جامعة بابل كلية

العلوم للبنات علوم

الحياة

Study of the level of the oxidation of lipids and state of oxidative stress in patients with renal failure.

أعداد الطالبات:_

1.آمنه هاشم حسن نعمه

2.آمنه محمد عبد علوان

3.أنتضار عالء عبد االمير حسن

أشراف / أ. م. د. داخل عبد الغاني

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إهداء

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إلهي لايطيب الليل إلا بشكرك ولايطيب النهار إلا بطاعتك ..
ولاتطيب اللحظات إلا بذكرك .. ولا تطيب الآخرة إلا بعفوك ..
ولا تطيب الجنة إلا برؤيتك

الله جل جلاله

إلى من بلغ الرسالة وأدى الأمانة .. ونصح الأمة .. إلى نبي الرحمة ونور العالمين

سيدنا محمد صلى الله عليه وسلم

إلى من كلله الله بالهيبة والوقار .. إلى من علمني العطاء بدون انتظار .. إلى من أحمل أسمه بكل افتخار ..

والدي العزيز

إلى ملاكي في الحياة .. إلى معنى الحب وإلى معنى الحنان والتفاني .. إلى بسمة الحياة وسر الوجود
إلى من كان دعائها سر نجاحي وحنانها بلسم جراحي إلى أغلى الحبايب

أمي الحبيبة

Abstract

Oxidative stress is at play in the progression of chronic renal failure (CRF) and in the genesis of atherosclerosis. The aim of the present study was to evaluate the factors that might influence the oxidative antioxidative balance in patients on hemodialysis. The study group was consisted of 64 hemodialysis patients due to CRF. Twenty-two healthy subjects constituted a control group. We measured changes in serum superoxide dismutase and glutathione peroxidase activity, and malondialdehyde levels in chronic renal failure patients and compared with healthy control groups. Superoxide dismutase and glutathione peroxidase activity, and malondialdehyde levels were assayed with spectrophotometric methods. Superoxide dismutase activity of CRF patients group were higher than those of control group ($p<0.001$). Glutathione peroxidase activity of CRF patients group were lower than those of control group ($p<0.001$). Malondialdehyde levels in hemodialysis patients were higher than those of control group ($p<0.01$). Several studies of SOD activity in chronic renal failure patients have found conflicting results. We propose that the increased SOD activity could be a protective mechanism for the cells due to the hyperproduction of free radicals in chronic renal failure. Decreased serum antioxidant activity in CRF patients on hemodialysis may contribute to the increased oxidative damage and in the development of renal complications. This study indicates the existence and increased production of oxidative stress resulting from hemodialysis and disturbance in antioxidant enzyme system. Our results supports that an increase in oxidative stress may be considered as one of the major risk factors in chronic renal failure patients.

Key words: SOD, GPx, Malondialdehyde, , Antioxidant enzymes

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1 | INTRODUCTION

Chronic Renal Failure (CRF) is a condition resulting from a permanent and typically progressive reduction in renal function, reaching a degree sufficient to adversely affect other bodily systems.[1] In developing countries, the awareness and societal impact of CRF have been highlighted in the last decade. In India, the incidence of CRF is not well-documented due to the absence of a national registry and data on its occurrence. It has been estimated that the prevalence of CRF in India may be as high as 785 people per million populations.[2]

CRF leads to various complications over time, with the most common ones being cardiovascular, cerebrovascular, and peripheral vascular diseases. Deaths due to cardiovascular complications are 4-20 times higher in CRF patients than any other cause in the general population.[3] These complications arise from metabolic and endocrine disturbances, with dyslipidemia being a constant feature of CRF. Lipid abnormalities can be detected as early as renal function begins to decline (Glomerular Filtration Rate (GFR) < 50 ml/min), but the type and severity vary among different patients. [3,4]. CRF patients are also subjected to oxidative stress.

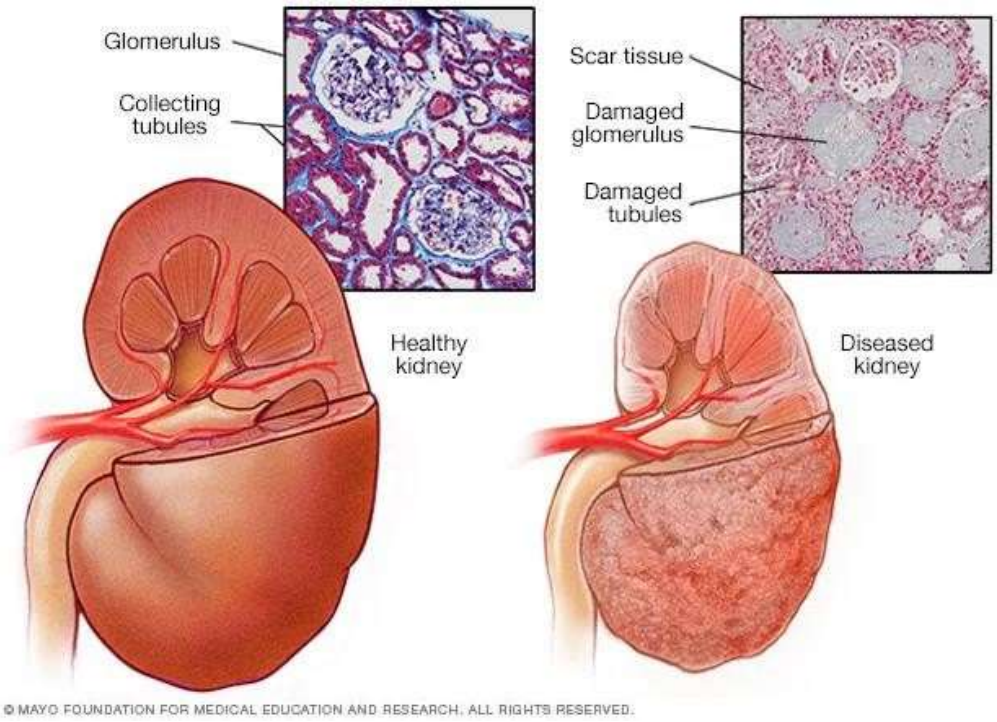


Figure 1. Healthy kidney vs. diseased kidney

oxidative stress:

Defined as tissue damage resulting from an imbalance between the excessive generation of oxidant compounds and insufficient antioxidant defense mechanisms.[9] The generation of oxidative compounds is physiologically relevant as an essential step in inflammation, tissue repair processes, and defense against invading microorganisms and malignant cells. However, in pathological situations like uremia, improper or maladaptive activation of oxidative processes may be chronically present, contributing to cell and tissue injury [10].

Sources of oxidative stress:

Include the mitochondrial respiratory chain, representing the most powerful cellular source of oxidants in the body. Mitochondrial oxidants may exert deleterious effects, contributing to cellular senescence and neurodegenerative diseases. However, to date, there is no available method to determine their potential contribution to cellular pathology.

The phagocyte oxidant generation system is based on the inducible production of reactive oxygen species (ROS) via univalent reduction of molecular oxygen (O2). Following exposure to appropriate stimuli, both

Polymorphonuclear neutrophils (PMNs) and monocyte-macrophages undergo activation and increase their oxygen (O2) consumption during a process known as the respiratory burst. The NADPH-oxidase enzyme system, situated on cellular membranes, reduces O2 to superoxide anion ($O2^-$), which is highly unstable and rapidly converts into hydrogen peroxide (H2O2). Both O2 and H2O2 serve as precursors for the production of more potent oxidants. O2 interacts with nitric oxide (NO) to generate highly reactive nitrogen species (nitrosamine stress), while H2O2 reacts with intracellular iron to produce hydroxyl radicals (OH^-), implicated in cell membrane lipid degradation, protein aggregation, and DNA damage. Additionally, H2O2 serves as the substrate for myeloperoxidase (MPO), leading to the production of chlorinated oxidants. In the presence of chloride ions (Cl^-), MPO converts H2O2 into hypochlorous acid (HOCl), a potent compound capable of oxidizing various molecules, including lipids, proteoglycans, and other membranous or intracellular constituents, particularly the Thiol groups of membrane proteins (chlorinate stress). Moreover, it may react with endogenous

amines (R-NH2) to produce chloramines (RNH-Cl). Reactive oxygen species (ROS) are released along with pro-inflammatory cytokines, which further amplify oxidant generation [8].

Markers of oxidative stress:

Oxidants are highly reactive compounds with a half-life of only seconds. Therefore, their in vivo determination is generally not feasible. In contrast, lipids, proteins, carbohydrates, and nucleic acids, after being modified by oxy-radicals, have lifetimes ranging from hours to weeks, which makes them ideal markers of oxidant stress (Table 1) [11].

Markers of Oxidative Stress	Antioxidants
• Lipid peroxidation Acrolein • Malonyldialdehyde 4-Hydroxynonenal • Thiobarbituric acid-reactive substances F2-isoprostanes • Advanced lipid oxidation products • Oxidized LDL antibodies • Protein oxidation • Advanced oxidation protein products • Carbohydrate oxidation • Advanced glycosylation endproducts • Nucleic acid oxidation 8-Hydroxy-29-deoxyguanosine	• Enzymatic • superoxide dismutase Catalase • Glutathione peroxidase • Non-enzymatic Glutathione • Vitamin E Vitamin C Ferritin • Transferrin Albumin • etc.

Table 1. Markers of Oxidative stress and antioxidants

During lipid peroxidation, unstable hydroperoxides, resulting from peroxyl radical-dependent chain reactions among unsaturated fatty acyl moieties, break down into smaller and more stable products, such as aldehydes (e.g., acrolein, malonyldialdehyde (MDA), 4-hydroxynonenal (HNE)), or thiobarbituric acid-reactive substances (TBARS). F2-isoprostanes primarily result from arachidonic acid oxidation and can serve as stable markers of free-radical attack on cell membrane phospholipids in vivo [10]. Additionally, increased levels of advanced lipid oxidation end-products (ALEs) and the presence of specific antibodies directed against oxidized low-density lipoproteins (LDLs) may serve as useful markers of enhanced oxidative stress [12]. Proteins are elective targets of oxidant-mediated injury, leading to cross-linking and aggregation products that may be resistant to proteolysis. Despite the historical difficulty in finding markers of protein oxidation, Witko-Sarsat et al. [13] identified advanced oxidation protein products (AOPPs) in uraemic patients. AOPPs, analogous to advanced glycation end-products (AGEs), share several homologies with AGEs. AOPPs, closely related to markers of monocyte activation, may serve as mediators of inflammation and positively correlate with dityrosine and AGE pentosidine plasma concentrations, indicating oxidant-mediated damage. The strong relationship between AOPPs and AGEs led to the concept of carbonyl stress, where oxidation acts together with glycation in the formation of AGEs [12].

Oxidative compounds may also interact with nucleic acids, contributing to mutagenesis and oncogenesis. Oxidative damage of leukocyte nucleic acids has been demonstrated in end-stage renal disease (ESRD). The determination of the 8-hydroxy-29-deoxyguanosine (8-OHdG) content by high-performance liquid chromatography (HPLC) was used to evaluate leukocyte DNA damage [14]. 8-OHdG levels were found to be elevated in chronic renal insufficiency (CRI), with the highest levels observed in ESRD.

To counteract the harmful effects of reactive oxygen species (ROS), both enzymatic and non-enzymatic anti-oxidant systems are naturally present. Superoxide dismutase (SOD) is the first line of enzymatic anti-oxidant defense, accelerating the dismutation rate of O2 to H2O2. Catalase reduces H2O2 to water, while selenium-containing glutathione peroxidase (GSH-Px) reduces organic lipid peroxides, requiring GSH as a hydrogen donor [12].

The most active non-enzymatic antioxidant is represented by GSH itself, acting as a scavenger for H2O2, OH, and chlorinated oxidants. Vitamin E protects the cell membrane from lipid peroxidation by forming a low-reactivity tocopheroxyl radical. Vitamin C directly scavenges O2 and OH. Inflammatory proteins such as ferritin, transferrin, and even albumin exert a non-enzymatic anti-oxidant effect by sequestrating transition metal ions [12]. Despite the lack of standards and conflicting results, the estimation of the anti-oxidant status of CRI patients comprises the measurement of different compounds of the antioxidative system in plasma and cells. The determination of plasma levels of vitamin C and GSH-Px, along with the erythrocyte content of SOD, GSH, GSH-Px, and vitamin E, has been applied successfully, revealing antioxidant deficiency in CRI.

2 | DISCUSSION

Each kidney possesses a characteristic bean-like shape, enveloped by a thin layer of fibrous tissue known as the capsule. The capsule intimately covers the kidney tissue, and in a healthy kidney, it can be easily stripped off. However, in certain diseases, the capsule becomes adherent.

The kidney exhibits a convex lateral margin and a concavity on the medial side known as the hilum. The hilum leads into a space called the renal sinus, occupied by the upper expanded part of the ureter called the renal pelvis.

Within the renal sinus, the pelvis divides into two (or three) parts known as major calyces. Each major calyx further divides into several minor calyces (Fig. 18.1), with the end of each minor calyx shaped like a cup. A projection of kidney tissue, called a papilla, fits into each cup. Kidney tissue comprises an outer part called the cortex, and an inner part called the medulla.

Medulla:

The medulla consists of triangular areas of renal tissue referred to as renal pyramids (Fig. 18.1). Each pyramid has a base directed towards the cortex and an apex (or papilla) directed towards the renal pelvis, fitting into a minor calyx. Pyramids exhibit striations passing radially towards the apex.

Cortex:

The renal cortex includes:

1. Tissue lying between the bases of the pyramids and the kidney's surface, forming the cortical arches or cortical lobules. This part displays light and dark striations, with the light lines known as medullary rays (Plate 18.1).
2. Tissue lying between adjacent pyramids, forming the renal columns.

Each pyramid is surrounded by a 'shell' of cortex, constituting a lobe of the kidney. This lobulation is evident in the fetal kidney.

The Uriniferous Tubules:

From a functional standpoint, the kidney can be viewed as a collection of numerous uriniferous tubules specialized for urine excretion. Each uriniferous tubule consists of an excretory part called the nephron and a collecting tubule. Collecting tubules from different nephrons join to form larger tubules called papillary ducts (of Bellini), each opening into a minor calyx at the apex of a renal papilla. Each kidney contains one to two million nephrons.Urinary tubules are held together by scanty connective tissue, with blood vessels, lymphatics, and nerves located within this connective tissue.

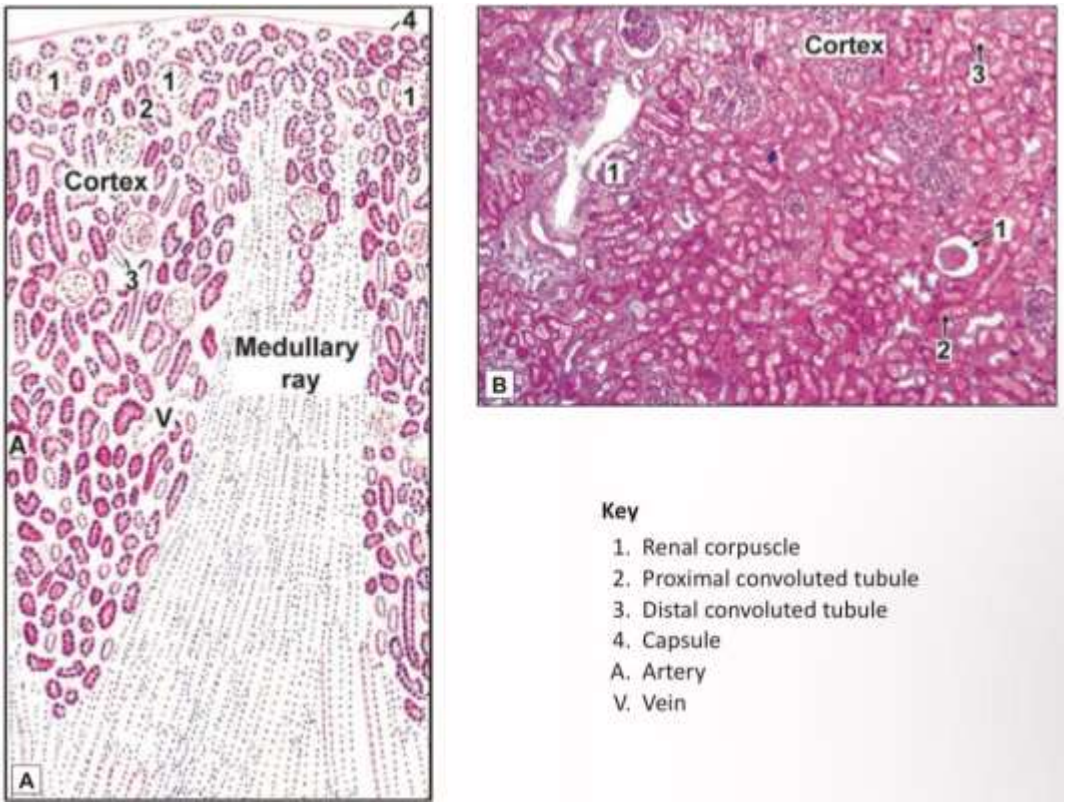
Added Information

Interstitial Tissue of the Kidney

Most of the interstitial space in the renal cortex is occupied by blood vessels and lymphatics. In the medulla the interstitium is composed mainly of a matrix containing proteins and glycosaminoglycans. Collagen fibres and interstitial cells are present.

It has been held that interstitial cells produce prostaglandins, but it now appears that prostaglandins are produced by epithelial cells of collecting ducts.

2.1 | The Urinary System



Kidney (Low Magnification)

A. As observed in the drawing,

B. Photomicrograph of cortex

The kidney is enveloped by a capsule. Beneath the capsule lies the cortex, and below the cortex is the medulla of the kidney. In the cortex, circular structures known as renal corpuscles surround tubules cut in various shapes.

The dark pink stained tubules represent parts of the proximal convoluted tubules (PCT), characterized by a small and indistinct lumen. These tubules are lined by cuboidal epithelium with a brush border. Lighter staining tubules, each with a distinct lumen, correspond to the distal convoluted tubules (DCT) and are lined by simple cuboidal epithelium. PCTs are more numerous than DCTs.

Within the medulla, elongated, parallel-running tubules with very light staining are visible. These tubules are identified as collecting ducts and the loop of Henle. Some extend into the cortex, forming a medullary ray. Collecting ducts are lined by

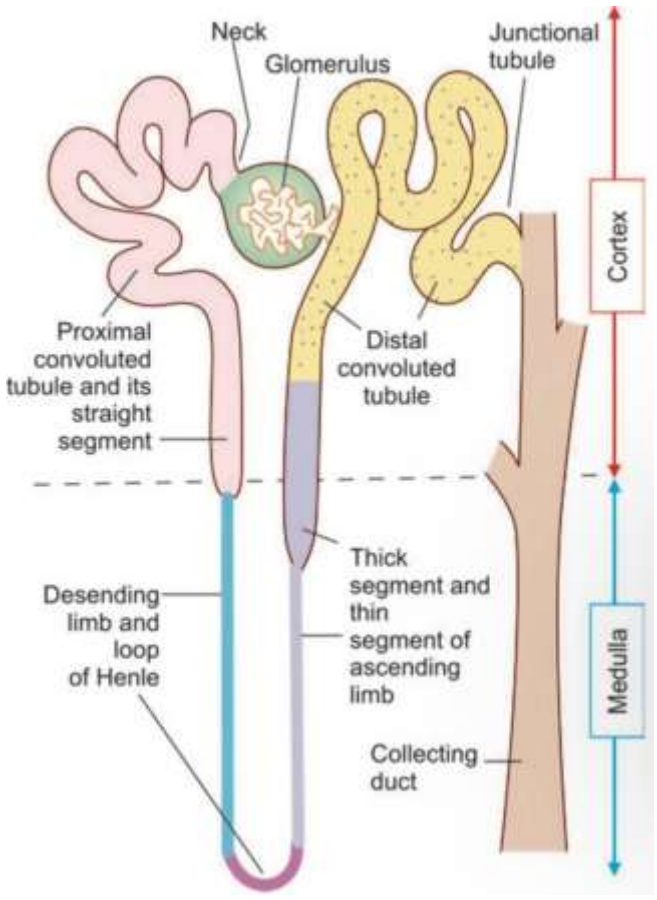
simple cuboidal epithelium, while the loop of Henle (thin segments) is lined by simple squamous epithelium.

Cut sections of blood vessels are evident both in the cortex and medulla.

2.2 | Nephron

The nephron serves as the structural and functional unit of the kidney, with approximately 1–4 million nephrons present in each kidney. This vital unit is composed of a renal corpuscle (or Malpighian corpuscle) and an intricate renal tubule. The renal tubule is divided into three essential segments: the proximal convoluted tubule, the Loop of Henle, and the distal convoluted tubule (Fig. 18.2).

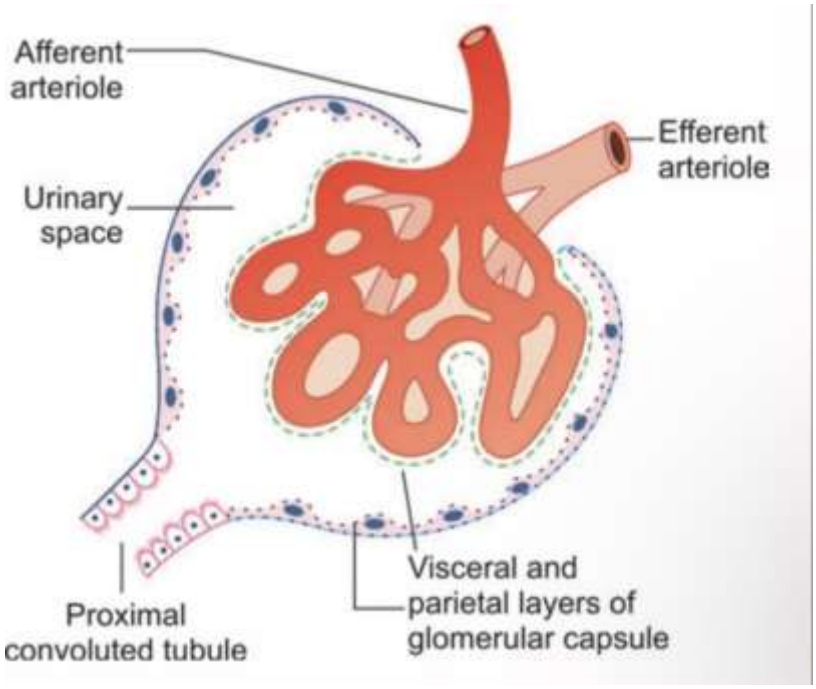
The renal corpuscle is situated in the kidney's cortex, positioned either near the periphery or close to the medulla. Depending on the location of the renal corpuscle, nephrons are classified into two types: Cortical nephrons or superficial nephrons (with corpuscles in the outer cortex) and Juxtamedullary nephrons (with corpuscles in the inner cortex near the medulla or corticomedullary junction).



Renal corpuscles, along with the greater portions of the proximal and distal convoluted tubules, are located in the cortex of the kidney. Conversely, the loops of Henle and the collecting ducts are situated within the medullary rays and the substance of the pyramids.

2.3 | The Renal Corpuscle

The renal corpuscle is a rounded structure consisting of (a) a rounded tuft of blood capillaries called the glomerulus and (b) a cup-like, double-layered covering for the glomerulus called the glomerular capsule (or Bowman's capsule) (Fig. 18.3). The glomerular capsule represents the cup-shaped blind beginning of the renal tubule. Between the two layers of the capsule, there is a urinary space that is continuous with the lumen of the renal tubule.



2.4 | Renal Cortex

In the high-power view of the renal cortex, large renal corpuscles can be identified. The renal corpuscle consists of a tuft of capillaries that form a rounded glomerulus and an outer wall, the glomerular capsule (Bowman's capsule). A urinary space between the glomerulus and the capsule is seen. Proximal convoluted tubules are dark staining, lined by cuboidal cells with a prominent brush border. Their lumen is indistinct. Distal convoluted tubules are lighter staining. The cuboidal cells lining them do not have a brush border, and their lumen is distinct.

2.5 | Kidney Problems

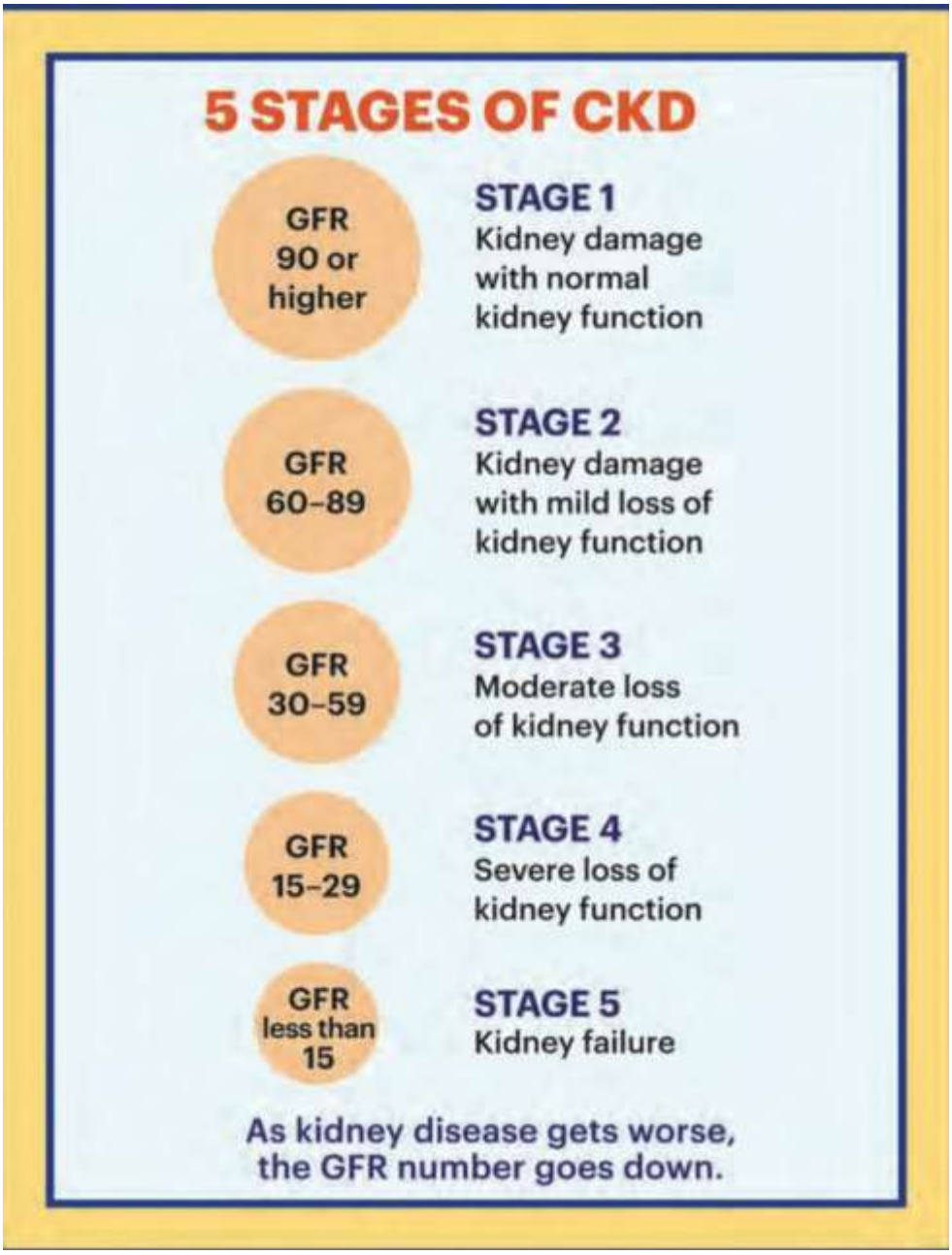
Kidneys can get damaged. When kidneys are damaged, they cannot do all the things they should. This is called chronic kidney disease or CKD. Chronic kidney disease can affect anyone—young or old! 1 in 7 American adults has kidney disease and most don't know it. Chronic kidney disease doesn't happen overnight! It happens slowly, and in stages. People with early kidney disease may not know anything is wrong. They cannot feel the damage before some kidney function is lost.

Risk factors include:

- Diabetes
- High blood pressure
- A family history of kidney failure
- Being age 60 or older
- African American, Hispanic, Asian, Pacific Islander, or American Indian
- Obesity

There are 5 stages of chronic kidney disease.

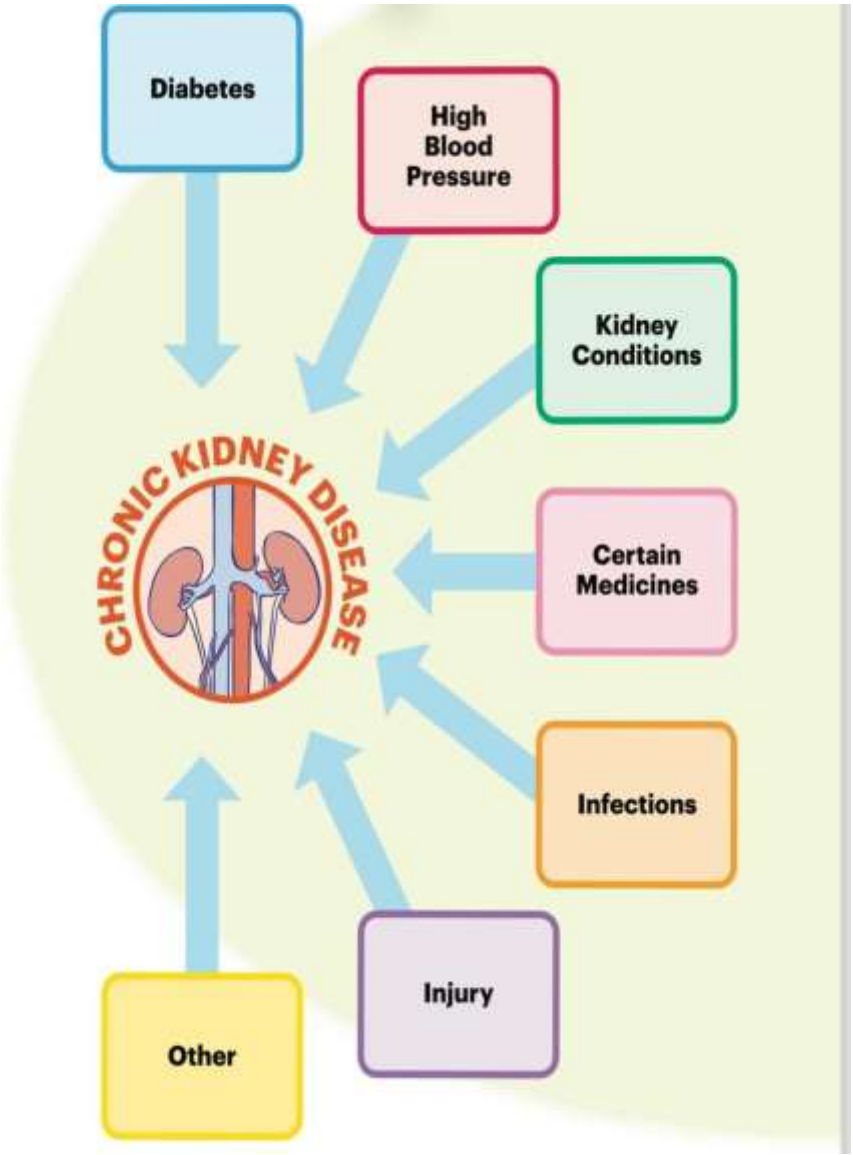
In each stage, the kidneys don't work as well as the stage before. The stages are determined by the level of kidney function. Kidney function is measured by a test called glomerular filtration rate, or GFR. This number tells how well the glomeruli are filtering waste and extra fluid. A person can lose a lot of kidney function before feeling symptoms of kidney disease. When kidneys fail, a person needs a kidney transplant or dialysis to stay alive. A kidney transplant replaces a failed kidney with a healthy kidney from someone else. Dialysis uses a machine or other equipment to filter the blood.



People with kidney damage for 3 months or more usually have chronic kidney disease.

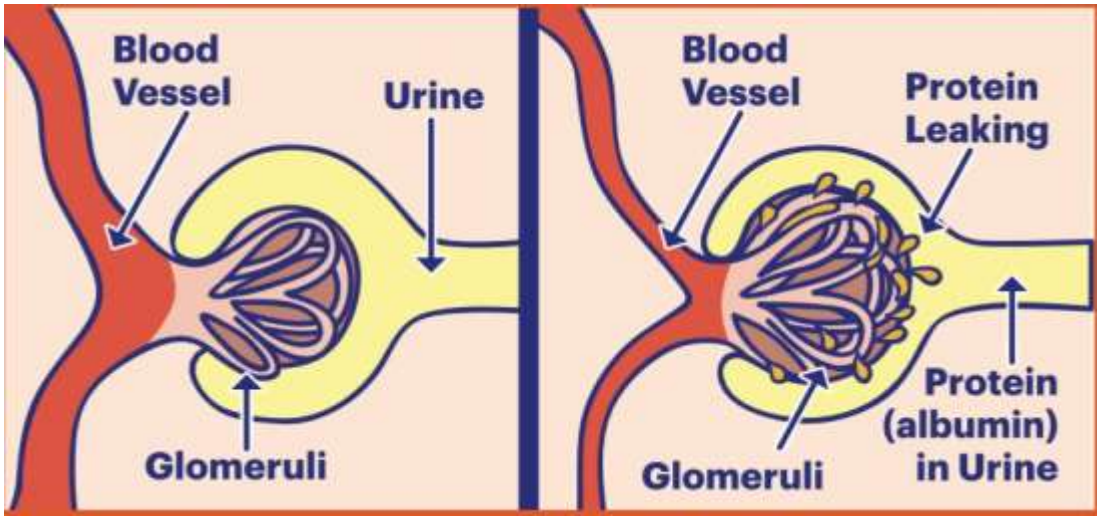
AND People with a GFR less than 60 for 3 months or more usually have chronic kidney disease.

What can Damage the kidneys?



Diabetes

Diabetes is the most common cause of kidney disease. • Diabetes is a chronic disease where the body cannot control sugar. A high sugar level in the blood damages the small filters (glomeruli) in the kidneys. • In people with diabetes, kidneys do not filter as well. They are more likely to filter out any amounts of albumin into the urine instead of keeping it in the blood. Albumin is a type of protein needed by the body. Having protein in the urine is a sign of kidney damage. When diabetes is controlled, there is less chance of damage to the kidneys.



High Blood Pressure

• High blood pressure is the second most common cause of chronic kidney disease.

• High blood pressure makes the kidneys work harder. This can damage the filters. Even a little rise in blood pressure is dangerous.

• People with high blood pressure can also have protein in the urine. Protein in the urine can mean the kidneys are damaged.

2.5 | Discussion & Conclusion

It was shown that ROS and other oxidants could be also formed in the normal physiological process (Onder and Gurer, 2001). Increased ROS, in turn, enhance LPO products, thus, lead to tissue injury (Yazic *et. al*., 2004). H2O2 and other derivatives of peroxides increase in some conditions, diffuse into plasma. Here, antioxidant components of plasma overwhelm them, and they are simultaneously consumed (Young and Woodside, 2001).In this study, a significant increase in plasma MDA level was observed in patients compared to control that were similar to findings of the investigators (Mimić-Oka *etal.,* 1999) who suggested that the increase in MDA levels could be due to increased oxidative stress in kidney from various sources or decrease in antioxidant defense mechanism and vice versa. The increase in serum iron is in agreement with the report of John *et al*., 1993 and this can also perpetuate the free radical damage. Our studies revealed a positive correlation of MDA with raised serum creatinine reflection of the degree of renal failure, which corroborates with similar work where it has been concluded that the level of severity of disease process is reflected by the serum lipid peroxide level (Martin-Mateo *et al*., 1999).Many antioxidant molecules found in blood prevent or inhibit the harmful effects of free radicals (Young and Woodside, 2001). Whenever there is a decrease in antioxidants and/or an increase in oxidants, oxidant/antioxidant balance is impaired in favor of oxidants and this is known as oxidative stress (Abuja and Albertini, 2001). It is known that oxidative stress is responsible for tissue injury in many diseases and contributes to the development of atherosclerosis (YlaHerttuala *et. al,* 1994). Evidence of an imbalance between appears to be a state of increased oxidative stress (Mimić-Oka *et al.,* 1999), has been abundantly documented in chronic renal failure. Massive oxidative stress might increase both lipid peroxidation and protein oxidation level. Alterations in lipid metabolism are well recognized as major risk factors for long-term complications in chronic renal injury (Haugen and Nath,1999). However, under the conditions of severe oxidative stress, radical generation at inappropriate sites also leads to protein modification since they are essential targets for free radical attack, both intracellularly and extracellularly Davies, 1987). Proteins may be damaged by specific interactions of oxidants or free radicals with particularly susceptible amino acids. The results presented in this study demonstrated that the concentrations of protein in plasma were markedly reduced ($p<0.001$) in individuals with renal failure compared with

healthy subjects. These results support hypothesis of Inagi *etal*., 1999, that uremia appears to be in a state of increased oxidative stress with potentially damaging proteins.Blood has an important role in the oxidant/antioxidant balance, as it carries and distributes antioxidants through the body (Ghiselli *etal*., 2000). Plasma has various antioxidant molecules. Antioxidants, like vitamin C, vitamin E, albumin and uric acid are the major antioxidant components of plasma (Erel, 2004).In normal healthy controls, albumin is an important chain breaking extracellular antioxidant (Mee-Kyuing and Il-Han, 1996) which has several biological functions, particular as a ligand binder (Sengupta *et al*., 2001). Since albumin is the main source of thiol groups in plasma (estimated to be as high as 500 µmol/L). Thiol groups, on the surface of albumin, bind oxidants. Albumin provides the bulk of"total plasma thiols". Although the thiol groups are oxidized during oxidative stress. Thus, under these conditions, free radicals mediated oxidation and poor degradation of albumin may lead to accumulation of oxidatively modified albumin with lowered capacity to bind uraemic toxins and other protein bound substances (Jasmina et. al., 2001). Low level of albumin can cause oxidative stress via leading to increase oxidants like homocystein (Sengupta *et al*., 2001).In this study, serum albumin concentrations were significantly reduced in renal failure patients when compared to controls Hypoalbuminemia was reported in end stage renal failure patients and was identified in many studies as the strongest mortality and morbidity predictor (Yeun and Kaysen, 1998).A negative correlationship was observed between MDA and albumin This supports the hypothesis of Sengupta et al., 2001 who suggested that decrease in the levels of this antioxidant accelerate the lipid peroxidation thereby generating more MDA.Low levels of serum vitamin E and plasma vitamin C($p<0.001$) were observed in study cases compared to control .This is in accordance with studies of (Evelyne *et al*.,1994),who demonstrated that there was a significant drop in vitamins E and C,whereas lipid peroxide were significantly higher in chronic renal failure patients,compared with control. A negative correlationship were bserved between MDA and Vitamin E ($r=-0.53$, $p<0.05$), Vitamin C ($=-0.47$, $p<0.05$) which is similare of Kato *et al*., 2007 who demostrated that the reduction in serum vitamin levels of E and C because of utilization during oxidative stress.Uric acid has a strong antioxidant activity and its concentration in the plasma is about ten fold than antioxidants like vitamin C and vitamin E (Ghiselli *et al*., 2000).As expected the serum uric acid levels were elevated in renal failure patients compared to controls The elevated of uric acid levels are thought to compensate for the high levels of ROS

(Racek.*et al*., 1997). Similarly some studies have found significant elevated levels of uric acid in patients of renal failure as compared to control group (Ghiselli *et al*., 2000). However, whereas uric acid is considered an antioxidant, it is also prooxidative under certain conditions, especially when other anti oxidants are at low level (Abuja, 1999).In summary, the findings in the present study suggested that the dynamic balance between Formation of ROS, and defense mechanisms that creates oxidative stress thereby, leading to the oxidative damage in the renal function (Templar *et al*., 1999; Sharma *et al*., 2000). There are a great number of metabolic derangements in the course of CRF which become intensified in end-stage of renal disease, when dialysis is required Impairments of enzymatic system have been reported such as glutathione peroxidase (GSH-Px) or superoxide dismutase (SOD), deficiency of selenium, zinc, copper, vitamins A, C, and E, and also diminished glutathione concentration in chronic renal failure Oxidative stress is another accompaniment of CRF. Gerardi et al and Marnett have found increased serum concentrations of lipid peroxidaion products malondialdehyde and 4-hydroxynonenal in hemodialysis patients. Mezzano et al have revealed increased concentration of thiobarbituric acid reactive substances , which is a product of lipid peroxidation, and a higher level of advanced oxidation protein products in patients with uraemia. Nguyen-Khoa et al have shown, in addition to high levels of thiobarbituric acid reactive substances and advanced oxidation protein products, a decreased activity of superoxide dismutase and glutathione peroxidase Biochemical parameters and the measured parameters are being presented in**.** Serum malondialdehyde levels, the biomarker of lipid peroxidation, were significantly increased in HD patients compared to healthy subjects ($p<0.01$)

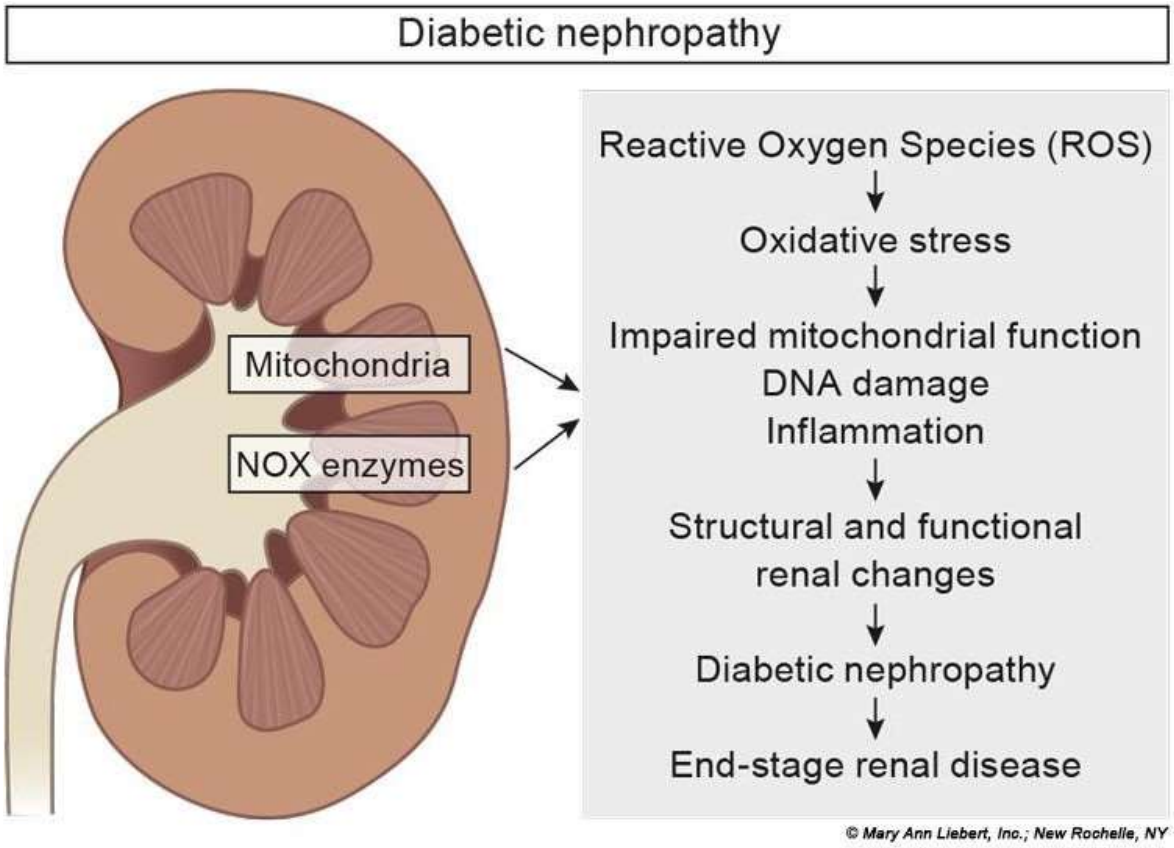


Figure 2. Renal Failure

Oxidative stress is defined as an imbalance between oxidant production and antioxidant protection. Oxygen free radicals induce damage to tissue in many clinical conditions. Also, it is well known that, in the diseases in which excessive immunoinflammatory responses are present, formation of free radicals are accelerated (6,7). Oxidative damage induced by reactive oxygen species (ROS) is thought to contribute to the development of macrophage foam cells in the walls of arteries, which may lead to lethal complications of chronic renal failure (CRF) patients such as cardiovascular disease (15,16). CRF is a disease caused by damage to renal parenchyma by chronic pathologic processes leading to decreased glomerular filtration rate (GFR). Causes of CRF are multiple, with the predominance of primary glomerulonephritis (26.4 %), diabetic nephropathy (19.2 %), tubulointerstitial nephritis (16.5 %), hypertensive nephropathy (8.9 %), and polycystic kidney disease (8.9 %) (11). There is increasing evidence about the

presence of oxidative stress in chronic renal failure patients, and particularly in those submitted to hemodialysis therapy. This seems to be due to multiple factors including an increase in the production of agents from oxidative metabolism and a decrease in antioxidant defenses. Besides, the use of low biocompatible membranes and purity of dialysis water has an influence on oxidative stress (6,17-19) When free radicals overwhelm the antioxidant barrier, they become available for interacting with phospholipidic structures producing lipid peroxidation. One of the major components of this reaction is the production of malondialdehyde, which identification would allow us having an idea of the magnitude of lipid peroxidation. The results found in our patients are in agreement with those of many other authors regarding increased lipid peroxidation (6,20-21).

Serum superoxide dismutase levels were significantly increased in CRF patients compared to healthy subjects ($p<0.001$) The plasma glutathione peroxidase levels of patient group have shown a significant decrease when compared with those of control group ($p<0.001$) In addition, no relationship was found between measured parameters and clinical parameters in both cases and controls. Several studies have shown decreased activity of glutathione peroxidase in patients with renal disorders and chronic renal failure (6-7,22-26). Glutathione peroxidase protects cells from oxidative damage by catalyzing the reduction of both organic and hydrogen peroxides, using glutathione as a reducing agent. GPx activity is an important test to assess the oxidative damage in patients with kidney diseases. Kidney proximal tubular cells are the main source of GPx activity in the plasma. The progression of renal disorders is accompanied by a decrease in GPx activity Blood 'Se' levels are frequently reported to be lower in hemodialyzed patients The integrity of GPX requires adequate intake of 'Se' and its deficiency causes low activity of GPX and reduction in GPX-protein (apoenzyme) synthesis During oxidative stress, inactivation of GPX may occur, and on the other hand superoxide anion itself can inhibit peroxidase function The previous studies indicate an impairment of antioxidant systems and augmentation of oxidants during hemodialysis sessions.

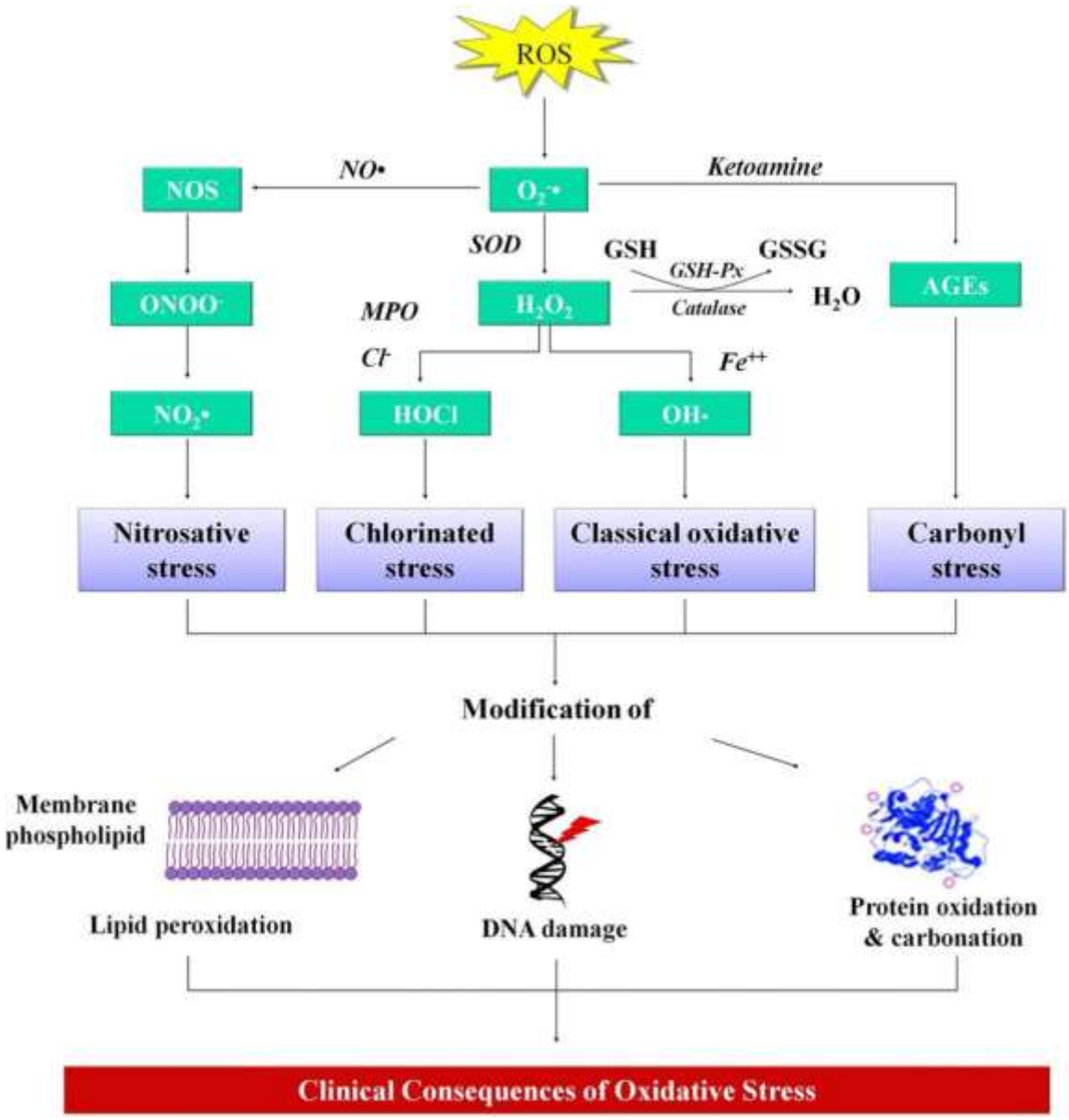


Figure 3. Pathways of oxidative stress in chronic kidney disease

The superoxide dismutase enzyme is the front line of defense against reactive oxygen species-mediated injury Several studies of SOD activity in chronic renal failure patients have found conflicting results We propose that the increased SOD activities could be a protective mechanism for the cells due to the hyperproduction of free radicals in chronic renal failure. The increase in superoxide dismutase activity

may originate from excess production of O2.- in macrophage. The lowering of plasma antioxidant activity in CRF patients on hemodialysis may contribute to the increased oxidative damage and in the development of renal complications. During hemodialysis, uraemic toxicity, malnutrition and the progressive worsening of clinical condition can lead to oxidative stress caused by an hyperproduction of oxidants including ROS and uraemic toxins with pro-oxidant function, and defective antioxidant protection. Losses of antioxidants via dialysis and the use of low biocompatible membranes are the factors that may be responsible for the imbalance between oxidative and antioxidative mechanisms in HD patients. All these factors contribute to the higher oxidative stress in hemodialysis patients included in our study. This study indicates the existence and increased production of an oxidizing stress resulting from hemodialysis and disturbance in antioxidant enzyme system. We found enhanced oxidative stress in all patient groups due to an increase in lipid peroxidation and reduced activities of glutathione peroxidase. Our results supports that an increase in oxidative stress may be considered to be as one of the major risk factors in chronic renal failure disease.

Disorders of lipoprotein metabolism, imbalance between generation of free radicals and antioxidant defense system during uremia and dialysis are important mechanisms of atherogenesis in CRF. The mean value of triglycerides is significantly increased in cases when compared to controls. This result is accordance with studies done by S M Alam et al Bharat Hypertriglyceridemia is a common feature of CRF. Presence of insulin resistance in renal failure activates hormone sensitive lipase causing increased FFA which stimulates the production of apoB-100 containing lipoproteins like VLDL leading to hypertriglyceridemia. Several authors also suggested that hypertriglyceridemia in CRF may be due to defective metabolism of TG rich lipoproteins by lipoproprotein lipase (LPL) and hepatic lipase The mean value of TC is significantly increased in cases when compared to controls. This is in accordance with the which is due to associated proteinuria and renal insufficiency per se. Proteinuria leads to alteration in gene expression for HMG-CoA reductase resulting in increased activity of HMG-CoA reductase leading to

hypercholesterolemia.

In our study mean value of HDL-C is significantly decreased in CRF patients when compared to controls. Many studies conducted by Ziad A Massy BS. have also observed the same results. The reason for decreased concentration of HDL-C in CRF

is not fully understood. It may be due to decreased activities of LPL, hepatic triglyceride lipase (HTGL), lecithin cholesterol acyl transferase (LCAT) and increased concentration of cholesterol ester concentrations.

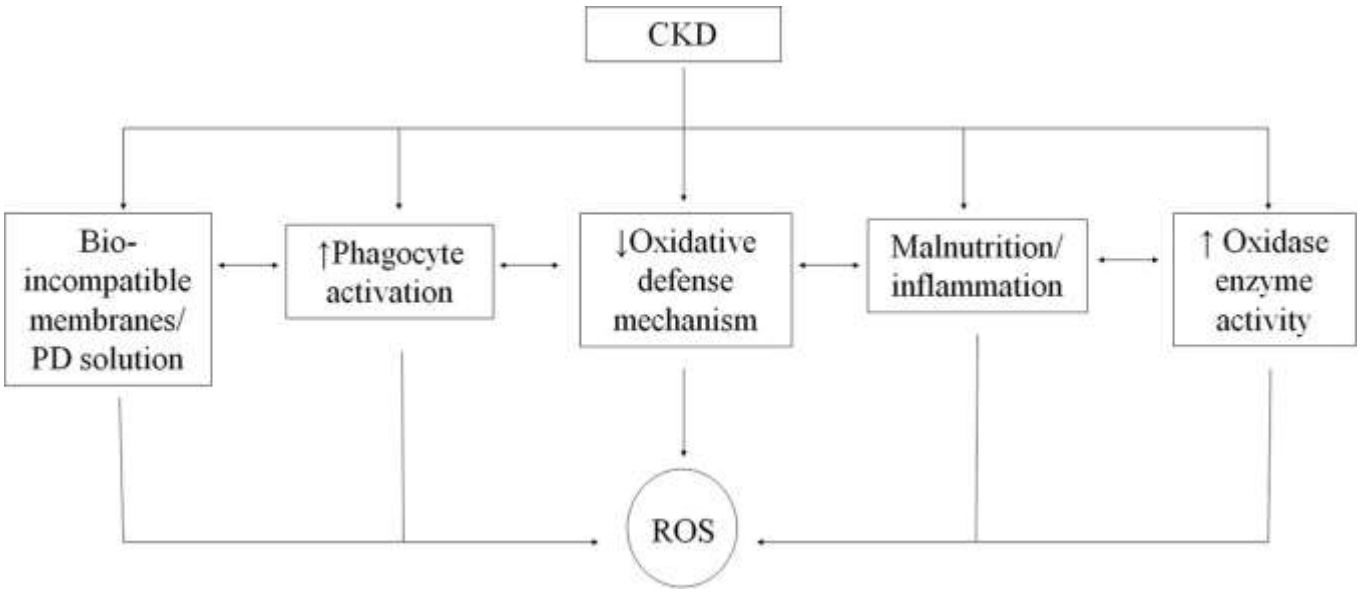


Figure 4. Pathogenic factors leading to oxidative stress. The oxidase enzymes include cytochrome P450, NADPH oxidase, and xanthine oxidase, and the oxidative defense mechanisms include vitamins C and E, glutathione, SOD, and catalase.

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