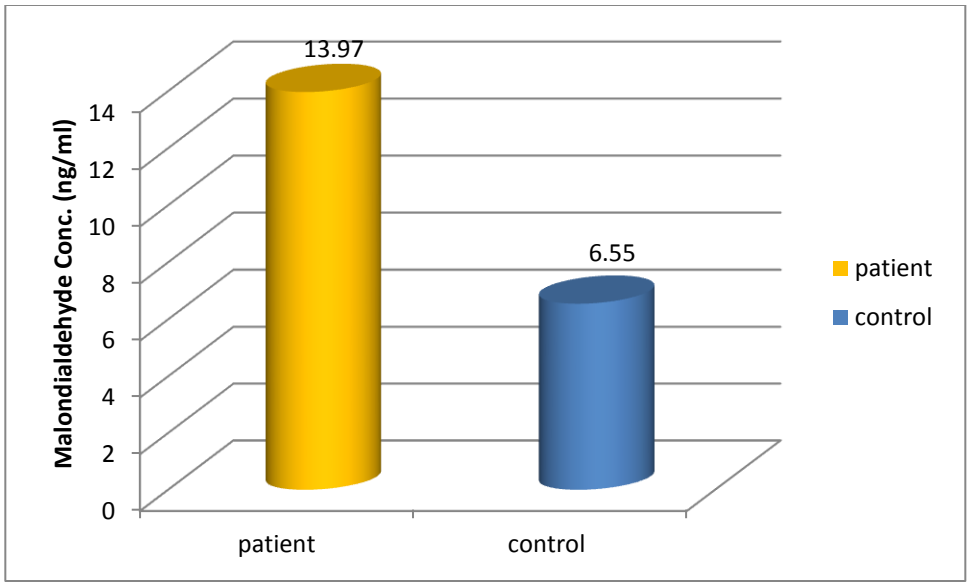


Fig 3.6 : The conc. of MDA in patients of CKD versus control subject

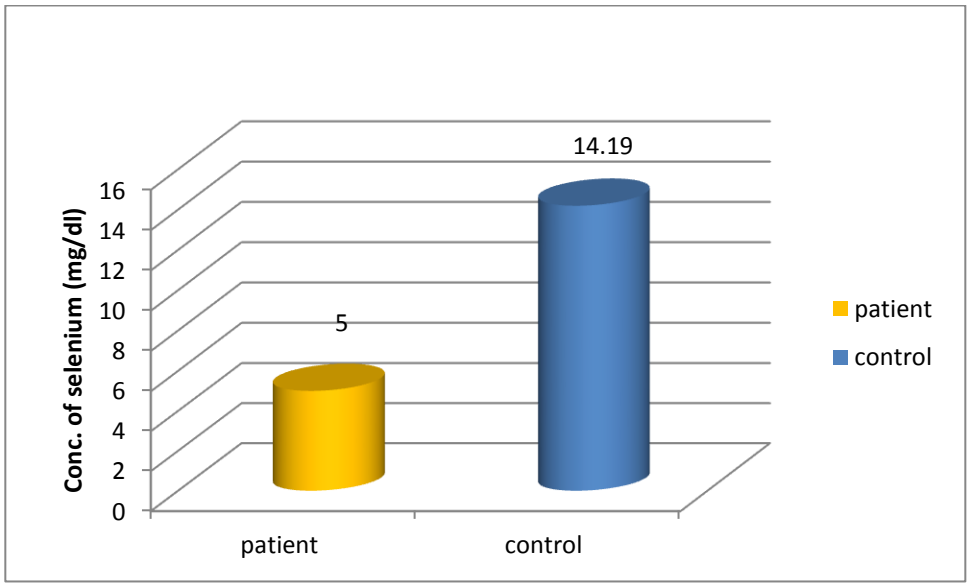


Fig 3.7 : The conc. of selenium in patient of CKD versus control subject

3.2. Correlation Study

Correlation study between blood lead levels and clinical characteristics were estimated by using Pearson ‘s correlation coefficient (r) for the evaluation of data .

As shown in table (3.2) If the value of the correlation is negative sign , then this

indicates the existence of an inversed relationship between the variables (negative correlation), while the positive sign indicates direct proportion (positive correlation) [173].

Table 3.2 : Correlation between BLL and other clinical characteristics.

Clinical parameters	BLL	
	r	P-value
B Ur	0.376**	0.001
S Cr	0.399**	0.000
e GFR	-0.463**	0.000
MDA	0.546**	0.000
TAC	-0.722**	0.000
C Se	-0.559**	0.000

r: Pearson's correlation coefficient

-significant correlation P≤0.05 ; **-highly significant P<0.01

There is a highly significant positive correlation between BLL and blood urea, serum creatinine P≤0.001. While BLL has a negative correlation with e GFR P<0.001. This results agreed with studies[188,194,195].

Previous epidemiologic study in vulnerable adult populations, such as those with CKD or hypertension, indicated that low-level ambient lead exposure was inversely related with kidney function in cross-sectional and prospective analyses. Even in the absence of other comorbidities, lead exposure at levels common in developed countries is linked to poor kidney function[196].

The figures (3.8- 3.10) show the correlation between blood lead level and blood urea and serum creatinine.

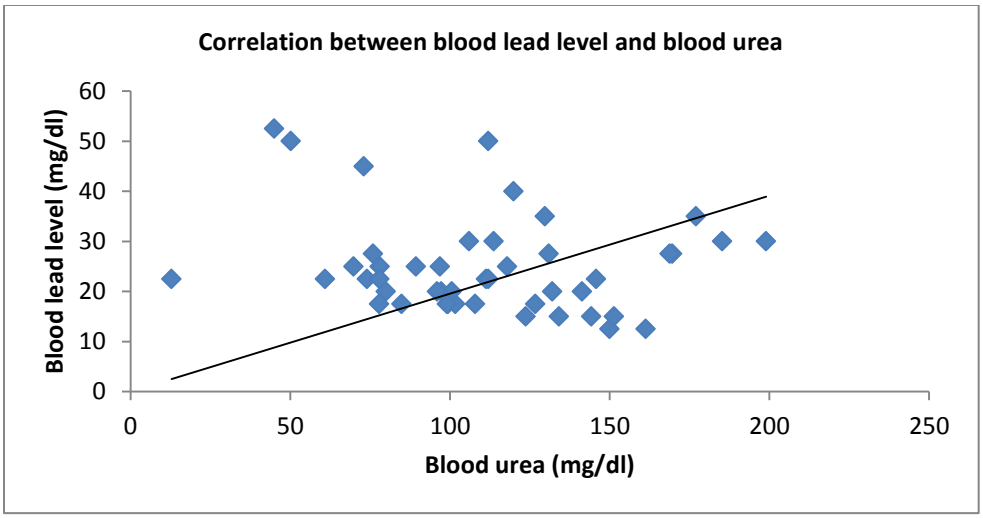


Figure 3.8: Correlatin between blood lead level and blood urea

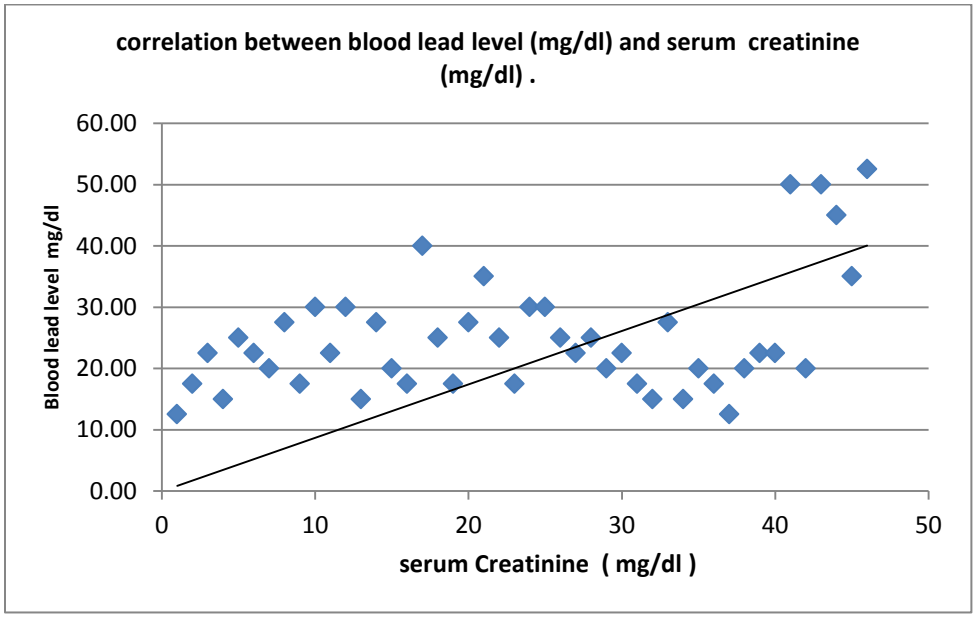


Figure 3.9: Correlation between blood lead level and serum creatinine

As well as, there was a positive correlation between BLL and MDA figure (3.10) this result agreed with studies by Kasperczyk *et al* and Singh *et al* which appear BLL had positive correlation with MDA (r=0.15-0.80; p<0.05)[197–199] . Lead has been demonstrated in various studies to cause oxidative stress and

damage by creating reactive oxidative species such as superoxide anion (O_2 •), hydrogen peroxide (H_2O_2), hydroxyl radical (•OH), and singlet oxygen ($1O_2$)[143,200–202], which could be the cause of the damage in patients that observed in this study.

BLL correlated negatively with TAC and selenium conc. P<0.001 as shown in figurer (3.11). This results agreed with other studies[197,203,204].

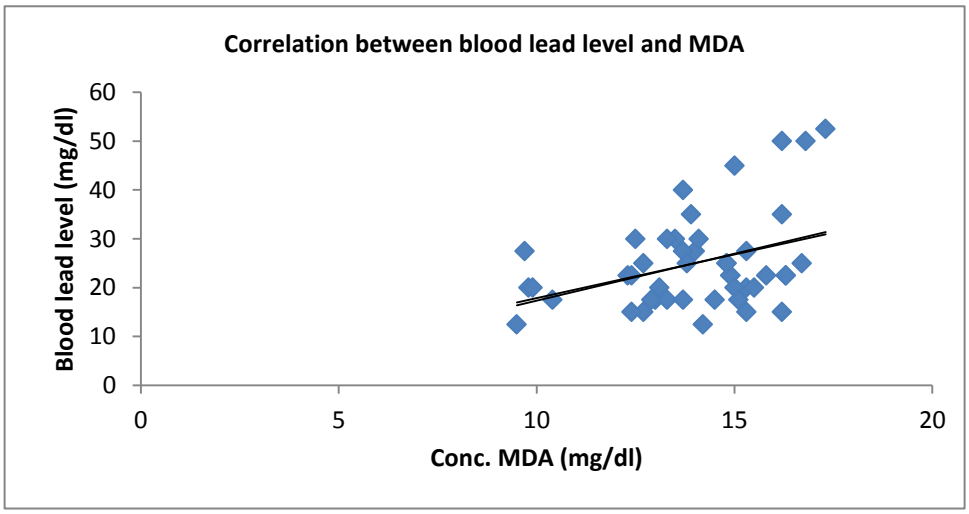


Figure 3.10: Correlation between blood lead level and malondialdehyde

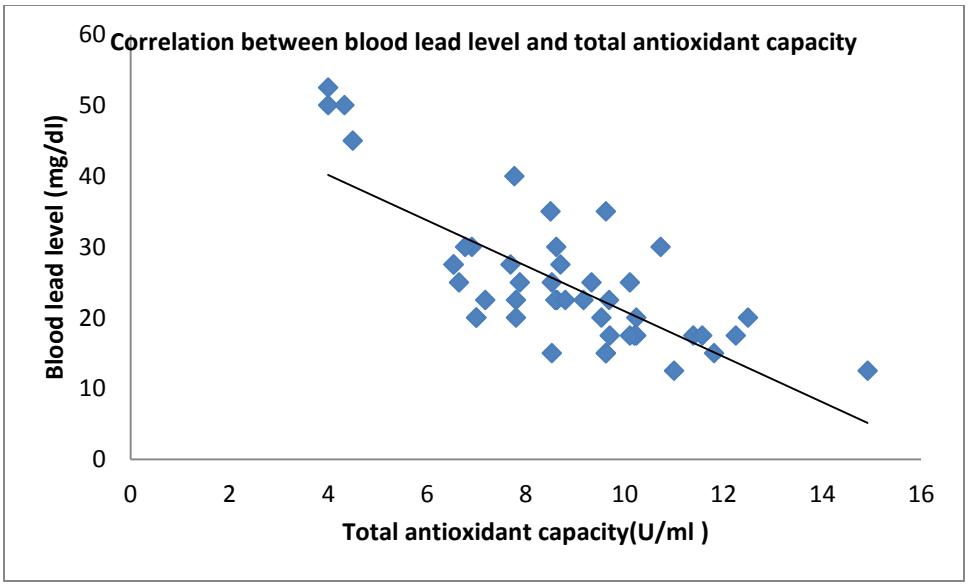


Figure 3.11: Correlation between blood lead level and total antioxidant capacity

CHAPTER FOUR:

CONCLUSION

&

RECOMMENDATION

4.Conclusion and Recommendation

4.1.Conclusion

In conclusion, this study confirmed that :

1- Low eGFR in CKD especially in those with elevated blood lead levels.

2- The rise in lead levels in the blood leads to a decrease in antioxidants and an increase in oxidants in patients, so it is involved in the development of chronic kidney disease.

3- Increased BLL levels were a major risk factor for CKD and may be clinically meaningful.

4- The high level of lead and low level of selenium in the blood of patients with chronic kidney disease have a relationship with oxidative stress, kidney failure and oxidative stress through a decrease in the level of antioxidants (TAC) and an increase in the final product of MDA.

4.2. Recommendation and Future work

- Future follow-up studies are necessary to clarify the causal relationships between heavy metals, proteinuria and CKD.
- In order to phase out lead sources from the environment, appropriate measures are needed to prevent exposure from lead sources such as batteries, electrical and electronic waste, and other waste containing lead.
- Regular monitoring of these patients is recommended once or twice a year.
- Clinical study to investigate blood lead levels in chronic kidney disease patients with and without hypertension.
- Study of the combined effect of lead and cadmium levels in the blood of patients with chronic kidney disease.

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الخلاصة

ضمنت الدراسة تقدير مستويات الرصاص في الدم (BLL) في مصل مرضى الكلى المزمنة ومقارنتها مع المجموعة الضابطة ، تقدير الرصاص في الدم كعنصر سام وارتباطه بتطورمرض الكلى المزمنة بين عينة من السكان العراقيين وللتحقق من العلاقة بين مضادات الأكسدة والإجهاد التأكسدي ومستويات الرصاص في الدم وتأثيرها على اختبارات وظائف الكلى التي يمكن رؤيتها في مرضى الكلى المزمن.

شملت دراسة الحالات والشواهد 77 شخصًا ، تراوحت أعمارهم بين (17-72) عامًا ، تم تشخيص 45 منهم بمرض الكلى المزمن ، بينما تم تشخيص 32 آخرين على أنهم أصحاء. تم جمع العينات من ديسمبر 2021 حتى مايو 2022.

تظهر نتائج هذه الدراسة أن متوسط BLL (mg / dl) كان زيادة كبيرة في المرضى الذين يعانون من مجموعة CKD مقارنة بمجموعة السيطرة ($p<0.001$) ، ووجدت أن الارتفاع الكبير في المؤشرات الحيوية SCr، B Ur، TC، HDL و Non-HDL-C و LDL و MDA في مجموعة المرضى مقارنة بالمجموعات الصحية في $p<0.001$ ، ووجدوا أن هناك تناقصًا كبيرًا في e GFR و TAC و Conc. Se في مجموعة المرضى مقارنة بالمجموعات الصحية في $p<0.001$ ، ووجدت أن غير مهم في العمر ، مؤشر كتلة الجسم ، TG ، و VLDL في مجموعة المرضى مقارنة بالمجموعات الصحية في $p\geq0.05$. كما يوجد ارتباط معنوي موجب بين BLL و B Ur و S Cr و MDA $p\leq0.001$ ، كما أن هناك علاقة ارتباط معنوية سالبة بين BLL و e GFR و TAC وConc Se. $p<0.001$.

من نتائج هذه الدراسة نستنتج إلى أن ارتفاع مستويات الرصاص في الدم يؤدي إلى انخفاض مضادات الأكسدة وزيادة المواد المؤكسدة لدى المرضى ، ويرتبط ذلك بانخفاض معدل الترشيح الكبيبي في المرضى المصابين بمرض الكلى المزمن ، ولذلك فهو يساهم في تطور الأمراض المزمنة. كانت أمراض الكلى ، وزيادة مستويات BLL عامل خطر رئيسي لمرض الكلى المزمن وقد يكون ذا مغزى سريريًا ، كما أن ارتفاع مستوى الرصاص وانخفاض مستوى السيلينيوم في دم المرضى المصابين بأمراض الكلى المزمنة له علاقة بزيادة مخاطر الإجهاد التأكسدي ، الفشل الكلوي ومن خلال انخفاض مستوى مضادات الأكسدة (TAC) وزيادة المنتج النهائي لـ MDA.

جامعة كربلاء

العلاقة بين تسمم بالرصاص و مرض الكلى المزمن في عينة من السكان العراقيين

رسالة مقدمة الى مجلس كلية التربية للعلوم الصرفة / جامعة كربلاء , كجزء من متطلبات نيل شهادة الماجستير في علوم الكيمياء

كتبت بواسطة

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