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Serum 4-Hydroxy-2-nonenal and Induced nitric oxide Synthase in Hypertension and Hypertension with kidney disease Patients

A Thesis

Submitted to the College of Science Al-Nahrain University as partial Fulfillment of the Requirements for the Degree of Master in Chemistry/(Biochemistry).

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بِسۡمِ ٱللَّهِ ٱلرَّحۡمَٰنِ ٱلرَّحِيمِ

﴿إِنَّا فَتَحْنَا لَكَ فَتْحًا مُّبِينًا (1) لِّيَغْفِرَ لَكَ اللَّهُ مَا تَقَدَّمَ مِن ذَنبِكَ وَمَا تَأَخَّرَ وَيُتِمَّ نِعْمَتَهُ عَلَيْكَ وَيَهْدِيَكَ صِرَاطًا مُّسْتَقِيمًا (2) وَيَنصُرَكَ اللَّهُ نَصْرًا عَزِيزًا (3)﴾

سورة الفتح:1-3

Dedication

To The Realest And Purest Love In The World

Mother

And To All My ***Family***

And to my country ***Iraq***

And to ***Iraqi soldiers****, those who are far from as now but keeping us continue life*

Ali Dhafer

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Summary

Hypertension (HT) And related diseases such as chronic kidney disease(CKD) share in that one of the main reasons for them is to increase the oxidative stress, which in turn increases the severity of the disease and exacerbation of symptoms. Reactive molecules produced from oxidative stress, in addition to causing tissue damage by oxidation of biomolecules like DNA, lipids, proteins and sugars; they are lead to the formation of mediators with potent inflammatory effect. The objective of this study was to investigate some markers of oxidative stress in hypertension (HT) and HT with CKD patients in addition to some biochemical parameters related to these diseases.

This study involved 84 male subjects aged between (25-60) year equally divided into three groups, first and second one belong to HT and HT with CKD patients respectively from Al-yarmouk Teaching hospital, while the third one for apparently healthy 28 subjects considered as control group. For each subject in the three groups these markers and parameters were evaluated; 4-hydroxy-2-nonenl(4HNE), induced nitric oxide synthase(iNOS), albumin, urea, creatinine ,total serum protein.

The results were compared to control; There was a significantly higher ($p<0.01$) in 4HNE, and iNOS levels in both HT and HT with CKD patients, while serum albumin and Total serum protein shows significantly ($p<0.01$) lower levels in both groups. The elevation levels of oxidative stress markers may be due to oxidative damage of tissues that caused by these inflammatory diseases. Was concluded that there was a positive relation between oxidation results from these diseases and their developments and suggest increase need to intake of antioxidants as precaution in front of these disease.

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Abbreviations

Ang II	Angiotensin II
BMI	Body Mass Index
BP	*Blood pressure*
cGMP	cyclic guanosine monophosphate
CKD	*chronic kidney disease*
CV	Cardiovascular
DNA	Deoxyribonucleic Acid
eNOS	Endothelial NO-Synthase
FAD	flavin adenine dinucleotide
FMN	Flavin mononucleotide
GWAS	Genome-wide association studies
H_2O_2	Hydrogen Peroxide
HSA	Human Serum Albumin
4HNE	*4-hydroxy-2-nonenal*
HT	Hypertension
iNOS	Indused nitric oxide synthase
KGDH	α-ketoglutarate dehydrogenase
$LOO^{\cdot}$	Lipid Peroxy Radical
NADH	Nicotinamide Adenine Dinucleotide
NADPH	Nicotinamide Adenine Dinucleotide phosphate
nNOS	neuronal nitric oxide synthase
NO	Nitric oxide
NOS	Nitric oxide synthase
$O_2^{\cdot}$	Superoxide

$OH^{\cdot}$	Hydroxyl Radical
ONOO	Peroxynitrite
PCR	Polymerase Chain Reaction
PDH	*pyruvate dehydrogenase*
RNS	Reactive Nitrogen Species
ROS	Reactive Oxygen Species
sGC	soluble guanylyl cyclase
SNP	Sentinel blood pressure
SOD	Superoxide Dismutase

Chapter one

Introduction

&

Literature Review

1.1 Hypertension:

1.1.1Definition of Blood Pressure :

Blood pressure is a measure of the force exercised circulation on the walls of the major arteries (1), and the pressure wave that moves along the arteries with each heartbeat and felt easily and pulse, is created higher (systolic) pressure from the measured contracting heart and lower (diastolic) pressure also fills the heart (2). The systolic pressure happens when the left ventricle is most contracted; the diastolic pressure occurs when the left ventricle is most relaxed prior to the next contraction. The normal blood pressure within the range of 100–140 mmHg systolic and 60–90 mmHg diastolic(3,4)

1.1.2 Definition of Hypertension :

Hypertension (HT), also known as high blood pressure or arterial hypertension, is a chronic medical case in which the blood pressure in the arteries is elevated. Hypertension is present if the blood pressure is constantly at or higher 140/90 millimeters mercury (mmHg) for most adults; different criteria apply to children.(3)

1.1.3 Symptoms of Hypertension:

Hypertension is a disease which in its early stages is almost without obvious symptoms it's identification is usually through screening. This symptoms includes a headache, heavyweights in the head, movement is slowly, a redness and warm to touch feeling the body, swelling and strained vessels, fullness of the pulse, swelling of the skin, colored and dense urine, Anorexia , weakness eyesight, impairment of thinking, yawning, lethargy, rupture the vascular , and stroke(5,6).

1.1.4 Hypertension Causes

Primary hypertension

Results of high blood pressure from a complex interactions between a genes and the environmental factors. It was a lot of common genetic variants to identify with small effects on blood pressure (7), as well as some genetic variants rare with large effects on blood pressure .Also, GWAS have identified 35 genetic positions related to blood pressure; it has been found on 12 of these genetic spatial affecting newly blood pressure (8). Sentinel each SNP positions and has shown a new genetic identification of the relationship with the DNA in several CPG sites nearby. This goalkeeper SNP is located inside the genes associated with vascular smooth muscle and kidney function. DNA can affect in somehow linking common variations in the genes to an alternative multiple phenomena, although the mechanisms underlying these associations are not understood. It showed variable single test carried out in this studying for 35 sentinel SNP (known and unknown) this genetic variants individually or in total share of the risk of clinical phenomena associated with hypertension(8).

Blood pressure increases with age and the risk of becoming hypertensive in life at a later time not too bad. There are several environmental factors that affect blood pressure A high percentage of salt increases blood pressure in salt individuals with sensitive . Lack of exercise, stress, obesity, (9) and gloom it can play a role in individual cases (10). It is believed that insulin resistance, which is common in obesity, a component of (metabolic syndrome), as well as contributing to the high blood pressure. events in early life, like low birth weight, maternal smoking pregnant women, lack of breast-feeding may be a risk for hypertension adults factors, although the mechanisms linking these exposures to high blood adult pressure is still not clear (11).

Secondary hypertension

Results of secondary high blood pressure from a specific reason. kidney disease it is the most common cause of secondary hypertension, it is also possible be caused by high blood pressure because of endocrine conditions, such as hyperthyroidism, hypothyroidism, acromegaly, hyperthyroidism and pheochromocytoma. (12.13) other reasons for secondary hypertension include sleep apnea, pregnancy, narrowing of the aorta, and excessive consumption of licorice and some drugs, herbaceous remedies and illegal drugs. (12.14) It was found exposure to arsenic via drinking water to bind with high blood pressure (15,16).

1.1.5Risk Factor of Hypertension(17)

*Age. The risk of hypertension raises as your aged. during early middle age, or almost age 45, hypertension is most common in men. Women are more likely to develop high blood pressure after reaching the age of 65.

*Race. hypertension is especially common among blacks, mostly developing in an earlier age much more than it does in whites. Serious complications, likes stroke, heart attack and kidney failure, also it's more common in blacks.

* Family history. hypertension tends to run in families. Genetic factors likely play some role in high blood pressure, heart disease, and other related conditions. However, it is also possibly that people with a family history of hypertension share common environments and other potential factors that increase their risk.

* Overweight or obese. The more weight you need more blood to supply oxygen and nutrients to your tissues. As the volume of blood circulation through blood vessels increases, so does the pressure on the walls of the arteries.

* Not to physical activity. People who are inactive tend to have highest heart rates. The higher your heart rate, the harder your heart should works with each systole and the more powerful the force on your arteries. physical inactivity also increases the risk of being obese .

* smoking . Not limited on smoking or chewing tobacco immediately increase your blood pressure temporarily, but the chemicals in tobacco products can damage the lining of your walls artery . This may cause narrow in your artery increasing your blood pressure.

* A lot of salt (sodium) in your diet. A large amount of sodium in your diet can cause your body to keep fluid, and this increases blood pressure.

* Very little potassium in your diet. Potassium helps for the balance the amount of sodium in your cells. If you don't take enough potassium in your diet or keep enough potassium, you may accumulate a large amount of sodium in your blood.

* specific chronic disease. specific chronic conditions also may raises your risk of hypertension, like kidney disease, diabetes and sleep apnea occasionally pregnancy contributes to hypertension.

High blood pressure (BP) is one of the main factors causing the disease, which contributes to the deterioration of kidney function. The presence of kidney disease is a medical reason by the subscriber to find and underappreciated from high blood pressure-resistant.(18) Therefore, treatment of hypertension has become the most important intervention in the management of all forms of chronic kidney disease (CKD).

The prevalence of chronic kidney disease (CKD) continues to increase worldwide as does end stage kidney disease. The most common, but not only, causes of CKD are hypertension and diabetes. CKD is linked with a significant increase in cardiovascular (CV) risk as most patients with CKD die of a CV cause. Moreover, CV risk increases in proportion to the decline in estimated glomerular filtration rate falls below 60 mL/min. CV causes of death in CKD are more common than those from cancer; as a result, the identification and reduction of CKD is a public health priority. Hypertension is a key pathogenic factor that contributes to the deterioration of kidney function(19). The kidney is both a cause and a victim of high blood pressure.

1.2Chronic kidney disease:

1.2.1 Difenition:

Chronic kidney disease, or CKD,(also called chronic kidney failure)(20) is a condition that affects the function of the kidney, which can progress over time to kidney failure. When the kidneys fail,dialysis or a kidney transplant is needed to support life, many diseases can causeCKD. The most common are diabetes and high blood pressure. High Blood pressure puts more pressure on the blood vessels throughout the body, including the kidney filters (nephrons). Hypertension is the number two cause of kidney failure (21).

1.2.2Symptoms of kidney disease:

Symptoms of chronic kidney disease development with time if kidney damage evolves slowly. symptoms of kidney disease may include:

- Vomiting
- Nausea
- Anorexia
- stress and weaknesses
- Trouble sleeping
- The changes in urine output
- Decline in mental sharpness
- Muscle cramps and spasms
- Hiccups
- Swelling of the feet and ankles
- Persistent itches
- Pain in Chest , if fluid builds around the lining of the heart
- Shortness of breathing, if fluid builds up inside the lungs

• High blood pressure that's difficult to control

The symptoms of kidney disease are often nonspecific, and that is meaning they can also be caused by other diseases. And that because the kidneys are highly adaptable and able to compensation for lost function, the symptoms may not appear until irreversible damage has occurred (22,23).

1.3 Oxidants & Antioxidant

1.3.1 Free Radicals :

A free radical is defined as any atom or molecule that contains unpaired electrons and has independent existence(hence, the term free).It uses a point to represent the types of free radicals. The abstraction or gain of one electron by a non radical molecule may (or may not) convert it to a radical species. Free radicals can have positive, negative, or neutral charges [reactions (1:3:1:1) and (1:3:1:2)].(24)

A → minus one electron → A+• (1:3:1:1)

B→ plus one electron → B−• (1:3:1:2)

A classic reaction is the radiation that is caused by homolysis of water yielding hydroxyl radical (•OH) and a hydrogen atom (H•), the simplest kind of free radical. . Examples Nitric oxide (NO.), superoxide($O_2^{\bullet-}$), hydroxyl radical (.OH), lipid peroxy radical (LOO.)(25).

NO, An free Radical agent signal transducing free, and synthesized natural · evolution by synthase nitric oxide (NOS) are similar enzyme that oxidizes L-arginine to L- citrulline and found in a variety of cell types, including vascular endothelial cells, nerve cells, smooth muscle cells, platelets , macrophages, and neutrophils (26,27). • NO is a small lipophilic molecule and has a biological half- life of few seconds.Thus, it can be easily spread to and through membranes and plasma lipoproteins. As a result of free radicals, · NO reacts easily with other types of extreme (eg, $O_2^{\cdot -}$, lipid alkoxyl [LO ·], and peroxyl [LOO ·] extremists) and centers metals from metalloproteins. The most well-characterized binding metal work • NO has an ability to linking heme iron from the soluble guanylyl cyclase (SGC) and stimulate the

formation of cyclic guanosine monophosphate(cGMP) (28) . This activates the movement of ion channels and compound-dependent, low calcium levels within cells membrane, and let the smooth muscles to relax. Thus, · NO it has been identified as a factor derived from the endothelium relaxation and as a result of the central modulator of blood pressure (28) . by stimulating the SGC NO· also prevents platelet aggregation and blood vessel wall adhesion of platelets.

1.3.2 Reactive Oxygen Species (ROS) & RNS

Reactive oxygen species (ROS) is a group which are either free radicals or molecular species capable of generating free radicals, Examples include singlet oxygen, superoxide, peroxides, , and hydroxyl radical In a biological context, ROS which is formed as a naturally byproduct of the natural metabolism of oxygen and have important roles in cellular signal and homeostasis. but, through times of environmental stress (like, UV or heat exposure), ROS levels can raise dramatically(29). And This may lead to severe damage to cell structures. Cumulatively, this is called oxidative stress. ROS are also created by exogenous sources like ionizing radiation (30). The limited of molecular oxygen (O_2) producing superoxide ($O_2^{\cdot -}$) and is the precursor for most other reactive oxygen species:(29).

In physiological conditions, low level ROS play a role in the protection of the organism, while high levels of ROS may cause damage to the structures of the cell, nucleic acids, lipids, proteins (32,38). Reactive nitrogen species (RNS) is a collective term that includes nitric oxide radical (NO •), peroxynitrite (ONOO −), nitrogen dioxide radical (NO_2 •), and other oxides of nitrogen and products arising when NO • reacts with O_2 •− , RO • , and H • NO • . NO • plays significant role in cellular signaling, vasodilation, and immune response. It is a highly reactive small uncharged molecule containing one unpaired electron, therefore considered a free radical. It has a half-life of 15 s and can promptly diffuse across the membrane because of it's uncharged state. Endogenous NO • is formed in the biological tissues via the action of NOS where l -arginine and oxygen are changed over into NO • and citrulline via a fi ve-electron oxidative process. The reaction requires the presence of many cofactors for example FAD, FMN, NADPH, tetrahydrobiopterin, and heme (33,34). An isoform of NOS which is subject to regulation by inflammatory

mediators is expressed in macrophages, and is termed as iNOS (35). iNOS is independent of calcium and calmodulin ions. Once activated, it produces a lot of NO • for as long as the inflammatory stimulus is available and kills or inhibits pathogens. iNOS is controlled by phosphorylation/dephosphorylation via protein kinases; in its phosphorylated form, the activity is decreased. eNOS can also be managed through phosphorylation/dephosphorylation. iNOS can also bind calmodulin, though calcium has little effect on its activity(36). rather than other signaling molecules which act through receptors, NO • diffuses out of the cell where it is produced and diffuses in target cells to transmit signals and interact with itis molecular target, e.g., proteins, nucleic acids, and other free radicals like superoxide (28,37,38,39).

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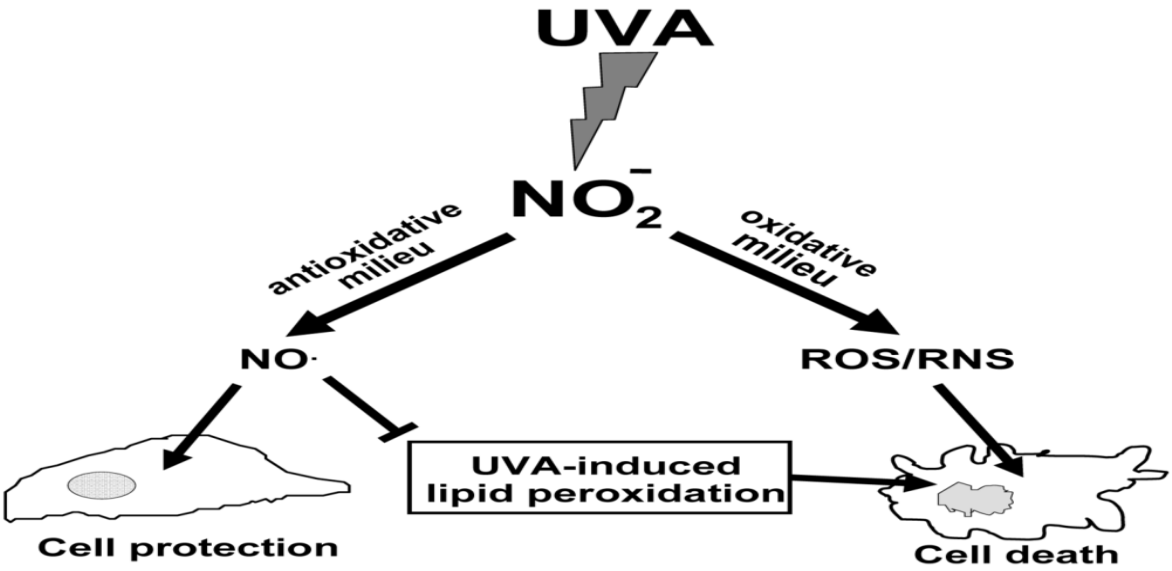


Figure (1-1) : Resources and the effects of reactive oxygen and nitrogen species (36)

1.4 Oxidative Stress

Oxidative stress it's reflects an imbalance between the systemic manifestation of reactive oxygen species and a biological system's capacity to quickly detoxification the responsive intermediates or to repair the damage(28, 40,41,42). Disturbances in the the normal redox condition of cells it can creating dangerous impacts amid the generation of peroxides and free radicals that harm all parts of the cell,for example, proteins, lipids, and DNA(42,43).

Oxidative stress leads to many pathophysiological conditions in the body(44). including neurodegenerative diseases such as Parkinson's disease and Alzheimer's disease, chronic fatigue syndrome , gene mutations and cancers, atherosclerosis,heart and blood vessel disturbance , heart, congestive heart attack and inflammatory diseases(45,46). Oxidative stress causes tissue damage through various mechanisms including promoting lipid peroxidation, DNA damage, and protein modification. These operations have resulted in the pathogenesis of many systemic diseases including kidney. The kidney is an organ highly vulnerable to damage caused by ROS, possibly because of the plenty of long-chain polyunsaturated fatty acids in the composition in the kidneys(47).

1.5Oxidative stress and Arterial Diseases:

oxidative stress change many functions of the endothelium, including modulation of vasomotor tone. Inhibition of nitric oxide (NO·) by superoxide and other reactive oxygen species (ROS) it seems that happening in cases like hypertension, diabetes , hypercholesterolemia, And cigarette smoking. You may lose NO · related to these traditional risk factors in part explain why they prepare to hardening of the arteries (48).

ROS are raise in hypertension in response to vessel stimulation by mechanical stretch or AngII. reaction of ROS with endothelium released NO inhibits vasodilatory or antisclerotic effects of NO and thus can aggravate the disease (49).

(Ang II) levels are raised but ROS is actually dicline , probably due to the accompanying increase in super oxide dismutase (SOD) expression(50). This ability to increase antioxidant defenses may be enough for protection the blood vessels from low levels of oxidant stress, allowing ROS to function as signaling molecules. However, when ROS yield becomes overwhelming, compensatory mechanisms are inadequate and pathophysiological consequence(51). Endothelial cells are able to synthesize and secrete a wide range of anti atherosclerotic substances, the most characterized of which is nitric oxide (NO), a gas produced from the metabolism of L-arginine by constitutive endothelial NO synthase(52).

Subsequently the bioavailability of NO, which leads to vasoconstriction, platelet aggregation and adhesion of neutrophils to the endothelium. overload ROS production may cause oxidative damage to biological macromolecules such as lipids, DNA , carbohydrates and proteins (53,54).

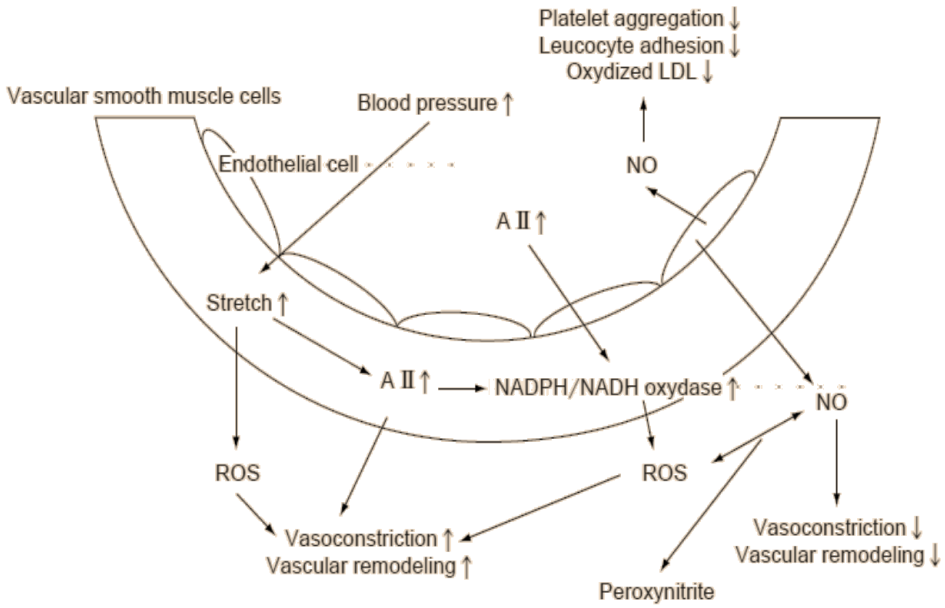


Figure (1-2) Reactive oxygen species in the arterial .(NO nitrogen oxide , AII angiotensin)(53).

1.6 Definition of Oxidative Stress Biomarkers:

A commonly used definition is which proposed in 2001 by the biomarkers definition working group by the national institute of health and food and drug administration (NIH/FDA) in the USA " a feature that is objectively measured and evaluated as an indicator of normal biological methods, pathogenic processes, or pharmacologic responses to therapeutic intervention (55).

There are three types of biomarkers:

(1) pharmacodynamics markers for monitoring pharmacological response .

(2) disease biomarkers-used to monitor and diagnose the development of a disease.

(3) The effectiveness of drug /toxicity biomarkers- used to monitor the effectiveness or toxicity of treatment regime.(56)

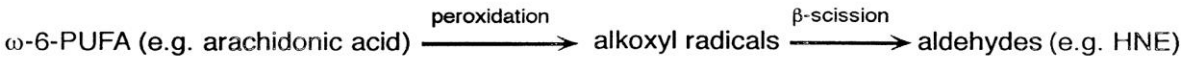
1.7 Oxidative Stress Biomarker

1.7.1 4-Hydroxy-2-nonenal

A. Definition:

4-hydroxy-2-nonenal (4HNE), a high toxicity product of lipid peroxidation, is an inhibitor of mitochondrial respiration. 4HNE exerts its influence on respiration by inhibiting α-ketoglutarate dehydrogenase (KGDH)(57,58).

A



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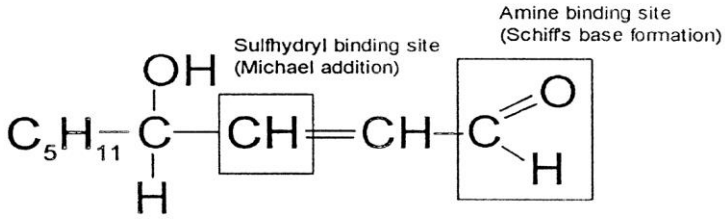


4-hydroxy-2-nonenal

Figure (1-3): Chemical structure of 4-hydroxy-2-nonenal (4HNE)(59)

Because of the KGDH of the central role in the metabolism and emerging evidence that free radicals contribute to mitochondrial dysfunction associated with many diseases, it is of great interest to further characterize the inhibition mechanism. It was found that free radicals and peroxide products generated during the process may be responsible for these effects because of its ability to

damage cellular components like membranes, proteins and DNA. (4HNE), a top end product of lipid peroxidation, a protein particle. Intraperitoneal treatment with one kidney carcinogen, nitrilotriacetate ferric, causing oxidative stress (60). KGDH and pyruvate dehydrogenase (PDH) are structurally catalytic complexes and multi-enzymes similar, suggesting a common pattern of suppression. To determine the mechanism of inhibition were examined for the effects of HNE to purify KGDH and PDH. This disruption by HNE was significantly enhanced in the presence of substrates that limit the sulfur atoms of lipoic acid covalently bound to the sub-units of the E2 KGDH and PDH. (61)

In addition, the loss of enzyme activity resulting from HNE closely linked with a decrease in the availability of lipoic acid group Silvhedril. The use of antibodies lipoic acid because the anti-HNE adjustment lipoic acid in each of the purified enzyme preparations mitochondria, and that this modification depends on the presence of substrates. These results identify a potential mechanism where the production of free radicals and subsequent lipid peroxidation leads to a certain modification of KGDH and PDH and inhibition of mitochondrial respiration-related NADH . HNE can contribute to improved accumulation of modified proteins oxidatively by the devaluation of ubiquitin / proteasome dependent on the intracellular proteolysis (58,61) .

1.7.2 Induced nitric oxide synthase(iNOS)

A. Definition

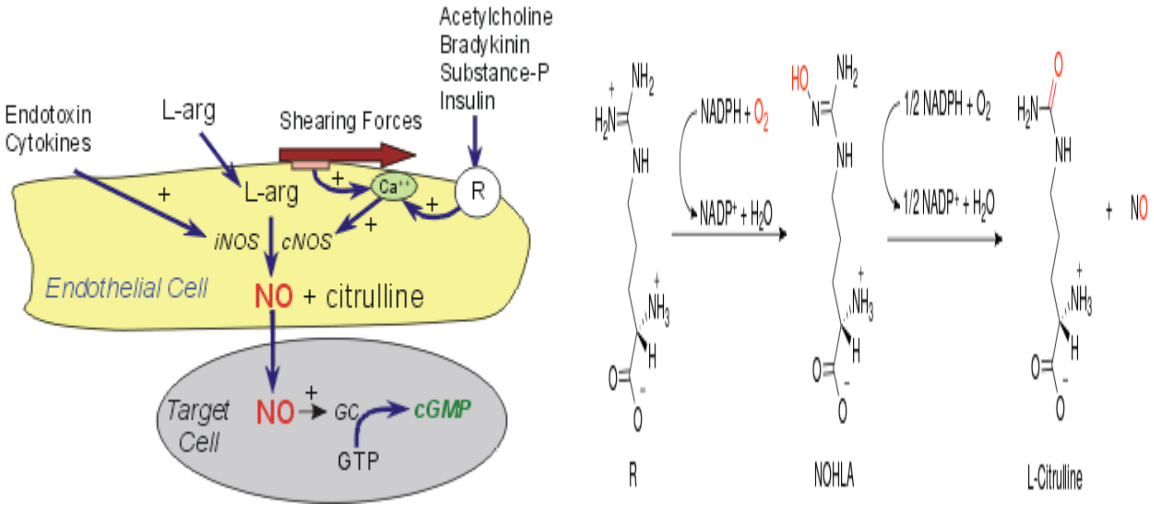
Nitric oxide synthases (NOSs) are a family of enzymes stimulating the production of nitric oxide (NO) from L-arginine.(73,74) NO is an important cellular signaling molecule. It also helps modulate vascular tone, airway tone ,insulin secretion , and peristalsis, and is involved in angiogenesis and neural development. It may work as a retrograde neurotransmitter. Mediated nitric

oxide in mammals that calcium calmodulin similarities enzyme controlled eNOS (endothelial NOS) and nNOS (neuronal NOS). Participates (67,69) and the inducible isoform, iNOS, in the immune response, connecting calmodulin in the concentrations of the relevant physiologically, and does not produce as an immune defense mechanism,, as NO is a free radical with an unpaired electron. It is the direct cause of septic shock and it may work in autoimmune disease. NOS catalyzes the reaction:(63) .

L-arginine + 3/2 NADPH + 1/2 H+ + 2 O_2 ↔ citrulline + nitric oxide + $2H_2O$ + 3/2 NADP+

NOS isoforms stimulate other leak and side reactions, like superoxide production at the expense of NADPH. In this way, this stoichiometry is not generally observed, and reflecting the three electrons supplied per NO by NADPH. The inducible isoform iNOS produces huge amounts of NO as a defense mechanism. It is synthesized by many types of cell in response to cytokines and is an important factor in the response of the body to attack by bacterial infection ,parasites , and tumor growth. It is also the cause of septic shock and may play a role in many diseases with an autoimmune etiology. eNOS is the primary controller of smooth muscle tone (65).

an increased iNOS production may reduce smooth muscle contractions and therefore affect embryo transport, which may consequently leads to ectopic pregnancy (64). Its chemical structure is below in figure(1-4).



(a) (b)

Figure (1-4): Producing (iNOS)(64).

1.8Antioxidant Molecules

An antioxidant is a molecule that prevent the oxidation of other molecules. Oxidation is a chemical reaction that can produce free radicals, resulting in chain reactions that may hurt cells. Antioxidants like thiols or ascorbic acid (vitamin C) terminate these chain reactions. To balance the oxidative state, plants and animals maintain complex systems of overlapping antioxidants, like glutathione and enzymes (e.g., catalase and superoxide dismutase) produced inside or vitamin C, vitamin A and vitamin E it was received by ingestion.(66)

Type of Antioxidant

There are three primary types of antioxidants exist in nature. Which included phytochemicals, vitamins, and enzymes:

*Enzymes are kinds of antioxidants that we get from the protein and minerals eat as part of our daily diets. These enzymes are made in the human body, and include superoxide dismutase (SOD), glutathione reductase,glutathione peroxidase , and catalases.

* vitamins: The human body does not produce antioxidant vitamins naturally, so it is necessary to include dietary sources of them in daily consumption of food, whether it through foods or supplements. Common antioxidant vitamins include vitamins C, A, E and folic acid.

*Phytochemicals are the antioxidants that are used in naturally by plants to protect themselves from free radicals(32,33).

1.8.1 Albumin

Human serum albumin (HSA) is an abundant multifunctional non-glycosylated, negatively charged plasma protein, with ascribed ligand-binding and tranfer facilities, antioxidant functions, and enzymatic activities. its normal concentration is between 35 and 50 g/l which form up to 60% of total plasma proteins. Its half-life is 20 days in normal conditions (28,67). It is manufactured primarily in the liver and is thought to be a negative acute-phase protein. Physiologically, albumin is in charge of maintaining colloid osmotic pressure and may affect microvascular integrity and aspects of the inflammatory pathway, such as neutrophil adhesion and the activity of cell signaling moieties(68,69) Albumin habit accounts for over 50% of total plasma protein content, being present at concentrations of almost 0.6 mmol/L. HSA is a small (66 kd) globular protein includes of 585 amino acids, with few tryptophan or methionine remains but an abundance of charged remains like lysine, and aspartic acids and no prosthetic groups or carbohydrate. X-ray crystallography has shown albumin to possess a heart-shaped tertiary structure, but in solution HSA is ellipsoid. About 67% of the tertiary structure of HSA is formed of helices. The antioxidant characteristics of HSA related to its structure(70,71) show in figure(1-5).

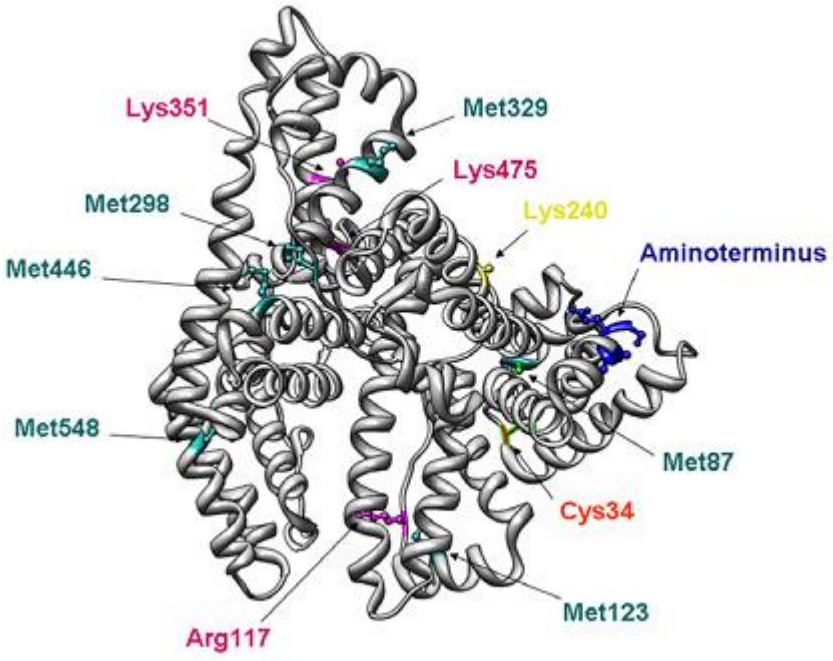


Figure (1-5) Structure of Albumin (72)

The decrease in serum albumin may be indicates to liver failure or diseases like cirrhosis or chronic hepatitis. Hypoalbuminemia can present as part of the nephrotic syndrome, where protein get lost in the urine because of kidney damage. The decrease in albumin levels can be an indicator of chronic malnutrition or protein losing enteropathy.

an increase in the albumin level are caused by(Kidney diseases, Severe infections,Congestive cardiac failure , Severe dehydration, Hepatitis,

Malnourishment, Chronic inflammatory diseases, Cancer)(72,73).

Generally, albumin represents the major and the dominant antioxidant in plasma, a body compartment known to be exposed to continuous oxidative stress. A large percentage of total serum antioxidant characteristic can be attributed to albumin.(74,75)

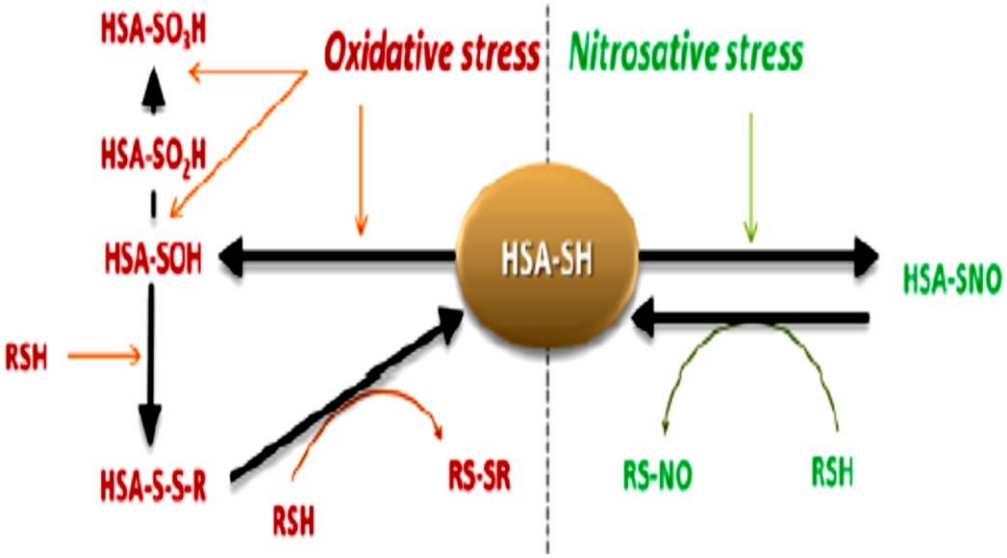


Figure (1-6) :Overview of steps leading to Cys-34 oxidation and thiolation (highlighted in red).(74)

1.8.2 Creatinine

Creatinine is the cyclic anhydride of creatine which is an end product of the dissociation of phosphocreatine (76). Creatine is manufactured primarily in the liver from the methylation of glycocyamine (guanidino acetate, in the kidney are manufactured from the amino acids arginine and glycine) by S - adenosyl methionine. Then transported through blood to the other organs, muscle, and brain, where, during phosphorylation, it becomes the high-energy compound phosphocreatine.(77)

Creatinine is removed from the blood mainly by the kidneys, in the first place by glomerular filtration, but also by proximal tubular excretion. with little or no tubular reabsorption of creatinine occurs. If the clearnace in the kidney is inertial, creatinine blood levels rise.(78) So, creatinine levels in blood and urine can be used to measure the creatinine clearance (CrCl), which is associated with the glomerular filtration rate (GFR). Blood creatinine levels may also be used alone to measure the estimated GFR (eGFR) (79,80). In human subjects, Creatinine reacts non-enzymatically with •OH to shape creatol (CTL: 5-hydroxycreatinine) and demethylcreatinin (DMC) in a one to

one ratio, and CTL partially dissociates to methylguanidine (MG) as shown in figure (1-7)

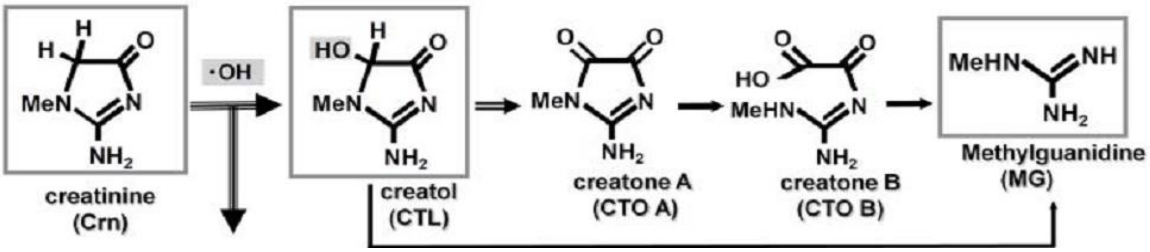


Figure. (1-7) Metabolic pathway of creatinine (81).

A high level of creatinine is not a direct cause of symptoms, and someone with more than-normal levels may notice no change. Symptoms associated with high creatinine level are most often caused by an primary illness that affects kidney function. The most common cause is kidney disease itself. Symptoms of kidney disease can include fatigue, itchy skin, weight loss, nausea or vomiting, loss of appetite, headaches, swelling In the hands and feet, frequent urination or painful or change in urine color. High creatinine level does not necessarily mean that the person is suffering from chronic kidney disease, but indicate the need for further tests. There is limited evidence that suggests that creatine can own antioxidant effect, which has not been tested directly. Because of oxidants such as free radicals can affect muscle fatigue and turnover of the protein, it is important to know whether creatine can neutralize free radicals and other reactive oxygen species.

Urea

Urea, also called Carbamide, the diamide of carbonic acid. Its formula is H2NCONH2.

Urea is the head nitrogenous end product of the metabolic breakdown of proteins in mammals and some fishes. The material occurs not only in the urine of mammals but also in their blood, milk, bile , and perspiration. During the collapse of the proteins are removed amino groups (NH2) of amino acids that make up proteins in part. The conversion of these amino groups to ammonia (NH3), which is toxic to the body, and therefore it must be

converted by the liver to urea. The urea then passes to the kidneys and is eventually secretion to the urine(83). .

Certain exogenous factors are known affect on urea concentration which includes high protein intake,reuptake of blood proteins dehydration , increased protein catabolism and after cortisol treatment(77). Decrease in renal blood flow results in decrease in globular filtration rate this leads to decline in distal tubular flow rate which leads to increase in urea reabsorption and reduce secretion which may be the reason for high serum urea concentration (82). In available references I cannot find a path for urea as antioxidant(82,84)

Urea level is increased because of(heart disease, heart failure, or heart attack,dehydration kidney failure , bleeding in the digestive tract, stress, urinary tract problems, like obstruction shock).

Aim of Study

Investigate oxidative stress effects using 4-hydroxy-2-nenol and Induced nitric oxide synthase as markers and albumin, protein, urea, creatinine as possible effective endogenous antioxidants, in serum of hypertensive and hypertensive with kidney diseases patients and Investigate possible associations among markers and parameters of oxidative stress in hypertension and hypertension with kidney diseases.

Chapter Two

Materials & Methods

2.1 Subjects

The subjects collected for this study include 56 male patients of two of diseases namely hypertensive (28) and hypertensive with chronic kidney diseases(CKD)(28) aged between 25–60 years referred to Al-yarmouk teaching hospital during the period from October 2015 to February 2016. The diagnosis of disease was made by physician with exclusion of presence of other diseases known to be associated with elevated oxidative stress (cancer, diabetes, arthritis, or cystic fibrosis and vitamin supplements taken in the last 4 weeks).

For comparative purpose, age and sex matching group of 28 healthy male devoid of conditions like diabetes mellitus, epilepsy, psychiatric disorders or history of any drug intake are selected as control group. A systematic questionnaire for the various etiological factors of relevant medical disorders (e.g. age, blood pressure, trauma, smoking) was administered (Appendix).

2.2 Materials &Instruments

2.2.1 Chemicals and biological materials

The chemicals and biological materials used in this work are listed in table 2-1 below:

Table (2-1) : Chemicals and biological materials used in the study

Chemicals	**Suppliers**
Albumin kit	Biosystem (France)
Creatinine kit	Randox (United Kingdom)
4HNE	Shanghai biological(China)
iNOS	Cusabio(United State
protein Kit	Spinreact (Spain)
Urea kit	Biomerieux (France)

2.2.2 Instruments

Table (2-2) Apparatus and Equipment used in this study

Instrument	**Company**
Centrifuge	Hettich (Germany)
Deep freeze	Froilabo (France)
Electronic sensitive Balance	DENVER (USA)
Microcenterifuge	Eppendrof (Germany)
Micropipette (Automatic)	Dragon (China)
Microwave	LG (Thailand)
Nano drop	Thermo (USA)
Refrigerator	Samsung (Thailand)

2.3 Methods

2.3.1 Blood Sampling

Blood sampling was performed at 8.30 – 12.30 a.m. in the fasting state. A 10mL of venous blood were obtained. Blood was allowed to clot for at least 10-15 min. at room temperature, centrifuged for (10) min. at (4000 rpm). Serum was divided into several parts by using sterilized eppendrof tubes, some for measuring the biochemical parameters and the other part was stored at -40 °C until the time of 4-HNE and iNOS assay.

2.3.2 Measurement of Blood Pressure(BP)

Blood pressure (BP) is the pressure exerted by circulating blood upon the walls of blood vessels. When used without further specification, "blood pressure" usually refers to the arterial pressure in the systemic circulation. Blood pressure

is usually expressed in terms of the systolic (maximum) pressure over diastolic (minimum) pressure and is measured in millimeters of mercury (mm Hg). It is one of the vital signs along with respiratory rate, heart rate, oxygen saturation, and body temperature. Normal resting systolic (diastolic) blood pressure in an adult is approximately 120 mm Hg (80 mm Hg), abbreviated "120/80 mm Hg.

Clinic blood pressures are usually categorized into three groups; low (90/60 or lower), normal (between 90/60 and 139/89), and high (140/90 or higher).

Long term hypertension is a risk factor for many diseases, including heart disease, stroke and kidney failure. The risk of cardiovascular disease increases progressively above 115/75 mm Hg.(86)

Various factors, such as age and sex, influence a person's blood pressure and variations in it. systolic pressure tends to rise and diastolic tends to fall. In the elderly, systolic blood pressure tends to be above the normal adult range, thought to be largely because of reduced flexibility of the arteries. Also, an individual's blood pressure varies with exercise, emotional reactions, sleep, digestion and time of day (circadian rhythm).

It is measured by Mercury manometer which is The auscultatory method (from the Latin word for "listening") uses a stethoscope and a sphygmomanometer. This comprises an inflatable (Riva-Rocci) cuff placed around the upper arm at roughly the same vertical height as the heart, attached to a mercury or aneroid manometer. The mercury manometer, considered the gold standard, measures the height of a column of mercury, giving an absolute result without need for calibration and, consequently, not subject to the errors and drift of calibration which affect other methods.(87)

2.3.3Measurement of Body Mass Index (BMI)

the length was measured to the nearest 0.5 cm and weight to the nearest 0.1 Kg. Body mass index is calculated by dividing subjects weight (Kg) by their height (m2). BMI is calculated as:

$$BMI = \frac{\text{Mass (Kg)}}{[\text{Height (m)}]^2}$$

According to [WHO;and Fost 2011] a BMI of (<16-18.5)indicates to underweight while a person with ~ 15-16 sever underweight and <15 is very sever underweight (88), 18.5-24.9 indicates a person of normal weight . A person with a BMI of 25-29.9 is overweight , while person with BMI > 30-35 is moderately obese, > 35-40 intensity obese and > 40 is very intensity obese (89).

2.3.4 Determination of Serum 4-hydroxy-2-nonenal Level (4HNE)

A-Principle:

This assay uses enzyme-linked immune sorbent assay(ELISA)based on biotin double antibody sandwich technology .To assay human 4-Hydroxynonenal was added (4HNE) to wells that are pre-coated with4-Hydroxynonenal (4HNE) monoclonal antibody and then incubated. after incubation ,add anti 4HNE antibodies labeled with biotin to united with streptavidin -HRP, which forms the immune complex. After removing unbound enzymes, then add substrate A and B. the solution will turn blue and changed to yellow with the effect of acid. the shaded of solution and the concentration of Human4-Hydroxynonenal (4HNE)are positively correlated.

B-Solutions:

Standards: (40, 80, 160, 320 and 640) ng/L

HRP-conjugate

Wash Buffer(Phosphate buffer saline)

Substrate A

Substrate B

Stop Solution

C-Procedure:

4-Hydroxy-2-nonenal measured in serum sampled were conducted according to manufacture protocol SHANGHAI YAHUA/China as the quantitative determination of endogenic human 4-HNE concentration (Test Kit No.YHB0034Hu). 50µL of standard or sample were added per well then 50µl of HRP-conjugated were added to each well (not to Blank well), Mixed well and then incubated for 1 hour at 37°C .

Wells were wished with washing buffer for(30 seconds) five times. Then 50µL of substrate A and Substrate B were added to each well, mixed well and incubated for 10 minutes at 37°C .
Reaction was stopped by added 50µL of Stop Solution. The concentration were determined by absorption at 450 nm using microplate reader.

D- Calculation:

Standard curve that was created by reducing the data using computer software and then by the program itself calculation the concentration of samples.

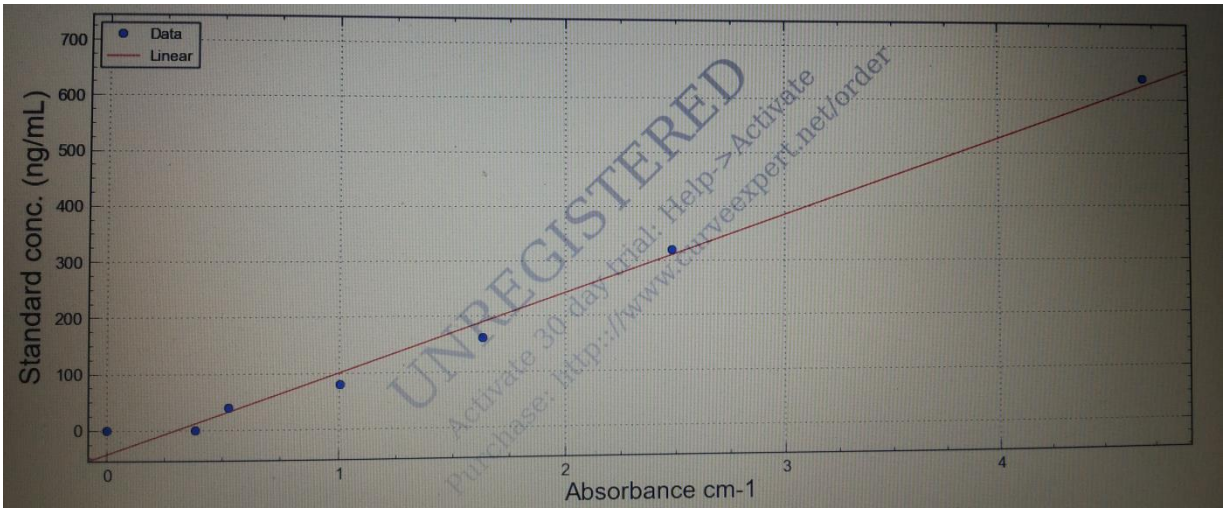


Figure (2-1) Standard curve for 4HNE

2.3.5 Determination of Serum Induced nitric oxide synthase (iNOS)

A-principle:

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific to iNOS has been pre-coated onto a microplate. Standards or samples were pipetted into the wells and any iNOS present is bound by the immobilized antibody. After removed any unbound substance, a biotin-conjugated antibody specific to iNOS is added to the wells. After washing, avidin conjugated horseradish peroxidase(HRP)is added to the wells. Followed a wash to removed any unbound avidin-enzyme reagent, asubstrate solution is added to the wells And the color develops in proportion to the amount of iNOS in the sample. The color development is stopped and the intensity of the color is measured.

B- solution:

Standards: (0, 0.9, 1.8, 3.75, 7.5, 15, 30 and 60) IU/mL

Biotin antibody

HRP-avidin

Wash Buffer

TMB Substrate contain (Tetra methyl benzidine)

Stop Solution

C- Procedure:

Inducible nitric oxide synthase measured in serum sampled of hypertension patient were conducted according to manufacture protocol CUSABIO/China as the quantitative determination of endogenic human iNOS concentration (Test Kit No.CSB-E08148h). 100µL of Standard or Sample was added per well then 100µl of biotin antibody were added to each well (not to Blank well), Mixed well and then incubated for 1 hour at 37°C. Wells were washed with wash buffer two times. Then 100µL of HRP-avidin were added to each well, mixed well and incubated for 1 hour at 37°C. after that washed for five times. then added 90µL of TMB Substrate to each wells mixed well and incubated for 15-30 minutes at 37°C .

The reaction was stopped by added 50µL of Stop Solution. The concentration were determined by absorption at 450 nm using microplate reader.

D-Calculation:

Standard curve created by reducing the data using computer software and then by the same program calculation the concentration of samples.

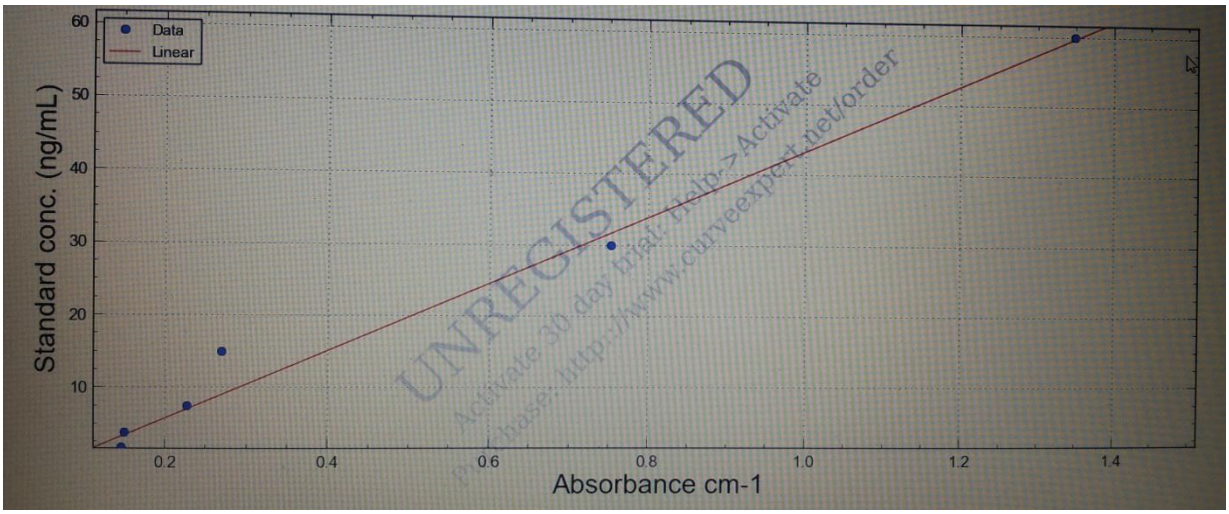


Figure (2-2) Standard curve for iNOS

2.3.6Determination of Albumin

A. Principle

The method is depend on the specific binding of bromocresol green (BCG), an anionic dye, and the protein at acid pH with the resulting shift in the absorption wavelength of the complex. The intensity of the color formed is proportional to the concentration of albumin in the sample.(90)

$$\text{BCG} + \text{Albumin} \xrightarrow{\text{pH 4.3}} \text{BCG-albumin complex}$$

B –Solution:

Reagent 1:

Succinate buffer (75mmol/L; pH 4.2)

bromocresol green(BCG) 0.12 mmol/L

Tensioactive 2 g/L (w/v)

Albumin standard: Bovine serum albumin (5 g/dL (50g/L)).

C- Procedure

Two tubes were labeled as blank, sample and standard firstly. were added10 µL of the serum and were added 2mL of BCG to sample tube.

In standard tube 10 µL of standard were added, , then 2ml of BCG were added, mixed and leaved to stand for 1 minute at room temperature. Read the absorbance (A) of the samples and the standard at 630 nm against the reagent blank(BCG).

2.3.7 Determination of Serum Creatinine

A-Principle

Creatinine under alkaline solution reacts with picric acid to form a colored complex. The amount of the complex formed is directly proportional to the concentration of creatinine (92)

B- Solution:

- **Reagent1:** Picric acid 25 mmol/L
- **Reagent 2:** Alkaline buffer
- **Phosphate buffer (300 mmol/L ; pH 12.7)**
- **SDS (2.0 g/L (w/v))**

Creatinine standard 177µmol/L (2 mg/dL)

Working reagent: Mix 1 volume of reagent1 and 1 volume of reagent 2.

C-Procedures:

Two tubes were labeled as sample and standard. were added100 µl of the serum , standard to labeled tubes. then by adding 1ml of reagent 1 to each tube.

In blank tube 1ml of reagent 1 mixture was added. after that mix well and the absorbance A1 was read after 30 seconds of the standard and sample. Exactly after2 minutes absorbance A2 of standard and sample was read all against blank.

D-Calculation:

$\Delta A_{sample} = A_2 - A_1$

Creatinine (mmol/L) = (Δ A sample / Δ A standard) * standard concentration(2.03 mmol/L)

2.3.8 Determination of Serum Urea

A-Principle

Urease stimulate the conversion of urea to ammonia. In a modified Berthelot reaction ,the ammonium ions react with a mixture of salicylate, hypochlorite and nitroprusside to produce a blue-green (Indophenol).The absorbance of this dye is proportional to the concentration of urea in the sample: (91).

$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} 2NH_3 + CO_2$$

$$NH_3 + \text{salicylate} + \text{hypochloritic} \xrightarrow{\text{Nitroprusside}} \text{2,2-dicarboxy indophenol}$$

B- Solution:

- **Reagent (1)** Standared Urea 8.3 mg/dL
- **Reagent (2)** (Enzyme) Urease 350 KU/L
- **Reagent (3)** Color reagent:

Phosphate buffer (50 mmol/L ; pH 8)

Sodium Salisylate (62 mmol/L)

Sodium Nitroprusside (3.35 mmol/L)

EDTA(1 mmol/L)

❖ **Reagent (4)**

Alkaline reagent

Sodium Hydroxide NaOH (0.5 mmol/L)

Sodium Hypocloride NaClO (24.8 mmol)

C-Procedures:

Three tubes were labeled as blank, sample and standard firstly. were added10 µLs of the serum to sample tube. then by adding 1mL of reagent 1. .
In standard tube 10 µLs of standard were added, after that diluted with 1mL of reagent 1.In blank tube 1ml of reagent 1 was added. All tubes were mix well and incubated for 5 minute at 37 oC. Finally, were added1mL of reagent 4 to all tubes, mixed well and incubated for 5 minute at 37 oC . a Sample and standard were read at 590 nm against blank.

D-Calculation:

$$\text{Urea (mmol/L)} = \frac{\text{A Sample}}{\text{A Standard}} \times \text{Standard Concentration(8.3 mg/dL)}$$

2.3.9Determination of Serum protein

A-Principle

In the biuret reaction, achelate is formed between the cu^{+2} ion and the peptide bonds of the protein in alkaline solution to form a coloured complex whose absorbance is measured photometrically. the intensity of the color produsd is directly proportional to the concentration of protein in the sample.(93)

$$\mathbf{Cu^{+2} + Serum\ protein} \xrightarrow[25\text{-}37\ ^{o}C]{\mathbf{ph>12}} \mathbf{Copper\text{-}protein\ complex}$$

B- Solution:

R1.Biuret reagent.
.Cupric sulfate 6 mmol/L
.Sodium-potassium-tartrate 21 mmol/L
.Potassium iodide 6 mmol/L
.sodium hydroxide 0.75 mol/L
Protein standard.
.Bovine searm albomin 7g/dl(70g/l)

C-Procedures:

Three tubes were labeled as blank, sample and standard firstly. were added 20 µLs of the serum to sample tube. then by adding 1mL of reagent 1.
In standard tube 20 µLs of standard were added, after that diluted with 1mL of reagent 1.

In blank tube 1ml of reagent 1 was added. All tubes were mix well and incubated for 10minute at 37 oC. a Sample and standard were read at 540 nm against blank.

D-Calculation:

$$\text{A Sample protein (mmol/L)} = \frac{\text{A Sample}}{\text{A Standard}} \times \text{Standard Concentration(7g/dL)}$$

2.4 Statistical Analysis

All statistical analysis in studies were performed using SPSS.Descriptive analysis was used to show the mean and standard deviation of variables. The significance of difference between mean values was estimated by Student T-Test. The probability $p \leq 0.05$ = significant, $p > 0.05$ = non-significant. Correlation analysis was used to test the linear relationship between parameters(94).

Chapter Three

Result & Discussion

3.1 Patient Descriptive Data:

3.1.1 Distribution of Groups :

The present study included 84 volunteers divided into three groups; first group is the hypertensive volunteers patients (28 subjects), the second group is the hypertensive with kidney disease volunteers patients (28 subjects), and 28 apparently healthy individuals as control group, all shown in figure (3-1) below with their percentage

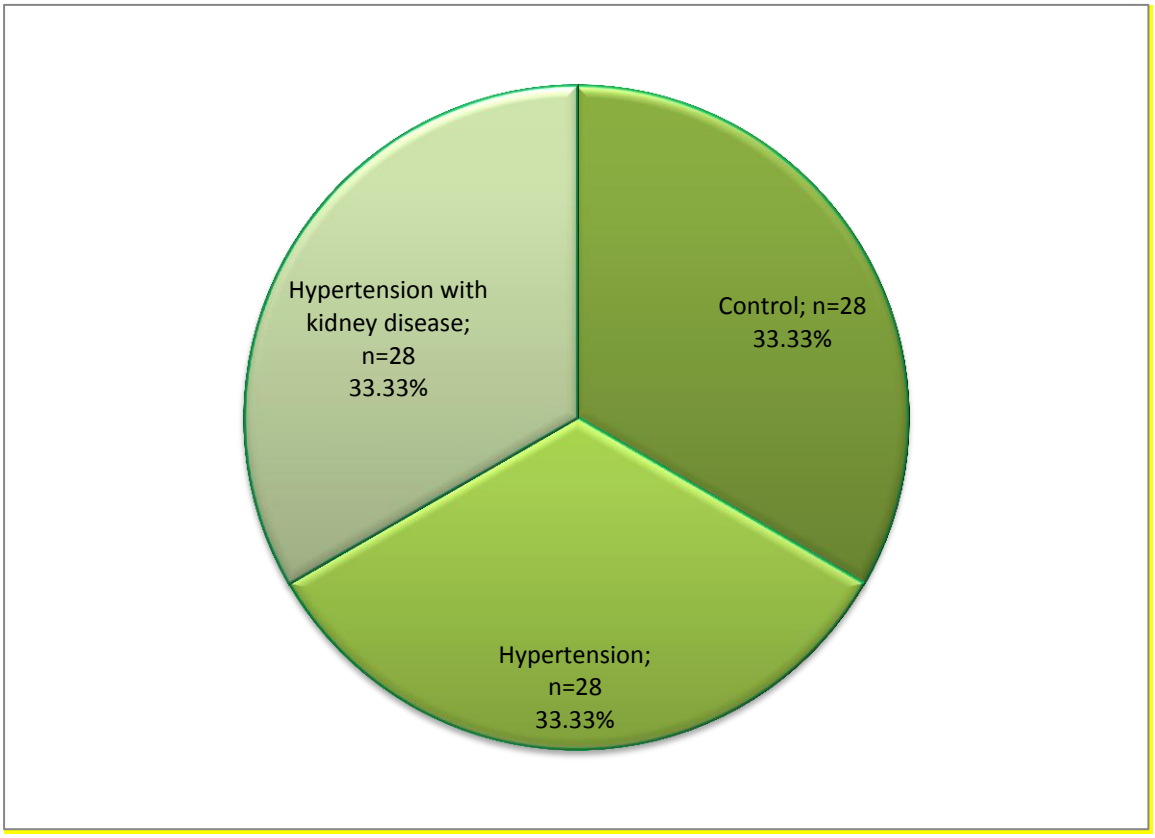


Figure (3-1): Distribution of studying group.

3.1.2 Clinical Feature of groups:

The clinical feature of the groups in the underlying study (shown in table 3-1) which include; ages , body mass index, systolic and diastolic blood pressure and duration of diseases.

Table (3-1): Clinical Feature of Studying Group (mean ± SD):

Characteristic	HT group n=28	Control group n=28	HT with CKD disease group n=28
Age(year)	55.46 ± 8.91**	23.82 ± 5.02	47.60± 11.71**
BMI (Kg/ m^2)	33.34 ± 4.68**	25.18 ± 2.89	28.17 ± 3.90**
Systolic blood pressure(mmHg)	14.60 ± 1.22**	11.96 ± 0.66	19.03 ± 3.23**
Diastolic blood pressure (mmHg)	9.23 ± 0.85**	8.63 ± 0.56	10.83 ± 1.25**
Duration of disease(years)	8.866 ± 1.48	-	5.133 ± 0.94

Significant **($p<0.01$)

The mean age for HT was (55.46 ± 8.91) year, HT with CKD (47.60 ± 11.71) years while the mean of the control group was (23.82 ± 5.02) years. This showed significant differences in age when comparing patients groups with control group. As shown in Table (3.1).

The significant difference in BMI ($p<0.01$) between HT and control group reveal the positive correlation between obesity and disease, this finding was agree with (Ahmed A. *et al*) and (Shugar L *et al)* who found a significant increase in BMI in hypertensive patients (95,96). Obese patients are more ready to be hypertensive than lean patients, and weight gain is typically associated with increases in arterial pressure(98).

The significant difference in BMI between HT with CKD patients and control group may be interpret the incident with that disease ($p<0.01$). This result was agree with (Michael E Hall. et al))(98)(who recorded this relation as a risk factor for HT with CKD incident. For HT with CKD disease the risk increases with increasing body mass indices BMI> 26.6kg / m^2)(99).

Obesity causes renal vasodilation and glomerular hyperfiltration, which act as compensatory mechanisms to maintain sodium balance despite increased tubular reabsorption. However, these compensations, along with increased arterial pressure and metabolic abnormalities, may ultimately lead to glomerular injury and initiate a slowly developing vicious cycle that exacerbates hypertension and worsens renal injury.(100)

3.2 Oxidative Stress Markers and Parameters in HT,HT with CKD, and Control.

3.2.1 4-Hydroxy -2-nonenal (4HNE) levels :

The mean levels of serum 4HNE showed a significant increase ($p < 0.01$) in hypertension patients group (160.22 ± 42.63) ng/L and hypertension with kidney disease group (273.71 ± 96.75) ng/L when compared to control group (136.46 ± 24.55) ng/L as shown in table (3-2) and figure (3-2).

Table (3-2): Values of 4HNE Between Groups (mean ± SD):

Patient groups	4HNE ng\L	Control group n=28
Hypertension patient n=28	160.22±42.63**	136.46± 24.55
Hypertension with kidney disease group n=28	273.71± 96.75**	

Significant with **(p<0.01)

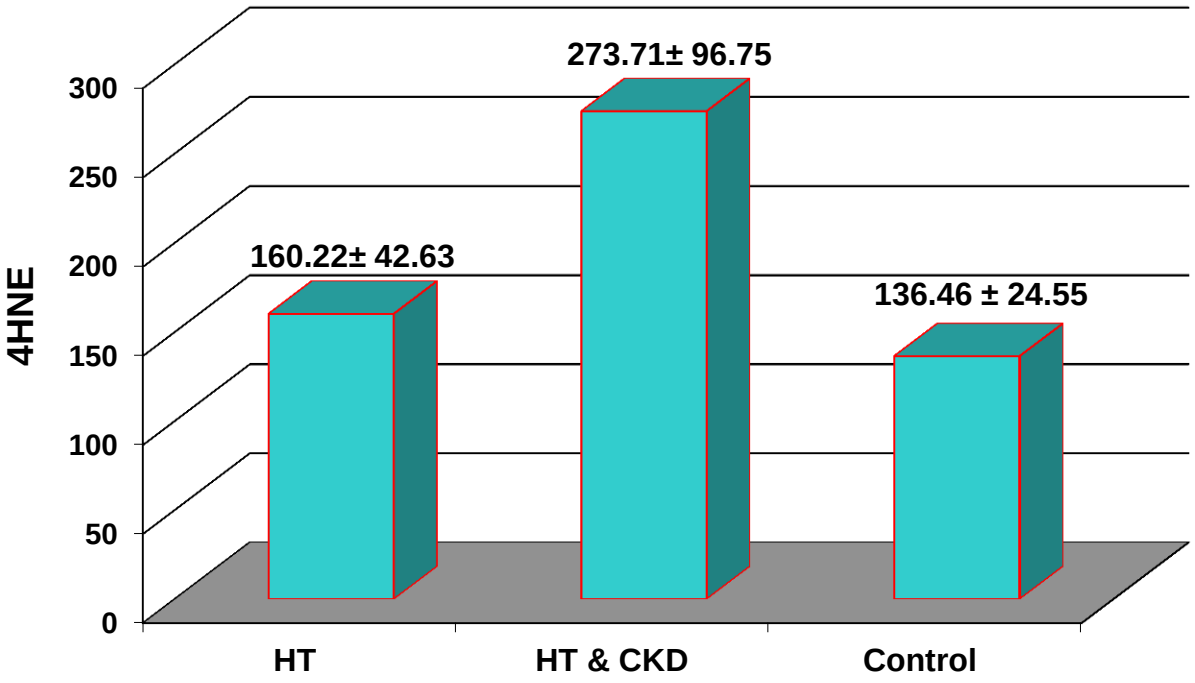


Figure (3-2): Mean Conc. (ng/L) of Oxidative Marker 4HNE for Studied Groups.

Findings of the present study in hypertension patient is also found by (Kamelija Z. *et al*) and(Ethan J. et al)(101,102) , and (Teresa Sousa *et al*)(103).

Significantly elevated (p<0.01) in diagnosed hypertension patient and this agree with (Mariso P. *et al*) (104). And agree with(Usberti M. et al)in hypertensive with kidney disease.(105)

The accumulation of 4-HNE-adducts is very high in the intimal aorta, mainly in older patients with high atherosclerosis grade. These data were expected since oxidized LDL and lipids accumulate in the intima in the early lesions and in the lipid core of advanced atherosclerotic lesions(106) . These data confirm that 4-HNE is a main marker of oxidative stress and LDL oxidation

which could contribute to the evolution of the lesions via its ability to modify proteins and generate cell dysfunction. 4-HNE expression was also increased in the adventitia of the elderly, probably associated with the vasa vasorum, which are involved in the supplying of nutrients and oxygen to atherosclerotic lesions, and the development of angiogenesis in the atherosclerotic plaque . The recently found effect of 4-HNE suggests a role for this aldehyde in the development of vasa vasorum and micro capillaries in atherosclerotic plaque(107,108).

It has been demonstrated that increased intracellular generation of ROS plays an important role in chronic inflammatory responses to arterial diseases , so this causes damage to the membrane polyunsaturated fatty acids leading to the generation of 4HNE cause elevation in 4HNE in these patients(109).

3.2.2 Induced nitric oxide synthase Levels:

The (mean ±SD) of iNOS for hypertension patients was(10.11 ± 7.08 IU\ml), and for hypertension with kidney disease patients was (15.58 ± 14.IU\ml) with regards to control group which is (6.42 ± 4.93IU/ml) as revealed by table (3-3) and figure (3-3)

Table 3-3: Values of iNOS in Patients and Control Group (mean ±SD):

Patient groups	iNOS [IU/ml]	Control group	*P* value
Hypertension group	10.11 ± 7.08 **	6.42 ± 4.93	<0.01
Hypertension with kidney	15.58 ± 14.44 **		<0.01

Highly significant **(p<0.01)

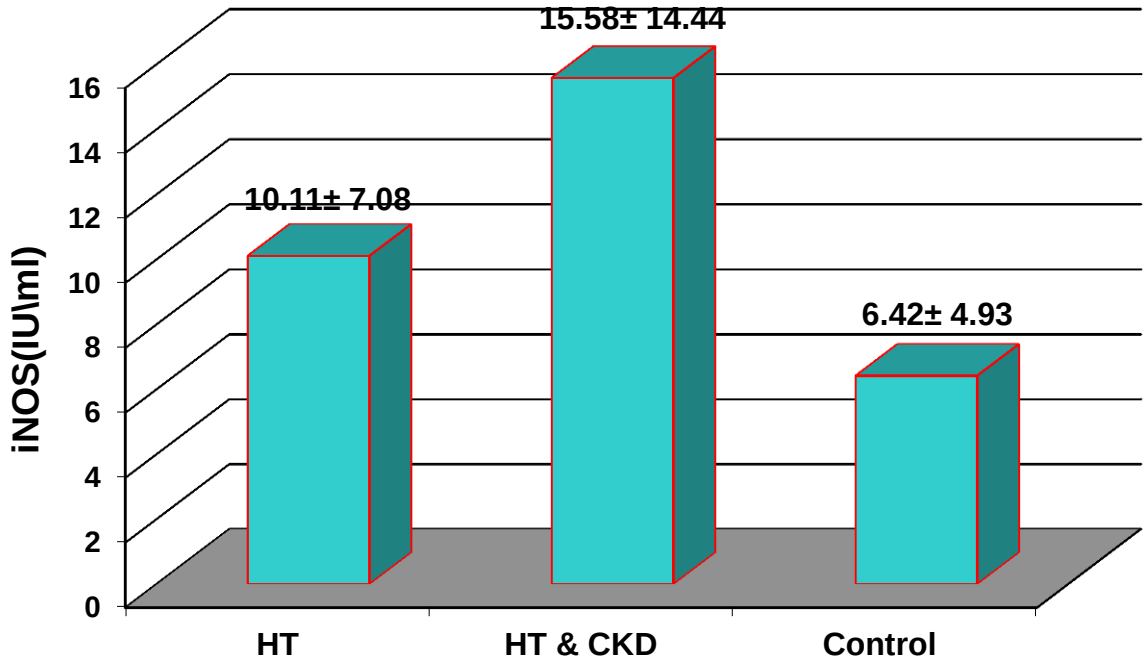


Figure (3-3): iNOS Levels in Patients and Control Group

The results show a significant difference between hypertension patient and control group ($p<0.01$). these results were agreed with (Caroline J. Smith *et al*)(110)who found a significant difference in iNOS levels in hypertensive patient. Also (Y Álvarez *et al*)(111)found an elevation in NO synthesis might be associated with elevations of vascular resistance and, thus with hypertension. Hypertension increases iNOS expression but decreases the bioavailability and the modulation elicited by iNOS-derived NO of contractile responses in aorta as a result of the increased O_2•− production from NAD(P)H oxidase(111).

The significantly increased in iNOS in hypertensive subjects as compared to the control group which may be due to the increased generation of ROS in certain type of white blood cells which contribute in reduction bioavailability of nitric oxide and thus to the endothelial dysfunction, as some of the hypertension-induced organ damage, which occur due to hyperactivity of

mechanisms that increase ROS production. There are other sources of oxidative stress in cardiovascular system that lead to heart disease and blood vessels involve NAD(P)H oxidase (multisubunit membrane complexes) and NOS as well as the mitochondrial cytochromes and hemoglobin . NOSs and hemoglobin are also principal sources of RNS, including NO which convey NO• bioactivity.(112)

iNOS produces large quantities of NO, which can then react with superoxide anion, produce highly reactive peroxynitrite, and cause oxidative damage to cellular components . The rate for the reaction of superoxide anion with NO to produce peroxynitrite is very high . It can be calculated that when NO production by eNOS is maximally stimulated and when there is significant induction of iNOS, even the relatively high level of EC-SOD in the arterial interstitium is probably insufficient to prevent peroxynitrite formation. Thus, it is likely that peroxynitrite or other nitrating species are formed in atherosclerotic lesions(113).

There was a significant correlation between iNOS and 4HNE in the study groups which related directly except control group , as shown in figures (3-4) (3-5) and (3-6).

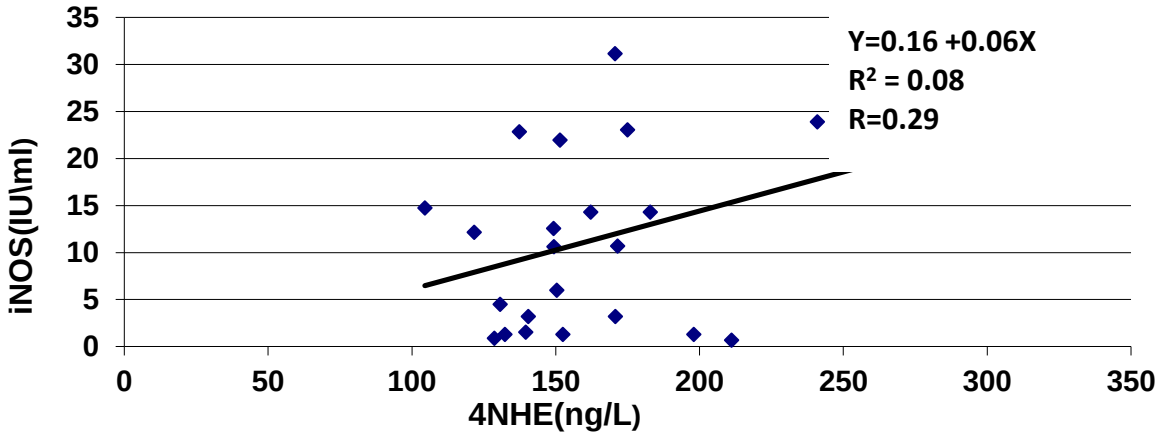


Figure (3-4): Correlation Directly Between 4HNE and iNOS for hypertension Groups.

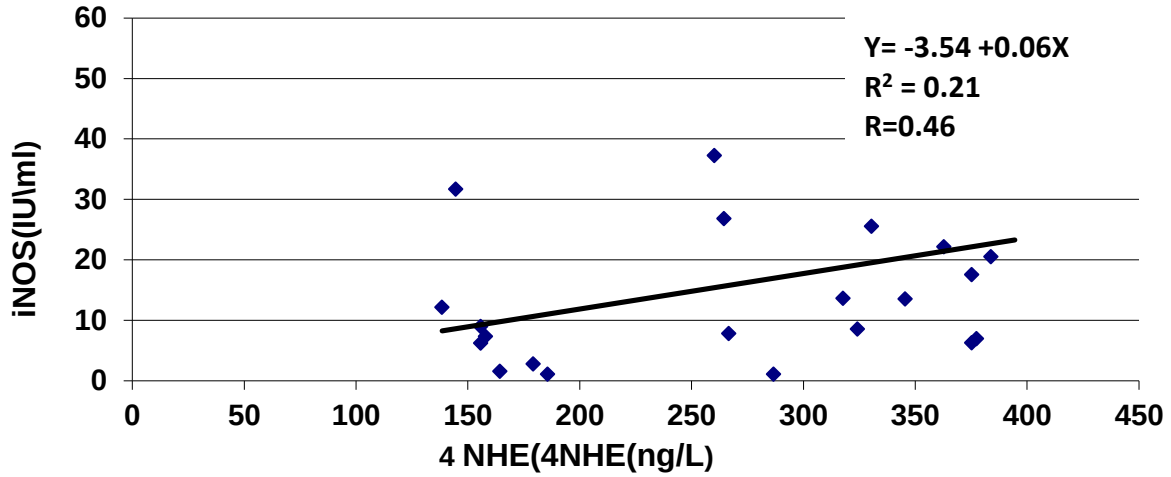


Figure (3-5): Correlation Directly Between 4HNE and iNOS for hypertension with kidney disease Groups.

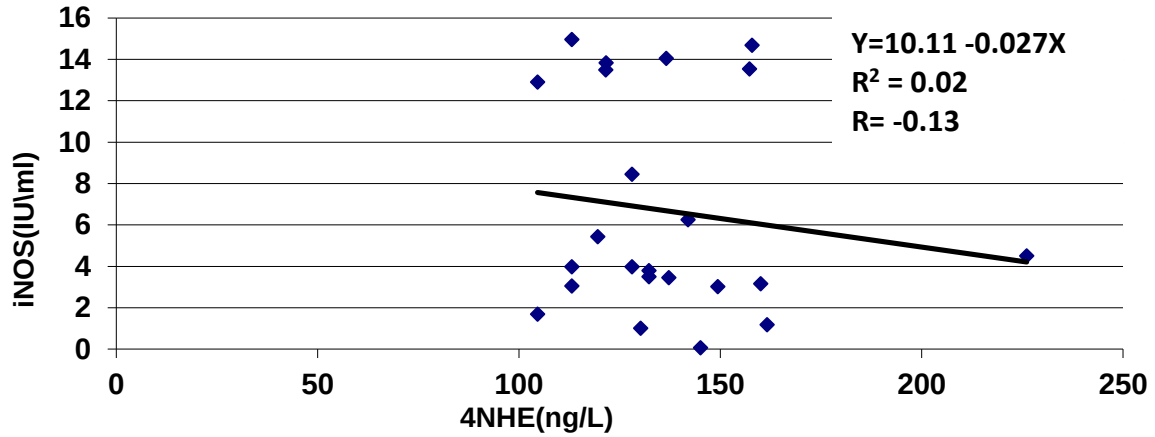


Figure (3-6): Correlation Directly Between 4HNE and iNOS for Control Groups.

The direct correlation between these two parameters shows increasing oxidative stress in all levels in the cell. The positive correlation between 4HNE and iNOS in hypertension was also found by (Jukka S. *et al*)and(Ying Sun. *et al*) in healthy supplier by antioxidant nutrition(114,115).

3.2.3 Serum Albumin Levels:

The mean levels of total serum albumin showed a significant decrease in both patients groups when compared to control group ($p<0.01$) table 3-4 and figure 3-5 show results :

Table (3-4): Values of Albumin of studied Groups (mean ± SD):

Characteristic	Albumin g /dL	Control group n =28	Comparison of Significant *p* value
Hypertension patient N=28	4.38 ± 0.27**	4.73 ±0.25	<0.01
Hypertension with kidney group N=28	3.90 ± 0.48**		<0.01

Significant**($p<0.01$)

Figure (3-7): Values for Albumin [g/dL] in Patients and Control Groups .

The results of the present study showed that the levels of albumin was significantly decrease ($p<0.01$) in diagnosed hypertension and hypertension with kidney patients which is agreed with (Oda E) in hypertension and (Vandana M. et al) in hypertension with kidney disease.(116,117)

Albumin is the most abundant circulating protein in the plasma, exerts important antioxidant activities. The molecule acts through its multiple-binding sites and free radical-trapping properties. In physiological or pathological conditions, function associated with changes in the redox status, the albumin structure, and its beneficial antioxidant properties can be altered. In general, albumin constitutes the major plasma protein target of oxidant stress. Critically, administration of albumin to patients confers robust

protection against oxidative stress and a favorable influence on redox-signaling processes regulating inflammation. Additional studies, especially in vivo, are needed to document the modifications in albumin structure and antioxidant properties in pathologic conditions(118).

Albumin is a hepatic protein, and its plasma concentration is mainly influenced by several factors, including the rate of albumin synthesis, exogenous albumin loss, and dilution. Synthesis of albumin is affected by nutritional intake, colloid oncotic pressure variations, and liver function. Plasma albumin levels are known to be decreased in inflammatory conditions, including infection, trauma, and surgery (119).

In chronic disease states such as end-stage renal disease or dialysis , hypoalbuminaemia is common, and it may be due to renal failure. There is also evidence that severe hypoalbuminaemia promotes fluid retention and oedema through a lowering of the plasma oncotic pressure, which may in turn aggravate both cardiac and renal failure(120).

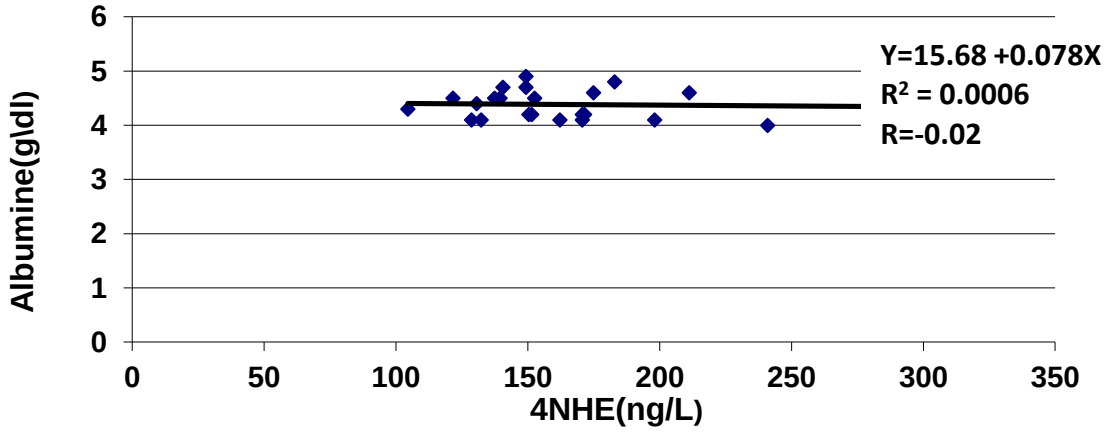
Hypoalbuminemia is a powerful predictor of mortality in patients with kidney failure. The interrelationships between inflammation, nutritional status, and outcomes in patients with kidney failure has led to speculate on the existence of a malnutrition, inflammation, atherosclerosis complex syndrome in this population. It has been suggested that the association between serum albumin levels and cardiovascular disease, in patients with kidney disease, may in part reflect the role of inflammation in inducing the malnutrition that is highly prevalent in these patients. Postulated mechanisms for the association between inflammation and malnutrition, include appetite suppression and enhanced protein catabolism by proinflammatory cytokines(117,121).

Other studies have however suggested that hypoalbuminemia is simply a reflection of the negative acute phase response, and that serum albumin levels

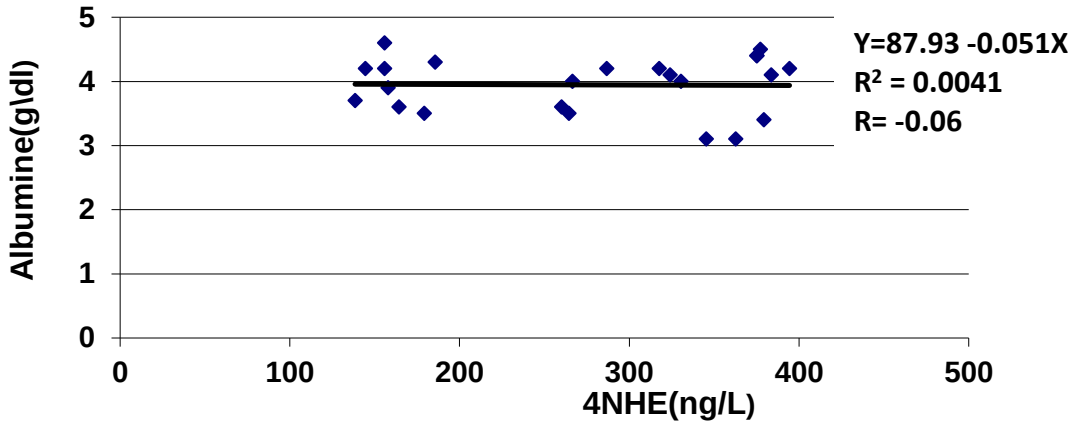
may be more indicative of underlying inflammation, rather than nutritional status, especially in patients with kidney disease. For example, a recent study demonstrated that although serum albumin levels ,measured as nitrogen index, were independent predictors of all-cause mortality in a cohort of patients starting dialysis(121).

The atherosclerosis has been considered as an inflammatory and immunizing disease. It is widely accepted that inflammation represents a risk factor for atrial fibrillation and for prothrombotic conditions. Different molecules behave differently during an inflammatory phase; albumin synthesis decreases, while other inflammatory globulins rise (122). In hypertension which is not inflammatory disease in basic, the decrease in albumin levels may be due to antioxidant activity more than its behavior as acute phase protein(123).

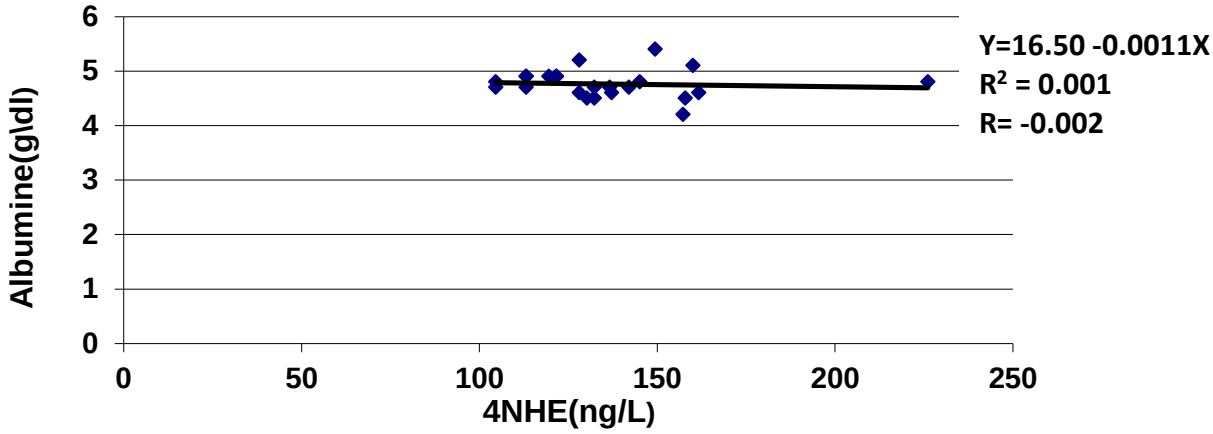
There was a negative correlation between albumin and 4HNE as shown in Figures (3-8)(3-9) and (3-10).



Figure(3-8): Correlation Between Albumin and 4HNE in HT group.



Figure(3-9): Correlation Between Albumin and 4HNE in HT with CKD group.

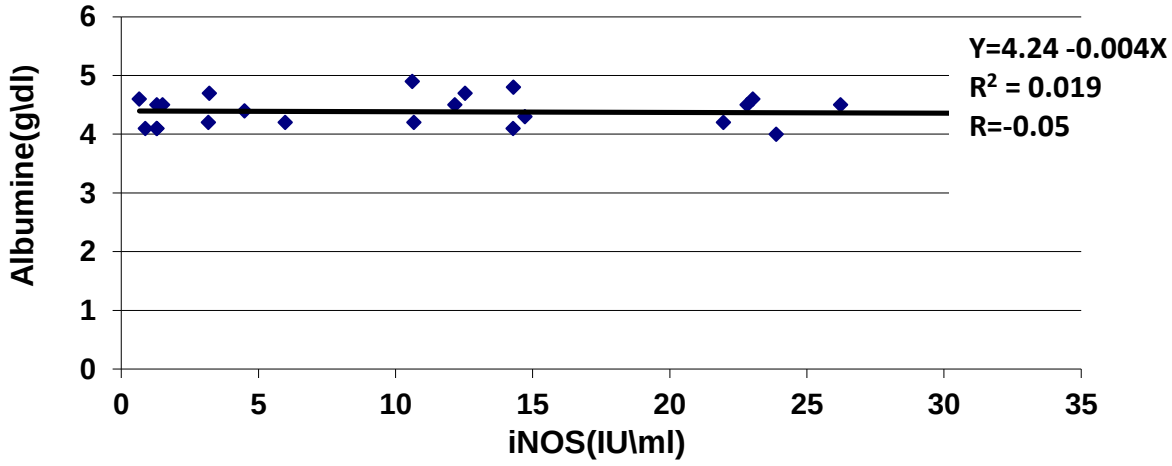


Figure(3-10): Correlation Between Albumin and 4HNE For Control group.

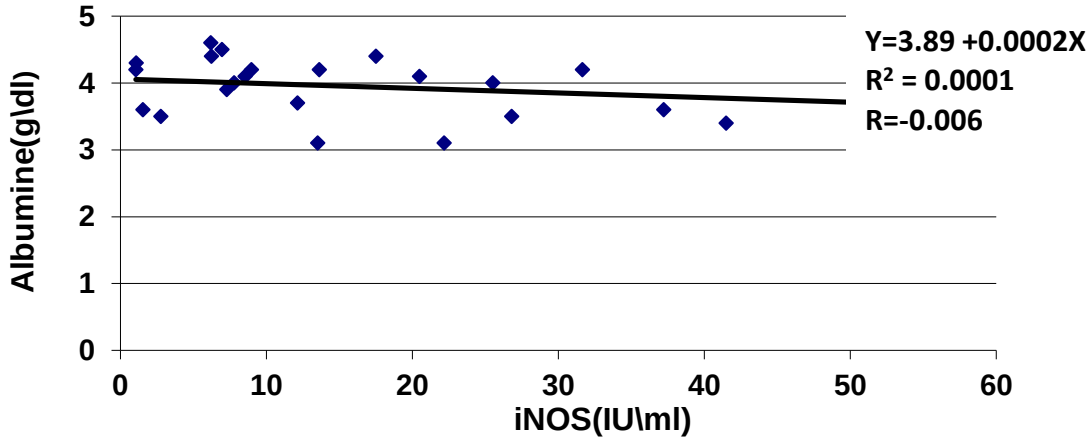
Weak negative correlation between 4HNE and albumin in patient and control group was observed in this study which agreed with (Giancarlo Aldini.*et al*) who found a negative correlation between 4HNE and Albumin(124) .

In humans, albumin is the most abundant serum protein. Its antioxidant activity is essential in maintaining physiological homeostasis because it binds and transports endogenous substances; such as lipids, ascorbate and divalent cations, scavenges oxygen free radicals and preserves microvascular integrity . The albumin molecule has been demonstrated to inhibit copper ion-dependent generation of hydroxyl radicals and lipid peroxidation, thereby preventing the oxidative injury of lipoproteins(125).

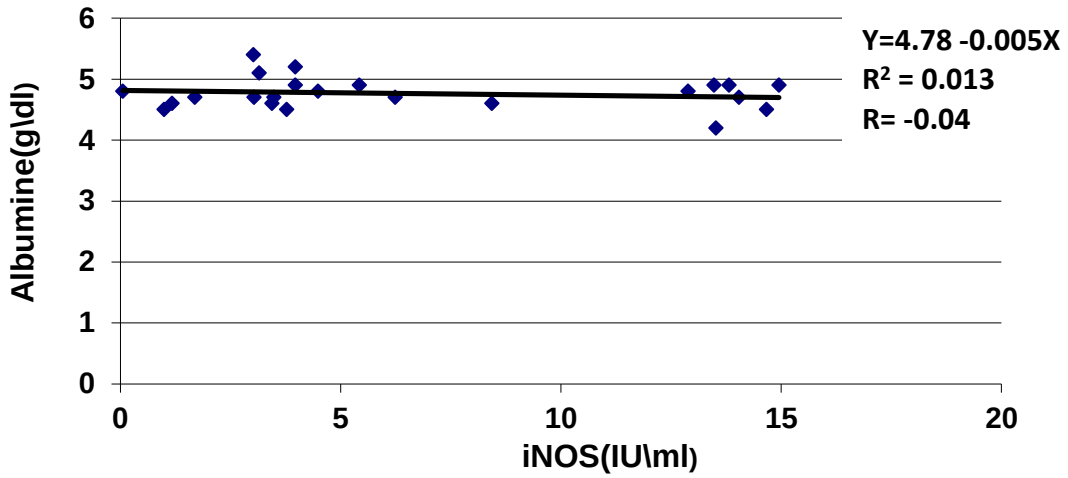
There is also negative correlation between iNOS and albumin in patient and control group was observed in this study Figure(3-11) (3-12) and (3-13) which agreed with (Michael Poteser.*et al*) who found a negative correlation between 4HNE and Albumin(126).



Figure(3-11): Correlation Between Albumin and iNOS in HT group.



Figure(3-12): Correlation Between Albumin and iNOS in HT with CKD group.



Figure(3-13): Correlation Between Albumin and iNOS For Control group.

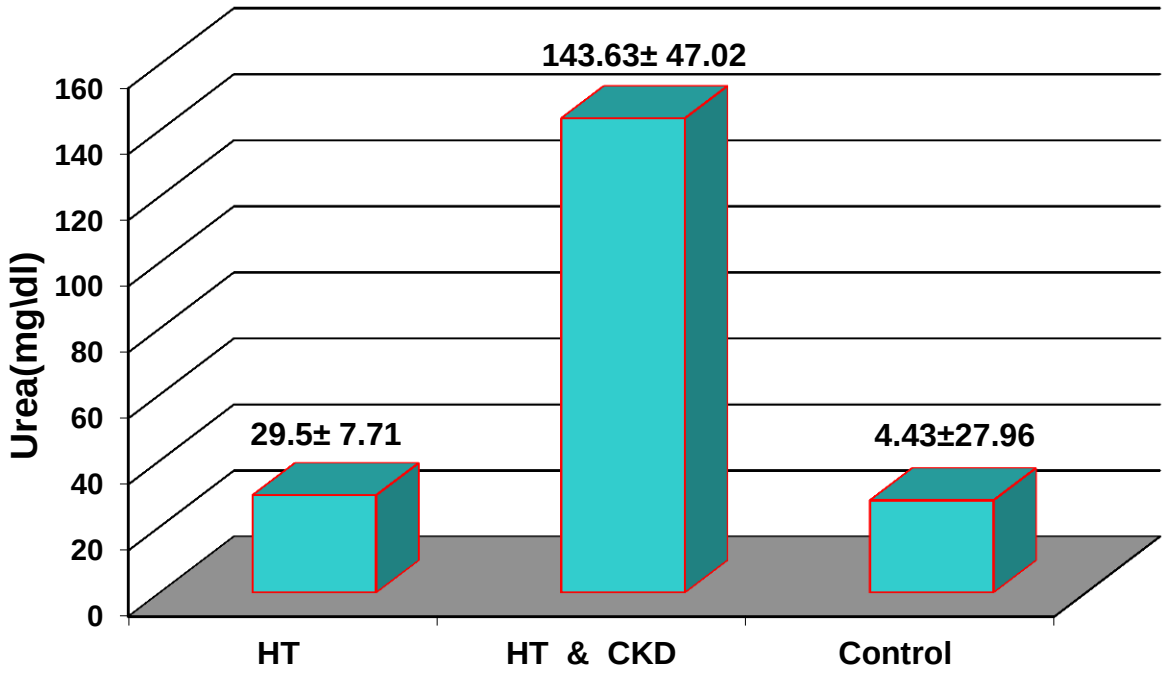
3.2.4 Serum Urea levels:

The mean levels of serum urea were significantly increased (*p<0.01*) in both patients groups as compared to control group as shown in Table (3-5) in figure(3-14)**.**

Table (3-5): Values of Urea in Studying Groups (mean±SD):

Group	Urea(mg\dl)	Control group n =28
Hypertension patient n=28	29.50 ± 7.71 N.S	27.96±4.43
Hypertension with kidney group n=28	143.63 ± 47.02**	

NS(Non-significant) **(p<0.01)



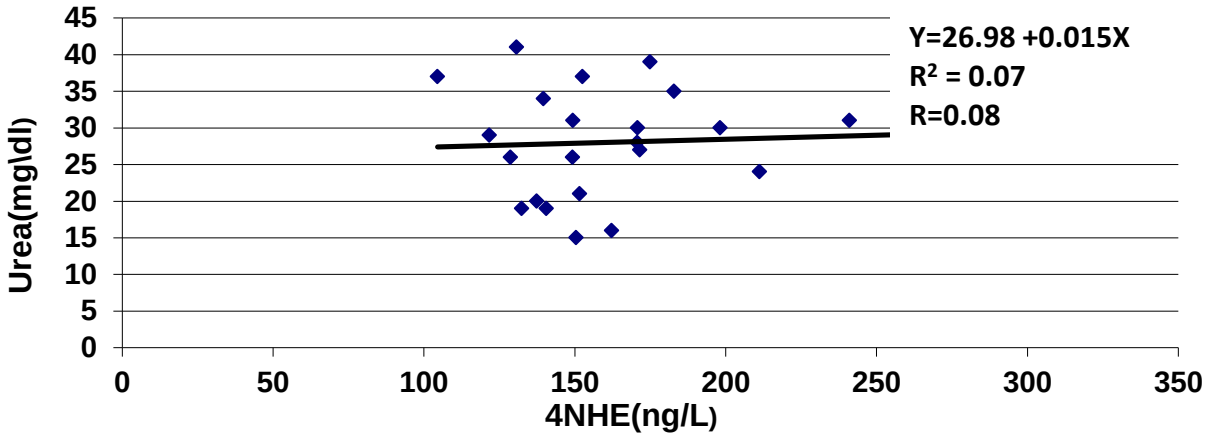
Figure(3-14): Values for Urea [mg/dl] in Patients and Control Groups .

The present study showed high level of the urea in patients groups when compared to control group and this result agreed with (Rakhee Y. et al)(in hypertension and with (Noor ul Amin *et al)* in hypertension with kidney disease.(127,128).

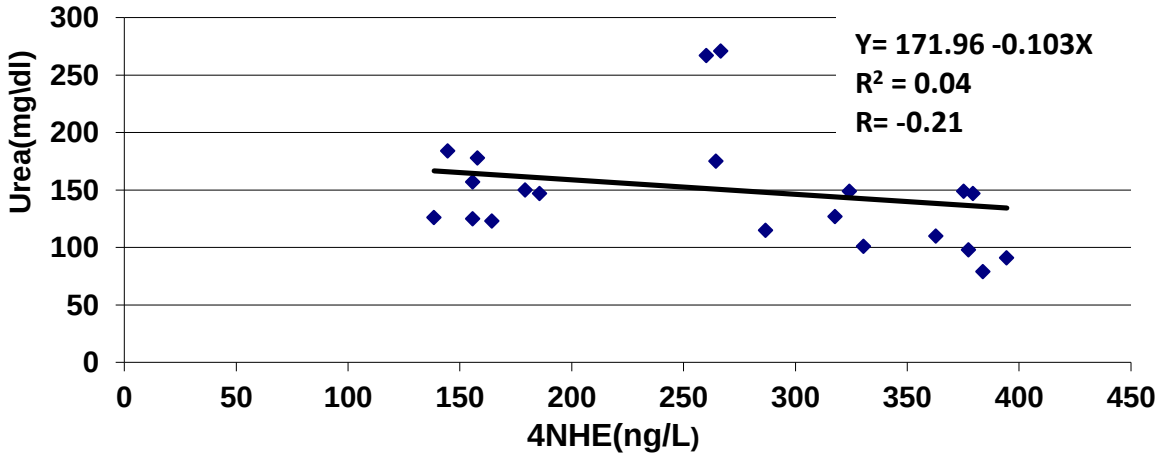
Urea is the major excretory product of protein metabolism. It is formed in the liver from amino groups (-NH_2) and free ammonia generated during protein catabolism(129). In large cohorts of patients with acute and chronic heart disease, an elevated urea has been shown to be a strong predictor of morbidity and mortality(130). In the heart failure and hypertension the blood flow decrease so less blood is delivered to the kidney; consequently, less urea is filtered(131). A reduction in renal blood flow leads to a decrease of glomular filtration, this is lead to a decrease distal tubular flow rate which lead to

increase of urea reabsorption and decreased secretion which may be the reason for elevated serum urea concentration.(132)

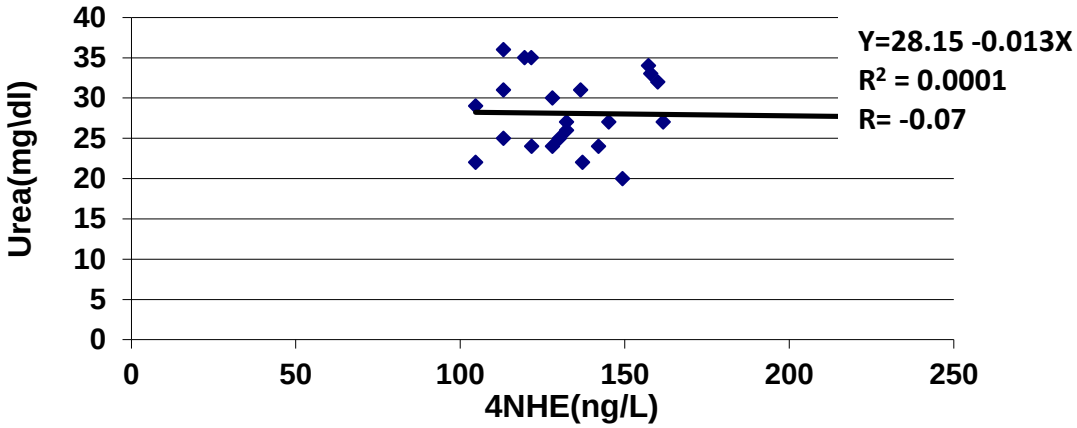
There is a positive correlation between 4HNE and urea in HT patient group was observed in this study Figure(3-15) but negative in groups HT with CKD and control Figure (3-16) and (3-17)which agreed with (Lisa M. Walker.*et al*) who found a positive correlation between 4HNE and urea(133).



Figure(3-15): Correlation Between Urea and 4HNE in HT patients.

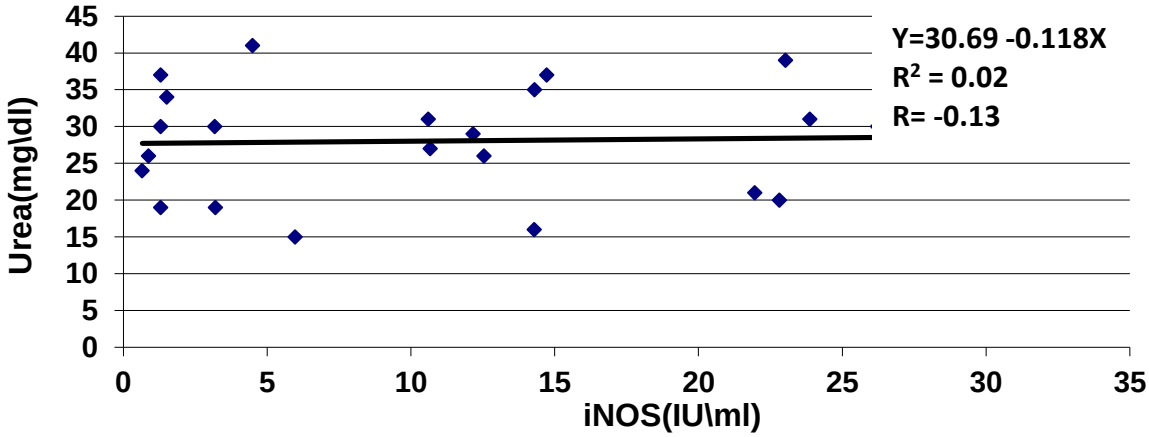


Figure(3-16): Correlation Between Urea and 4HNE in HT with CKD patients.

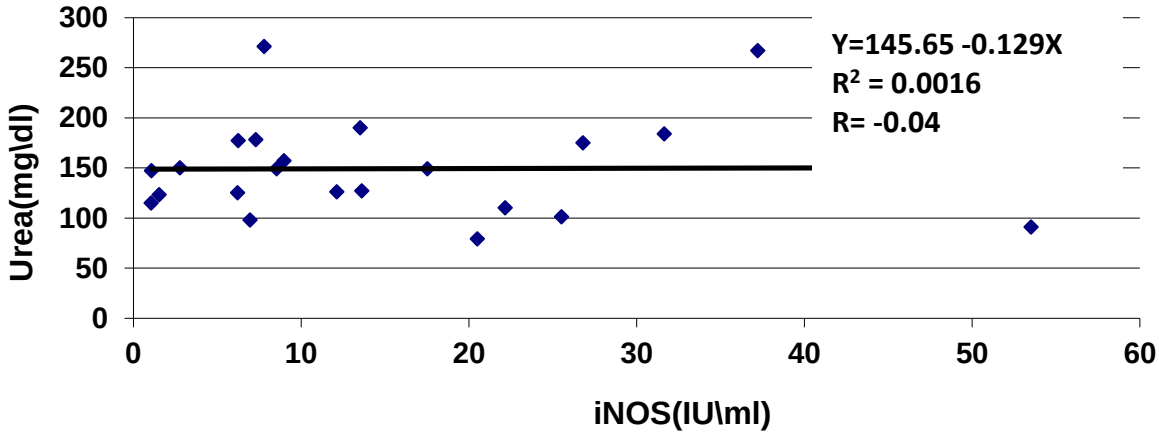


Figure(3-17): Correlation Between Urea and 4HNE For Control group.

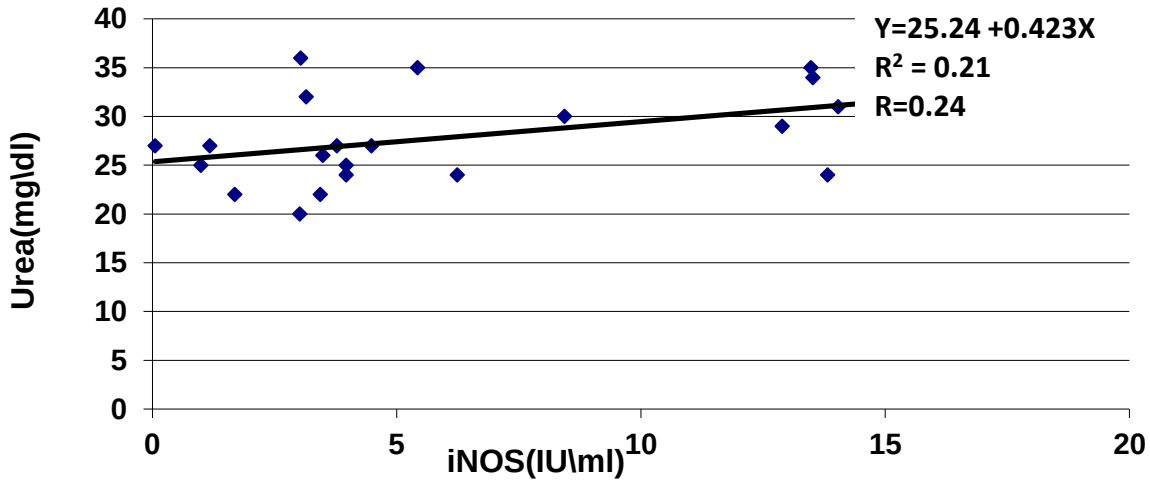
There is a positive correlation between iNOS and urea in patient and control group was observed in this study Figure(3-18) (3-19) and (3-20) which agreed with (S. R. Meenakshi.*et al*) who found a positive correlation between iNOS and urea(134).



Figure(3-18): Correlation Between Urea and iNOS in HT patients.



Figure(3-19): Correlation Between Urea and iNOS in HT with CKD patients.



Figure(3-20): Correlation Between Urea and iNOS For Control group.

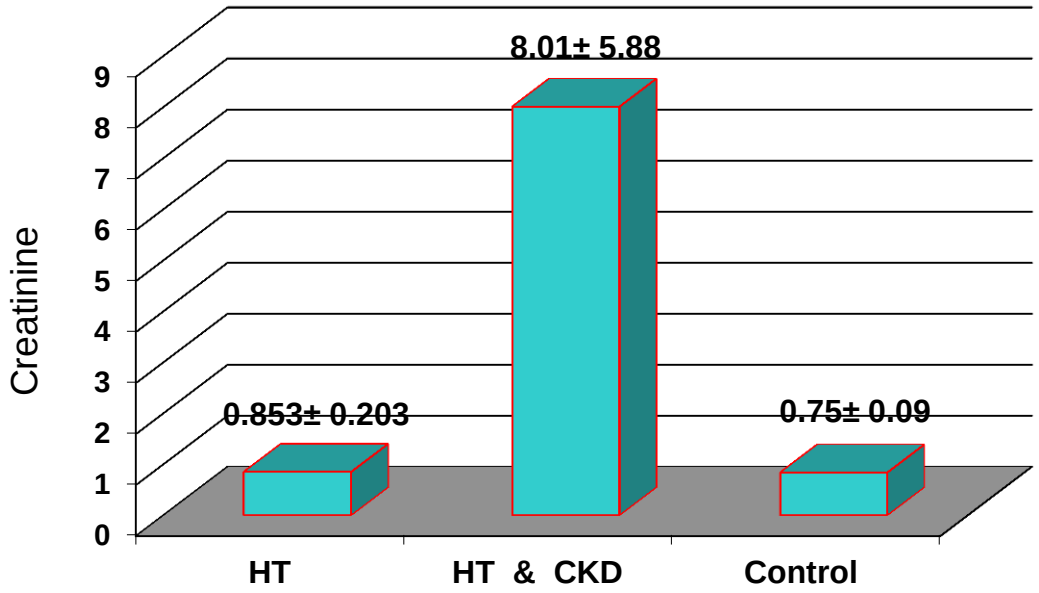
3.2.5 Serum Creatinine Level:

The mean levels of serum creatinine of both patients groups compared to control group are shown in table (3-6)in figure(3-21).

Table (3-6): Values of Creatinine in Studying Groups (mean±SD):

Group	Creatinine mg/dl	Control group n =28
Hypertension patient n=28	0.853 ± 0.203N.S	0.75 ± 0.09
Hypertension with kidney group n=28	8.01 ± 5.88 **	

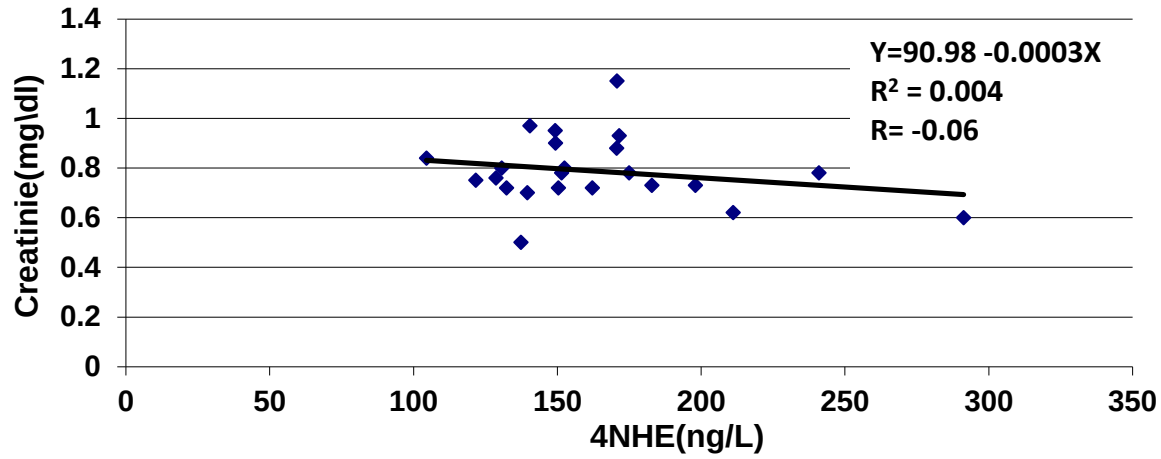
NS(Non-significant) **(p<0.01)



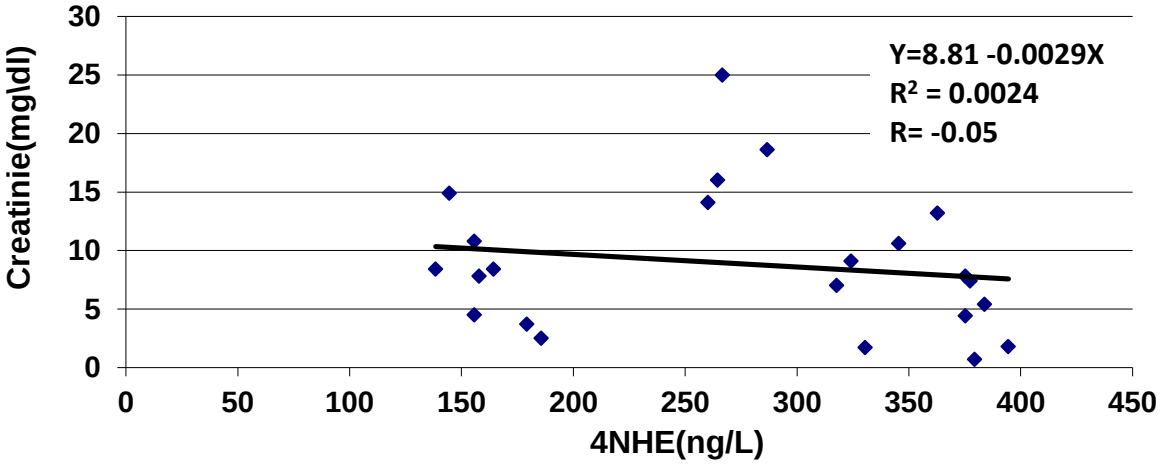
Figure(3-21): Values for Creatinine [mg/dl] in Patients and Control Groups .

The results showed a significant in Creatinine levels in patients groups when compared to control group and this agree with(Isra'a H.) (135).

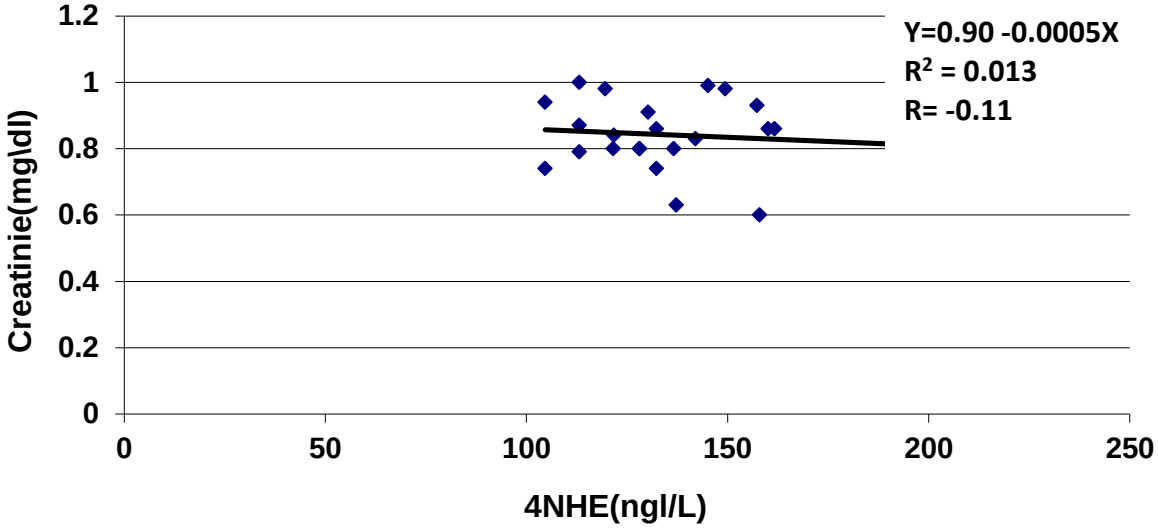
There were significant differences between patients and control in creatinine concentration This elevation of serum creatinine concentration may be attributed to the decrease in creatinine clearance due to the decrease in the GFR (136) , Creatinine produced as a waste product of muscle creatine, about 1 % of the total muscle creatine pool is converted daily creatinine through the spontaneous, nonenzymatic loss of water. Since it is released into the blood at a constant rate, and since its excretion is closely matched to glomerular filtration rate(137). The elevation of serum creatinine concentration may be attributed to the decrease in creatinine clearance due to the decrease in the GFR .



Figure(3-22): Correlation Between Creatinine and 4HNE in HT patients .



Figure(3-23): Correlation Between Creatinine and 4HNE in HT with CKD patients .

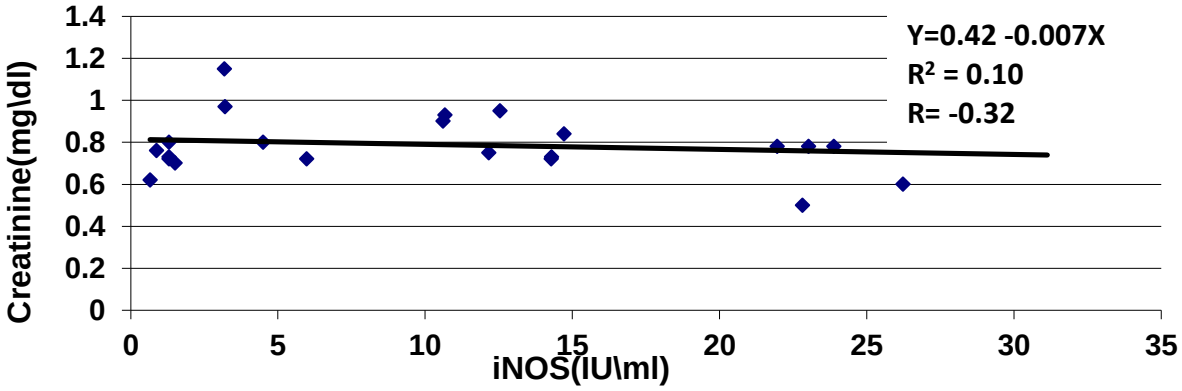


Figure(3-24): Correlation Between Creatinine and 4HNE For Control group .

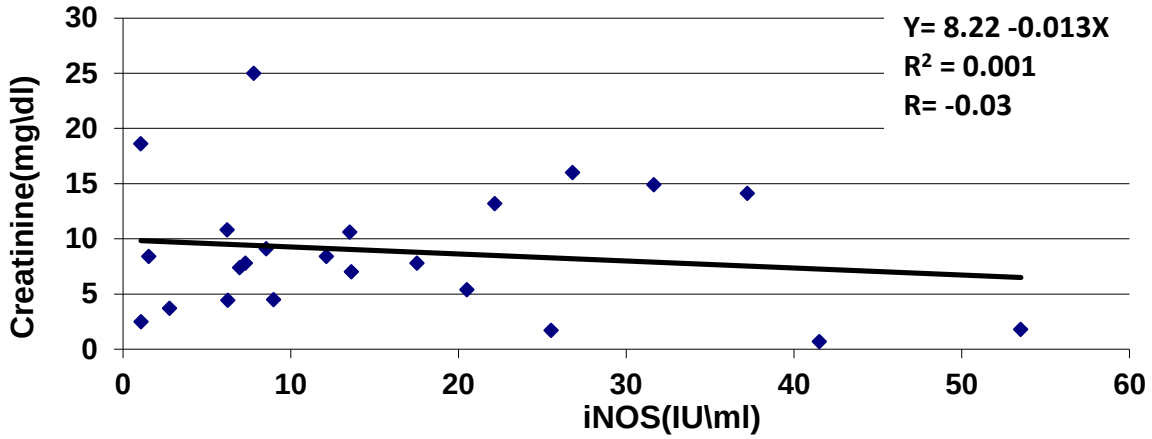
There is a negative correlation between 4HNE and creatinine in patient and control group was observed in this study which agreed with (Hiroyuki Kobori.*et al*) who found a negative correlation between 4HNE and creatinine(138) .

Creatinine, a by-product of muscle energy metabolism that, similar to urea, is filtered from the blood by the kidneys and excreted into the urine Elevated serum creatinine might be to direct effect of hypertension and its related complications on renal function. Creatinine test also provide a base line measurement of kidney function that can be used as monitoring for side effects of certain antihypertensive drugs on kidney function(139).

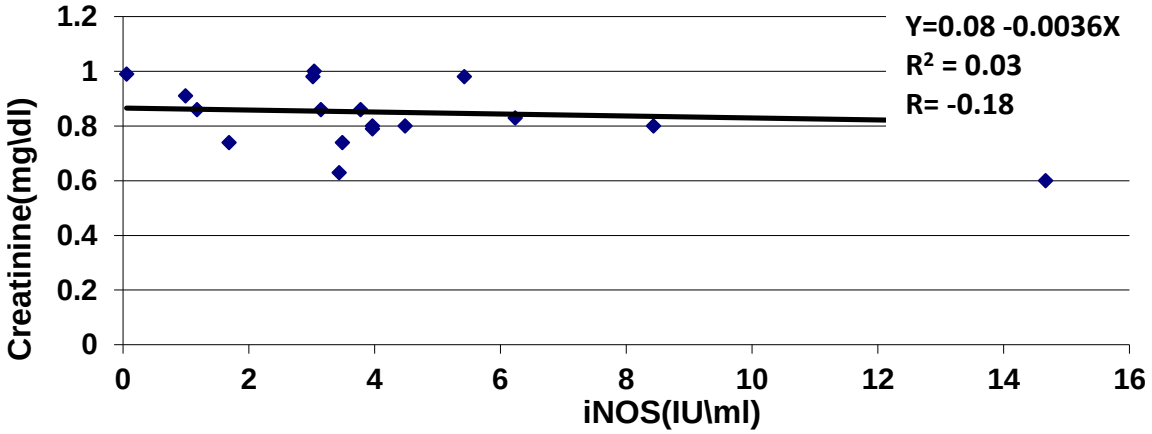
Elevation of blood creatinine is a more sensitive indicator of impaired kidney . creatinine is only minimally altered during transit through the tubule, the excretion rate approximately equals the GFR times the plasma concentration. Thus, each time the GFR decreases to one-half its starting level, the plasma concentration doubles. This estimate breaks down a little at the very lowest GFR levels where tubular secretion of creatinine plays a larger role(140).



Figure(3-25): Correlation Between Creatinine and iNOS in HT patients .



Figure(3-26): Correlation Between Creatinine and iNOS in HT with CKD patients .



Figure(3-27): Correlation Between Creatinine and iNOS For Control group .

There is also negative correlation between iNOS and creatinine in patient and control group was observed in this study which agreed with (Kwon Moo P.*et al*) who found a negative correlation between iNOS and creatinine(141) .

3.2.6 Total Serum protein Level(TSP):

The mean levels of serum Total protein of both patients groups compared to control group are shown in table (3-7).

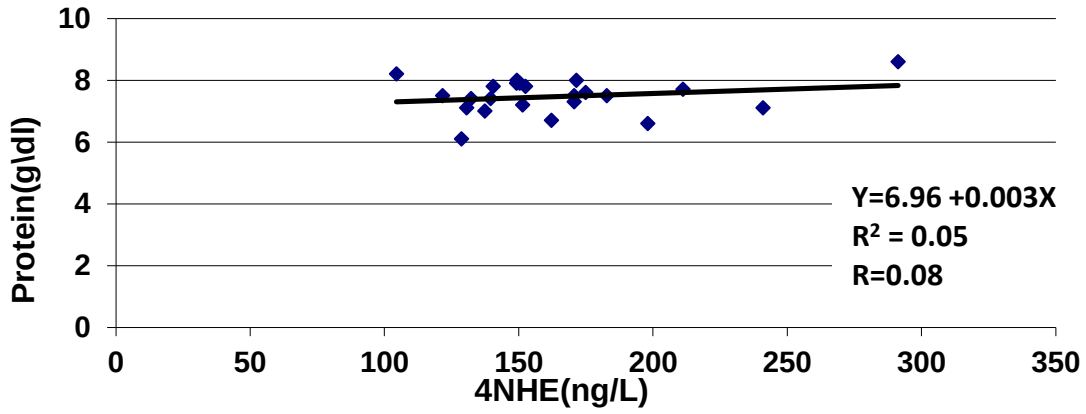
Table (3-7): Values of Total serum protein in Studying Groups (mean±SD):

Groups	Total serum protein(g\dl)	Control group n =28
Hypertension patient n=28	7.24 ± 0.55 N.S	7.80±0.49
Hypertension with kidney group n=28	6.94 ± 1.06 **	

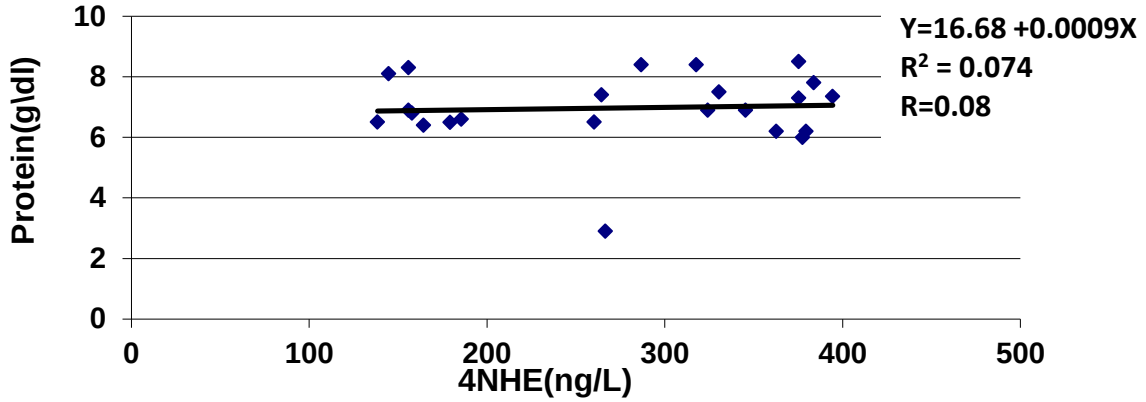
NS(Non-significant) **($p<0.01$)

The results showed a significant in TSP levels in patients groups when compared to control group and this agree with(Koenig W.et al) and(Marisol Peña S. et al) (142,143). .

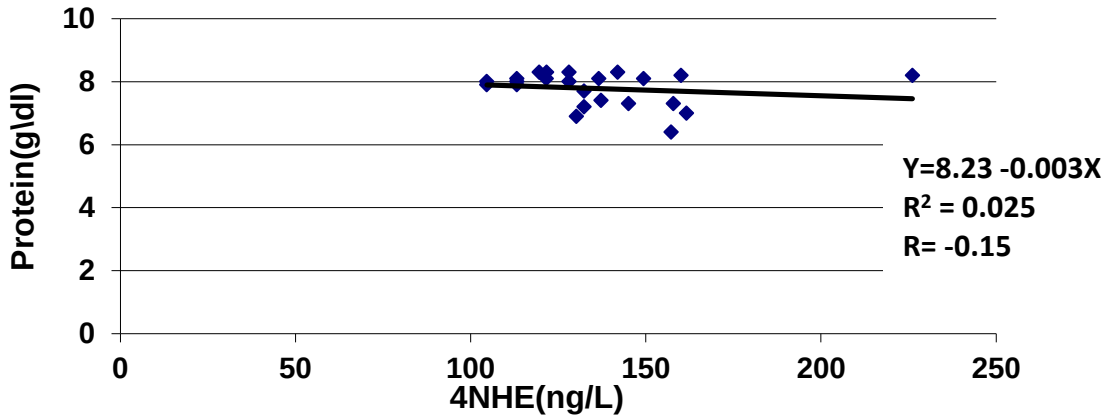
There is a positive correlation between 4HNE and Protein in patients group (p-value 0.01) and negative correlation in control group was observed in this study and this agreed with(Carlos K. et al) (144) show in Figure(3-28) (3-29) and (3-30).



Figure(3-28): Correlation Between Protein and 4HNE in HT patients .



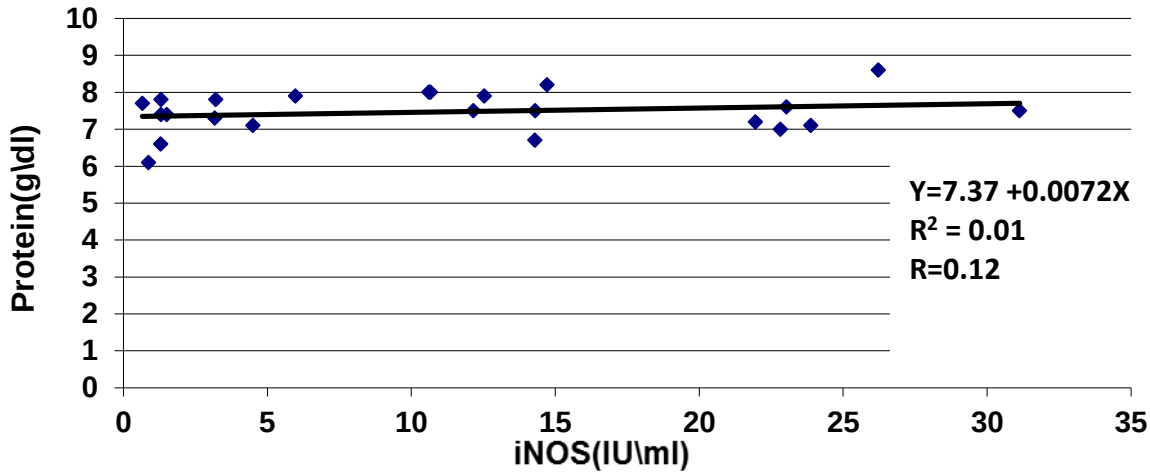
Figure(3-29): Correlation Between Protein and 4HNE in HT with CKD patients .



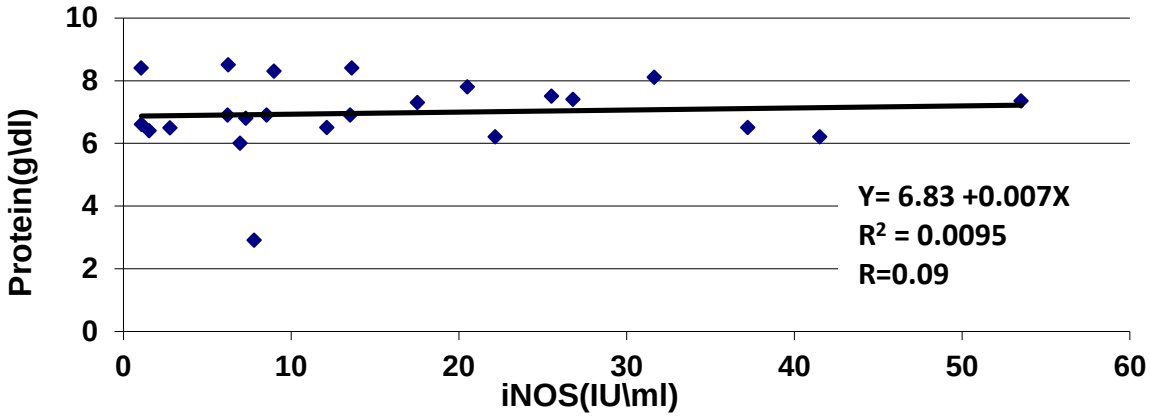
Figure(3-30): Correlation Between Protein and For Control group .

There is a positive correlation between iNOS and Protein in patient and control group was observed in this study and this agreed with (Caroline J. Smith. et al) (110) show in Figure(3-31) (3-32) and (3-33).

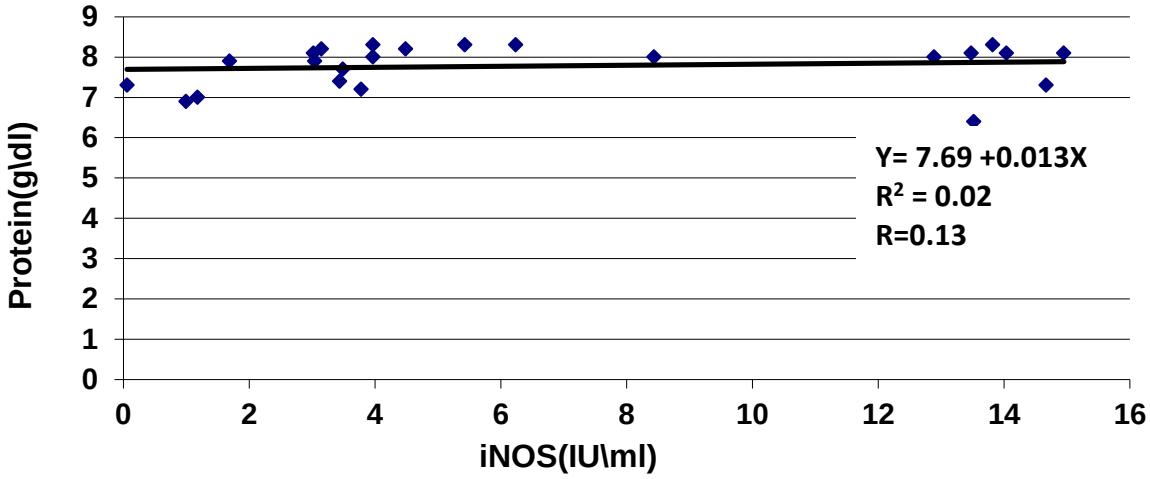
Antioxidant therapy ameliorated the CRF-induced hypertension, improved vascular tissue NO production, lowered tissue nitrotyrosine burden, and reversed down regulations of NOS isoforms. In contrast, antioxidant therapy had no effects in the controls. CRF is associated with oxidative stress which promotes NO inactivation by ROS leading to functional NO deficiency, hypertension, and widespread accumulation of protein nitration products. Amelioration of oxidative stress by high-dose vitamin E enhances NO availability, improves hypertension, lowers protein nitration products, and increases NOS expression and vascular NO production in CRF(145,146).



Figure(3-31): Correlation Between protein and iNOS in HT patients .



Figure(3-32): Correlation Between protein and iNOS in HT with CKD patients.



Figure(3-33): Correlation Between protein and iNOS Control group.

Several observational studies did not find an association between total serum protein and 4HNE,INOS. It has been suggested that the relation between total serum protein ,INOS and 4HNE may vary across sex, age

Table (3-34)Correlation coefficient between parameters in patients group

Parameters correlated in HT	**Correlation coefficient (r)**	**Parameters correlated in HT with CKD**	**Correlation coefficient (r)**
4-hydroxy-2-nenol & Urea	0.08 NS	4-hydroxy-2-nenol & Urea	-0.12 *
4-hydroxy-2-nenol & Creatinine	-0.06 NS	4-hydroxy-2-nenol & Creatinine	-0.05 NS
4-hydroxy-2-nenol & Albumin	-0.02 NS	4-hydroxy-2-nenol & Albumin	-0.06 NS
4-hydroxy-2-nenol & Total serum protein	0.08 NS	4-hydroxy-2-nenol & Total serum protein	0.08 NS
Induced Nitric oxide & Urea	-0.13 *	Induced Nitric oxide & Urea	-0.04 NS
Induced Nitric oxide & Creatinine	-0.32 *	Induced Nitric oxide & Creatinine	-0.03 NS
Induced Nitric oxide & Albumin	-0.05 NS	Induced Nitric oxide & Albumin	-0.06 NS
Induced Nitric oxide & Total serum protein	0.12*	Induced Nitric oxide & Total serum protein	0.09 NS

* (P<0.05), NS: Non-significant.

Conclusion

&

Recommendation

Conclusions

1. Oxidative stress in its markers and parameters is the main chemical manifestation of hypertension and cardiovascular diseases and related diseases in patients of this study. This conclusion was obvious in high significant difference between serum 4HNE, iNOS and albumin in patients of both HT and HT with CKD. urea, creatinine, protein field in achieve this results.
2. The association between albumine and 4Hydroxy-2-nonenal(4HNE) leads to conclude that low serum levels of albumine is very risky due to high probability of its oxidation which is exacerbate the diseases of underlying study. Also the association between 4HNE and iNOS leads to conclude the deep effect of cells from membrane to the nucleus by oxidative species.
3. Chronic renal failure(CRF) results in down regulation of renal and cardiovascular NOS isoform expressions and depressed NO production capacity. Acquired NO deficiency in CRF is compounded by ROS-mediated oxidation/inactivation of NO. These events contribute to the pathogenesis of CRF associated hypertension and the formation of protein nitration products.And we concluded from the above finding that there is adverse effect of the hypertension on renal function

future work

Future work:

1. study the BMPR2 gene in hypertension patients to define the type of disease in each patients
2. Study the effect of pollution, bombing, modern life style on the incidence of the disease
3. stud the effect of antioxidant supplement on the oxidative stress parameters in HT patients
4. study the effect of exercising on the oxidative stress statute in HT patients

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Appendix

(Subjects Questioners)

التاريخ:	
تسلسل المريض:	
رقم المريض:	
الأسم:	
العمر:	
الوزن:	الطول:
نوع المرض:	
مدة المرض:	
هل هناك أمراض ثانية؟	
هل المرض وراثي؟	
العلاج المتعاطي	
السكن:	
العمل:	
تدخين:	

الخلاصة

ارتفاع الضغط والامراض ذات الصلة بها مثل امراض الكلى تتشارك في ان احد الاسباب الرئيسية المؤدية للاصابة بها هو تزايد الجهد التاكسدي والذي بدوره يزيد من شدة المرض ويفاقم اعراضه. الجزيئات الفعالة الناتجة من الجهد التاكسدي بالاضافة الى انها تسبب تلفا بالانسجة باكسدة الجزيئات الحيوية مثل الشريط النووي والدهون والبروتينات والسكريات، فهي تؤدي الى توليد وسائط ذات تاثيرات التهابية كامنة. الهدف من هذه الدراسة هو لبحث بعض مؤشرات الجهد التاكسدي في مرضى ارتفاع ضغط الدم ومرضى ارتفاع ضغط الدم المرتبط بالفشل الكلوي المزمن . بالاضافة الى بعض المؤشرات الكيموحيوية المتعلقة بهذه الامراض.

هذه الدراسة استلزمت 84 رجل تتراوح اعمارهم من (25-06) قُسمتْ بشكل متساوي الى ثلاث مجاميع, الاولى والثانية هي لمرضى ارتفاع ضغط الدم ومرضى ارتفاع ضغط الدم مع الكلى على التوالي من مستشفى اليرموك التعليمي، والثالثة فلرجال اصحاء مثلوا مجموعة السيطرة. لكل عينة في المجموعات الثلاث, تم قياس هذه المؤشرات: 4-هيدروكسي-2-نونينال,انديوسد نايترك اوكسايد , الالبومين, اليوريا، الكرياتنين, البروتين الكلي.

قورنت النتائج بمجموعة السيطرة فمستويات 4-هيدروكسي-2-نونينال, انديوسد نايترك اوكسايد في الدم سجلت ارتفاعا معنويا ($p<0.01$) في كلا المرضين مقارنة بمجموعة السيطرة. بينما سجل الالبومين انخفاضا معنويا ($p<0.01$) في مستوياته. يظهر البروتين الكلي في الدم بشكل ملحوظ المستويات اقل ($p<0.01$) في كل من المجموعتين.

الارتفاع في مستويات مؤشرات الجهد التاكسدي يعزى الى التلف التاكسدي في الانسجة مما يسبب هذه الامراض الالتهابية. استنتج من هذه الدراسة بان هناك علاقة ايجابية بين الاكسدة الناتجة من هذه الامراض وتفاقمها وان التلف الناتج من الاكسدة يشمل النظام الدفاعي والجزيئات الحيوية . وتقترح الحاجة لزيادة اخذ مضادات الاكسدة كاجراء وقائي من هذه الامراض.

جمهورية العراق

وزارة التعليم العالي والبحث العلمي

جامعة النهرين

كلية العلوم

قسم الكيمياء

مصل 4-هيدروكسي-2-نونينال واوكسيد النتريك المستحث في مرضى ارتفاع ضغط الدم ومرضى ارتفاع ضغط الدم مع الكلى

رسالة

مقدمة إلى كليه العلوم ,جامعة النهرين , وهي جزء من متطلبات نيل درجه الماجستير في علوم الكيمياء/(الكيمياء الحياتية).

من قبل

علي ظافر حميد

بكالوريوس علوم كيمياء/كلية العلوم/جامعة النهرين

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بأشراف

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علاء حسين جواد

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