Role of Oxidative Stress in Chronic Kidney Disease

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Abstract

Kidneys are important organs of the renal system. Besides being one of the principal excretory organs of the body, these perform many important biological functions. Any impairment or dysfunction of the kidney persisting for more than a 3-month duration is termed as chronic kidney disease (CKD). There are many causes of CKD, the most important ones being diabetes and hypertension. The grading of CKD is done depending on the changes in the glomerular filtration rate (GFR). Oxidative stress is a common accompaniment to CKD. Increased production of oxidants, including reactive oxygen species (ROS), in CKD may be due to associated inflammation, abnormality of iron metabolism, or disturbed high-density lipoprotein (HDL) metabolism besides other causes. The most common cause of mortality in CKD or end-stage renal disease (ESRD) is cardiovascular disease, and oxidative stress plays the main culprit in that. Various consequences of increased oxidative stress include endothelial dysfunction, left ventricular hypertrophy, and cardiac fibrosis. Mounting of inappropriate defense system involving nuclear factor erythroid 2-related factor 2 (Nrf2) and other antioxidants also adds up to the condition. This chapter is an attempt to throw light on these aspects, as well as important enzymatic markers for this disease. The therapeutic role of various measures to counter this oxidative stress in CKD will also be discussed.

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15.1 Kidney and Its Normal Physiology

The kidneys are the primary functional organs of the renal system. These are a pair of bean-shaped organs covered by the renal capsule present in the abdominal cavity behind the peritoneum. The right kidney sits just below the diaphragm and posterior to the liver, the left below the diaphragm and posterior to the spleen. Internally, each kidney is divided into three major regions, viz., cortex, medulla, and pelvis. The renal cortex is a space between the medulla and the outer capsule. The renal medulla contains the majority of the nephrons. The renal pelvis connects the kidney to the circulatory and nervous systems of the body. The basic structural and functional unit of the kidney that filters the blood in order to regulate chemical concentrations and produce urine is known as a nephron [1].

A nephron is the smallest functional unit of the kidney. Each kidney is made up of more than one million nephrons. Each nephron is composed of glomerulus, proximal convoluted tubule (PCT), loop of Henle (LOH), distal convoluted tubule (DCT), and a series of collecting ducts. Kidneys filter blood in a three-step process. First, the nephrons filter blood that runs through the capillary network in the glomerulus. Almost all solutes, except for proteins, are filtered out into the glomerulus by a process called glomerular filtration, producing an ultrafiltrate consisting of the other smaller circulating elements. Second, the filtrate is collected in the renal tubules. Most of the solutes get reabsorbed in the PCT by a process called tubular reabsorption. In the loop of Henle, the filtrate continues to exchange solutes and water with the renal medulla and the peritubular capillary network. Water is reabsorbed during this step, and additional solutes are secreted into the kidney tubules during tubular secretion. The collecting ducts collect filtrate coming from the nephrons and fuse in the medullary papillae. From here, the papillae deliver the filtrate (now called urine) into the minor calyces, which eventually connect to the ureters through the renal pelvis. Ureters finally drain the urine into the urinary bladder [1].

The main physiological functions of the kidney include excretion of waste products, e.g., urea, creatinine, drugs, etc. (excretory function); regulation of electrolytes, serum osmolality, and acid-base balance within narrow limits (homeostatic function); and formation of erythropoietin, renin–angiotensin system, and activation of vitamin D (endocrine function) [1].

15.2 Chronic Kidney Diseases

Chronic kidney disease, also known as chronic renal failure, chronic renal disease, or chronic kidney failure, is defined as an abnormality of the kidney structure or function, present for more than 3 months, with implications for health. It is not unusual for people to realize that they have chronic kidney failure only when their kidney function is down to 25% of normal. Chronic kidney failure, unlike acute kidney failure, is a slow and gradually progressive process. Even if one kidney stops functioning, the other can carry out normal functions. It is not usual for signs and symptoms to be noticeable until the disease is fairly well advanced and the condition has become severe, by which time most of the damage is irreversible [2].

Further, persons with CKD are defined as all individuals with markers of kidney damage or those with an estimated glomerular filtration rate (eGFR) of less than 60 mL/min/1.73 m^2 on at least two occasions, 90 days apart (with or without markers of kidney damage). The markers of kidney disease may include albuminuria (albumin creatinine ratio ACR $>$ 3 mg/mmol), hematuria of renal origin, electrolyte abnormalities due to renal tubular dysfunction, histological abnormalities of renal tissue, structural abnormalities of the kidneys detected by imaging, or a history of kidney transplantation [3].

15.2.1 Causes

The most common causes of CKD are diabetes and hypertension, termed as diabetic and hypertensive nephropathies, respectively. Other causes include [3]:

- Polycystic kidney disease (PKD)
- Renal artery stenosis
- Infection
- Systemic autoimmune diseases such as systemic lupus erythematosus (lupus nephritis), Goodpasture's syndrome, etc.
- IgA glomerulonephritis
- Drugs or toxins
- Alport syndrome, rare genetic condition
- Hemolytic uremic syndrome in children
- Henoch–Schönlein purpura

15.2.2 Clinical Features

The common signs and symptoms of chronic kidney disease include [3]:

- Anemia
- Hematuria/proteinuria
- Discolored urine

- Abnormal urine output
- Edema
- Easy fatigability
- Disorientation/decreased mental alertness/unexplained headache
- Hypertension
- Insomnia
- Loss of appetite
- Erectile dysfunction
- Polyuria
- Persistent itching
- Muscle cramps/twitches
- Nausea/vomiting
- Pain in the abdomen radiating to the lower back
- Shortness of breath
- Sudden change in bodyweight

15.2.3 Grading of CKD

The grading of chronic kidney disease may be done by noting changes in the GFR rate as follows [3]:

GFR category	GFR (mL/min/1.73 m^2)	Terms used for the grading of CKD
G1	≥90	Normal or high
G2	60–89	Mildly decreased
G3a	45–59	Mildly to moderately decreased
G3b	30–44	Moderately to severely decreased
G4	15–29	Severely decreased
G5	<15	Kidney failure

15.2.4 Diagnosis [4]

- Blood tests: serum levels of creatinine, urea, uric acid, electrolytes, and estimated GFR (eGFR), calculated by the abbreviated Modification of Diet in Renal Disease (MDRD) equation—$186 \times (\text{creatinine}/88.4)^{-1.154} \times (\text{age})^{-0.203} \times (0.742$ if female$) \times (1.210$ if black)
- Urine tests: urine albumin to creatinine ratio (UACR) (normal is 30 mg/g or less)
- Imaging tests like ultrasound, computerized tomography (CT) scan, magnetic resonance imaging (MRI), etc.
- Kidney biopsy

15.2.5 Treatment [4]

Treatment usually includes curing the cause and employing measures to control signs and symptoms, reduce complications, and slow the progression of the disease. It includes following a diet chart with low-protein diet. Different drugs used include:

- Antihypertensive
- Anti-inflammatory
- Antihyperlipidemics
- Antihistaminics
- Antiemetics
- Erythropoietin
- Calcium and vitamin D supplements

For end-stage renal disease (ESRD), treatment options include:

- Dialysis
- Renal transplant
- Regenerative medicine approaches

15.2.6 Mortality and ESRD

A very high rate of mortality has been reported in patients with end-stage renal disease, approaching approximately 9% per year. It is also notable that most prominent causes of death (approximately 50%) have been attributed to cardiovascular disease (CVD) [5].

15.3 Oxidative Stress and CKD

Oxidative stress (OS) is defined as an imbalance between oxidant molecules and the antioxidant systems of the body in favor of oxidants. Oxidant compounds such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) are continually formed under physiological conditions and get removed by several antioxidant defense mechanisms. OS leads to certain metabolic derangements and/or oxidation of macromolecules like lipids, deoxyribonucleic acid (DNA), and proteins, bringing about oxidative damage in cells, tissues, or organs, culminating in several disorders [6].

The kidney is a metabolically active organ and is thus quite vulnerable to damage caused by perpetrators of OS. Several complications of CKD, such as inflammation and CVD, are also linked to enhanced OS. This association between CKD and its complications is achieved through several mechanisms like induction of activities of certain enzymes responsible for producing oxidants and less availability of antioxidants due to dietary restrictions, diuretic use, protein energy wasting, and/or decreased intestinal absorption. Patients with CKD are otherwise also more predisposed to oxidative stress because of increased propensity of the presence of

other comorbid conditions like diabetes mellitus and hypertension. These patients generally belong to advanced age, which is again associated with oxidative stress. Further, with end-stage disease, because of the risk of hyperkalemia, patients are advised to have a restricted intake of fresh fruits that are a rich source of minerals and vitamins with antioxidant activity, especially vitamin C. The human body is unable to synthesize vitamin C by itself. In addition, treatment in the form of hemodialysis aggravates the generation of ROS by the activation of polymorphonuclear leucocytes (PMNLs), as well as loss of antioxidants during the procedure. Low levels of antioxidants (both enzymatic and nonenzymatic) have been observed in this disease. In addition to vitamin C, plasma vitamin E concentrations are also reported to be lower in these patients, making the defense against oxidation of low-density lipoprotein (LDL) particles still poorer. Oxidized LDL particles are one of the major culprits in the pathogenesis of CVD. Plasma levels of major scavengers of ROS, like superoxide dismutase, glutathione peroxidase, etc., have also been reported to be low due to the downregulation seen in patients with CKD [7].

Inflammation is the natural defense mechanism of the body against any kind of insult. It is a common accompaniment of CKD and contributes substantially to morbidity and mortality associated with this disease. It involves the recruitment of immune cells and the release of inflammatory mediators in the form of cytokines and interleukins. CKD has been found to lead to the activation of PMNLs, important component of the immune system, as well as different mediators and markers of inflammation, such as CRP, IL-6, TNF-α, fibrinogen, etc., suggesting their strong association. Inflammation enhances the oxidative status of the body by a number of mechanisms. In this process, an enzyme, myeloperoxidase, is activated by PMNLs to combat any pathogen invasion by generating ROS, adding further to oxidative stress [2].

15.3.1 Mechanism of Increased Oxidative Stress in CKD

Different mechanisms may be enumerated as responsible for the increased production of oxidants and the decreased concentration of antioxidants in patients suffering from CKD [8].

1. Mechanisms of Increased Oxidant Production
   (a) Increased expression of ROS-producing enzymes (e.g., nicotinamide adenine dinucleotide phosphate (NADPH oxidase), cyclooxygenase, lipoxygenase, etc.)
   (b) Decreased generation of NO by reduced activity of NO synthase (NOS) (via NOS inactivation, depletion of tetrahydro-biopterin (BH4), accumulation of asymmetric dimethylarginine (ADMA), an endogenous competitive inhibitor of NOS)
   (c) Leakage from the mitochondrial respiratory chain
   (d) Respiratory burst with the help of myeloperoxidase
   (e) Recruitment and activation of PMNLs and other immune cells

(f) Oxidation of important macromolecules of the body, including lipids, proteins, LDL particles, etc. and initiating a chain reaction

2. Impaired Antioxidant Defense System
   (a) Decline in the generation of endogenous antioxidants, including antioxidant enzymes, reduced glutathione (GSH), uric acid, etc.
   (b) Defective or insufficient expression of genes encoding different antioxidant molecules
   (c) Improper activation or mounting of antioxidant enzymes
   (d) Depletion, by way of consumption, of antioxidant molecules to counter increased ROS levels
   (e) Impaired activity of good cholesterol carrier, i.e., high-density lipoprotein (HDL)
   (f) Insufficient inclusion of fresh fruits and vegetables in routine diet
   (g) Loss of water-soluble antioxidant molecules during hemodialysis procedure
   (h) Anemia leading to compromised antioxidant status

15.3.1.1 Role of HDL Disturbances in Oxidative Stress of CKD Patients

HDL is also termed as "good cholesterol" as it transports cholesterol from the peripheral organs to the liver (reverse cholesterol transport) in contrast to low-density lipoprotein (LDL), also known as "bad cholesterol," which transports cholesterol from the liver to the peripheral tissues, including the coronary arteries. Any defect or decline of HDL molecules is going to contribute to CVD. Various antioxidant and antiatherogenic actions of HDL are well known. These may be enumerated as follows [8]:

- Reverse cholesterol transport by HDL
- Endothelial cell migration followed by repair (via scavenger receptor B1)
- Inactivation of platelet-activating factor (PAF) and PAF-like phospholipids by action of PAF acetyl hydrolase
- Various antioxidant and anti-inflammatory actions:
  - ApoA-I-mediated scavenging of oxidized phospholipids present in lipoproteins and cell membrane
  - Lecithin cholesterol acyl transferase (LCAT)-mediated hydrolysis of oxidized phospholipids
  - Prevention of LDL oxidation
  - Elimination of oxidized phospholipids by action of paraoxonase-1 and glutathione peroxidase (GPX)

Patients with CKD tend to have alterations in both HDL quantity and HDL quality. Even a mildly impaired GFR is associated with low HDL cholesterol (HDL-C) concentration, which becomes progressively worse through ESRD. Moreover, in CKD, HDL particles tend to be smaller and denser due to metabolic defect. It has been observed that patients with renal dysfunction have significant disturbances in lipoprotein metabolism, and HDL in these patients becomes dysfunctional. Patients with CKD have lower plasma levels of HDL-C and reduced ability of

HDL to bind to ATP-binding cassette transporter A1 (ABCA1), resulting in slowing down the reverse cholesterol transport and disturbances in HDL maturation due to decreased LCAT. Studies have demonstrated that the HDL of CKD patients loses its vasoprotective, antioxidative, and anti-inflammatory properties and turns into a noxious particle that promotes endothelial dysfunction via stimulating superoxide production and limiting NO bioavailability. Alterations of HDL at the molecular and functional levels have also been observed in renal transplant recipients, even in those with excellent graft function [9].

15.3.2 Role of Iron Metabolism in Oxidative Stress in CKD

Iron is a redox-active transition metal. As a part of iron and heme containing proteins, which perform variety of important functions in the body, it is highly essential for body. Besides this, free or active iron catalyzes the formation of highly reactive and toxic ROS in the body. Thus, iron homeostasis is brought about by cells through a tightly regulated mechanism making use of iron regulatory protein (IRP) and hepcidin. Increased intracellular iron has been demonstrated in polymorphonuclear leucocytes isolated from patients on hemodialysis and has been associated with vascular calcification. Vascular calcification induces stiffness of vessel walls, decreasing their vascular compliance. Iron-induced calcification is found to be mediated by interleukin 24 (IL-24) and is, hence, linked to oxidative stress. CKD patients are frequently found to suffer from anemia and are treated with erythropoiesis-stimulating agents (ESA) and iron (oral or parenteral). Excessive iron treatment has a risk of overloading the cells with free iron. The accumulation of iron in mitochondria, a highly redox-active place in the cell, may potentiate the leakage of ROS from the electron transport chain and the production of highly reactive and damaging hydroxyl radicals ($OH^{\bullet}$) through Fenton and Haber–Weiss reactions (Fig. 15.1).

Intravenous iron has been shown to increase biologic markers of oxidative stress in cell cultures, animal models, and ESRD patients on hemodialysis. It has been reported that intravenous administration of iron sucrose in dialysis patients results in an increase in total peroxide, free iron, and markers of lipid peroxidation, which gets significantly improved with administration of the antioxidant vitamin E. Intravenous

Fig. 15.1 Fenton and Haber–Weiss reactions

Fenton Reaction:

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + OH^{\bullet} + OH^-$$

$$Fe^{3+} + H_2O_2 \longrightarrow Fe^{2+} + OOH^{\bullet} + H^+$$

Haber-Weiss Reaction:

$$O_2^{\bullet-} + H_2O_2 \longrightarrow O_2 + OH^{\bullet} + OH^-$$

iron has been reported to add to cytotoxicity and tissue injury, as well as to exacerbate oxidative stress, promoting endothelial dysfunction, inflammation, and the progression of both CKD and cardiovascular disease. This generation of oxidative stress, tubular injury, glomerular permeability, and renal inflammation may be mitigated by therapy with the antioxidant *N*-acetyl cysteine (NAC). It is also possible that the transient injury that may be caused by intravenous iron sucrose is outweighed by the benefits of iron repletion and repair of anemia.

15.3.3 Inflammation in CKD and Oxidative Stress [10]

It is quite a known fact that inflammation plays a key role in CKD progression and its outcome. Inflammation is another important cause of increased oxidative stress observed in patients with advanced renal disease, with malnutrition, chronic volume overload, and autonomic dysfunction being among some of the factors implicated in the increased inflammatory state seen in renal impairment. Systemic or intrarenal inflammation contributes to the deregulation of microvascular response to its regulators and sustains the production of an array of tubular toxins, including ROS, leading to tubular injury, nephron damage, and the onset of CKD. Circulating pro-inflammatory cytokines activate intrarenal microvasculature, including endothelial cells and leukocytes, resulting in the local amplification of pro-inflammatory factors and ROS. These processes affect cell surface adhesion molecules and disrupt the glycocalyx layer. Endothelial barrier function, activation of coagulation system, and receptor-mediated vasoreactivity are also compromised. These inflammation-mediated alterations can induce irreversible tubular injury and nephron failure. Oxidative stress and inflammation are inseparably linked, being major characteristics of CKD and drivers of CKD progression. The presence of systemic inflammation and its severity contribute to CKD-associated oxidative stress, which represents a condition in which the generation of ROS overrides the capacity of the antioxidant defense system. Activation of polymorphonuclear neutrophils is a well-recognized feature in CKD patients, with proven association between renal dysfunction and the different mediators and markers of inflammation, such as C-reactive protein (CRP), interleukin 6 (IL-6), tumor necrosis factor alpha (TNF-α), and fibrinogen, suggesting that CKD is a low-grade inflammatory process in itself. Myeloperoxidase, generated in response to the activation of polymorphonuclear neutrophils, triggers ROS activation and the inactivation of NO.

15.3.4 Adaptive Response to Oxidative Stress

When the body is subjected to increased oxidative stress, various defense mechanisms are mounted, which includes the upregulation of antioxidant status. An important role is played by a factor called nuclear factor erythroid 2-related factor 2 (Nrf2) [8].

15.3.4.1 Role Played by Nrf2

Normally, imbalance of oxidant–antioxidant status in favor of the former triggers an adaptive defense mechanism, resulting in an enhanced expression of antioxidant cytoprotective enzymes and other molecules. In mammals, an increased expression of these molecules is brought about by nuclear factor erythroid 2 p45-related factors 1 and 2 (Nrf2). Nuclear factor erythroid 2-related factor 2 (Nrf2) is present in the cytoplasm as an inactive complex bound to Kelch-like ECH-associated protein 1 (Keap1), a repressor molecule that facilitates Nrf2 ubiquitination. Keap1 contains several reactive cysteine residues that serve as sensors of the intracellular redox state. Oxidative or covalent modification of thiols in some of these cysteine residues leads to conformational changes in Keap1, which results in the disruption of its interaction with Nrf2. Inside the nucleus, Nrf2 binds to regulatory sequences (known as antioxidant response elements or electrophile response elements) in the promoter regions of genes responsible for expressing different antioxidants and molecules used in detoxification. Therefore, Nrf2 plays a central role in the defense against oxidative stress by its effect on the antioxidant and anti-inflammatory systems [11].

Glutathione is the principal and most abundant cellular endogenous antioxidant and plays a major role in the regulation of the cellular oxidative state. It is a tripeptide made up of glutamate, glycine, and cysteine (γ-glutamyl-cysteinyl-glycine). Glutathione directly helps in scavenging ROS and other oxidized substances by serving as a substrate for a number of antioxidant enzymes such as glutathione peroxidase. During the process, glutathione itself gets oxidized to G-S-S-G, or oxidized glutathione. It needs to be reconverted to its reduced form (GSH) with the help of an NADPH-requiring enzyme, glutathione reductase. Any impairment of the glutathione redox cycle increases the risk of ROS-mediated cell injury and consequences like atherosclerosis, diabetes mellitus, chronic liver disease, and cerebrovascular diseases. An association of these occurrences has been reported with elevated glutamate cysteine ligase enzyme, due to induced expression by Nrf2 and resulting in reduced GSH levels. Hence, impaired Nrf2 activation is associated with the severity of oxidative stress and inflammation and consequent disease progression in CKD [8].

15.4 Consequences of Increased Oxidative Stress in CKD

The principal consequences of increased oxidative stress in CKD pertain to the cardiovascular system. These mainly include dysfunction of the endothelium, hypertrophy of the left ventricle, and fibrosis of cardiac tissue [12].

15.4.1 Endothelial Dysfunction

Increased oxidative stress in the form of excessive ROS production is a major cause of endothelial dysfunction, the most important consequence. LDL molecules get easily modified in the presence of increased oxidative stress. These oxidized LDL

molecules are internalized by macrophages (via scavenger receptor class A), which then get transformed into foam cells. These foam cells are the principal components of fatty streak, one of the initial steps in the formation of atheromatous plaque. This initiates an antigenic reaction involving T lymphocytes and mounts an immunological response. The foam cells accumulating in the arterial intima produce an inflammatory response in the vessel wall. This involves inducing the expression of various chemotactic factors, including leukocyte adhesion, leading to the migration of a variety of circulatory inflammatory cells into the subendothelial space. Various cytokines and growth factors are also released at the site. The result of all these processes includes endothelial dysfunction, platelet aggregation, metalloproteinase expression, collagen deposition, fibrosis, and the consequent thrombogenesis. Ultimately, an occlusive thrombotic plaque develops in the vessel wall.

Endothelial dysfunction is an early indicator of atherosclerosis. It is an early predictor of cardiovascular events/disease, including unstable angina, myocardial infarction, heart failure, and death, and may also be associated with restenosis after a multitude of coronary interventions. Ischemia–reperfusion phenomenon is the main culprit in producing ROS in this event.

15.4.2 Left Ventricular Hypertrophy

Left ventricular hypertrophy (LVH) is considered to be the lesser appreciated adverse cardiovascular consequence of oxidative stress. Depending on the severity of renal dysfunction in CKD patients, the estimated LVH prevalence vary between 40% and 75%. Though the appearance of LVH in CKD is multifactorial, still factors like hypertension and volume overload (especially in patients undergoing dialysis) play an important role. The role of oxidative stress in the development of LVH in these patients cannot be overlooked. A variety of growth factors and hypertrophy signaling kinases (e.g., tyrosine kinase Src, GTP-binding protein Ras, protein kinase C, mitogen-activated protein kinase, and Jun-nuclear kinase), as well as transcription factors (like kB, Ets, and activator-protein-1), which stimulate matrix metalloproteinase expression, get induced by ROS. G-protein-coupled hypertrophic stimulation, as well as apoptosis induction, is also brought about by ROS. It has been reported that the activation of apoptosis signaling kinase-1 activates nuclear factor kB, which is an important mediator in the hypertrophy of cardiac tissue.

NO also pays a significant role in endothelial dysfunction. In conditions of increased oxidative stress, with oxidation of BH4, NOS starts to produce superoxide anion, further aggravating oxidative stress. This process is notably seen in endothelial dysfunction associated with hypertension, diabetes, and chronic smoking. Further, increased oxidative stress can inactivate endogenous NO, and NO deficiency has been reported to exacerbate LVH in experimental conditions.

15.4.3 Cardiac Fibrosis

The event mentioned above ultimately stimulates cardiac fibroblast proliferation and activates matrix metalloproteinases in the cardiac tissue, culminating in fibrosis.

15.4.3.1 Oxidative Stress and Pressure Overload: A Vicious Cycle

Increased ROS generation produces a hypertrophic stimulus and also acts as a mediator in bringing about tissue hypertrophy. A vicious cycle gets created as pressure overloading results in increased oxidative stress, which in turn leads to a hypertrophic response of the tissue, and the cycle goes on. Different causes of pressure overload in CKD include the expansion of extracellular volume, increased sympathetic activity, enhanced expression of endothelin protein, increased renin–angiotensin system activity, and exaggerated action of pump inhibitors like Na^+-K^+ ATPase and Ca^{2+}-ATPase inhibitors.

15.5 Important Enzymatic Markers of Oxidative Stress in CKD

Besides being produced in the mitochondrial respiratory chain, there are other metabolic reactions leading to the generation of ROS, e.g., nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, xanthine oxidase (XO), myeloperoxidase (MPO), and endothelial nitric oxide synthase (eNOS). These may be termed as markers of oxidative stress as their levels correlate with the degree of oxidant/antioxidant imbalance in the body [13].

15.5.1 NADPH Oxidases

NADPH oxidase is the major source of ROS production in different tissues, mainly endothelial cells, vascular smooth muscle cells, and renal parenchymal cells. This enzyme belongs to a big family, which consists of seven members: five different types of NADPH oxidases (NOX) and two dual oxidases (DUOX1–2). All the NOX enzymes consist of two heme containing oxidoreductases present as six transmembrane domains. The principal function of NOX is to catalyze the transfer of electrons intracellularly, i.e., within specialized compartments of the cell or from the cytosol to the extracellular space.

Cytosolic NADH or NADPH acts as the electron donor for the action of NOX. The different isoforms of NOX differ in their tissue distribution, intracellular localization, regulation, and binding proteins. The role played by NOX1 and NOX4 in a broad range of diseases has drawn the attention of researchers worldwide. NOX4 is the most important isoform present in renal tissue and has been isolated from renal tubules, glomerular mesangial cells, fibroblasts, and podocytes in the kidney, as well as from endothelial cells and fibroblasts in the vasculature. Under basal conditions, NOX have been found to show low activity but turn highly active in the presence of cytokines and growth factors and under the influence of conditions associated with

oxidative stress, like high glucose or cholesterol levels. Once active, these enzymes are responsible for producing ROS and setting in a chain reaction of initiation and propagation of oxidation and peroxidation.

15.5.2 Glucose-6-Phosphate Dehydrogenase (G6PD)

Glucose-6-phosphate dehydrogenase (G6PD) is the rate-limiting enzyme of the pentose phosphate pathway. The principal significance of the pentose phosphate pathway/hexose monophosphate (HMP) shunt pathway is the generation of ribose and NADPH. NADPH is required to keep glutathione in its reduced form, and because of this reason, altered activity of G6PD (enzymopathy) is associated with increased oxidative stress. Interestingly, high activity of G6PD is found in kidneys of rodents with experimental diabetic nephropathy, warranting its measurement in human models also.

15.5.3 Endothelial Nitric Oxide Synthase

The isoform of nitric oxide synthase synthesized in the endothelium is known as endothelial nitric oxide synthase (eNOS). There are three different isoforms of NO synthases (NOS): neuronal NOS (nNOS), inducible NOS (iNOS), and eNOS. L-arginine is metabolized by NOS to form L-citrulline and NO requiring NADPH and oxygen as cosubstrates and BH4 as a cofactor. NO performs a number of important functions like neurotransmission and the regulation of vascular tone. In vasculature, eNOS is the most abundant of the NOS isoforms, and NO synthesized in the endothelium acts as the major vasodilator or endothelium-derived relaxing factor (EDRF). Under certain pathological conditions, because of "eNOS uncoupling," i.e., electron transfer within the active site is uncoupled from L-arginine oxidation and oxygen gets reduced to $O_2^{\bullet -}$. Thus, eNOS can produce ROS in the form of superoxide radicals under these circumstances. This superoxide anion combines rapidly with NO to produce peroxynitrite (ONOO−), a highly reactive radical with great oxidative potential.

The uncoupling of eNOS may be brought about by a variety of mechanisms like deficiency of L-arginine or BH4 and by the accumulation of asymmetric dimethylarginine (ADMA), a naturally occurring L-arginine analog and endogenous NOS inhibitor. ROS, once generated, produce oxidation of either BH4 and protein arginine *N*-methyltransferase (PRMT type 1) or dimethylarginine dimethylaminohydrolase (DDAH), generating increased levels of ADMA. Thus, this may also become a vicious cycle.

15.5.4 Myeloperoxidase

Myeloperoxidase (MPO) is a heme-containing peroxidase that is synthesized during myeloid differentiation and gets stored in the azurophilic granules of different leucocytes. Under normal circumstances, MPO catalyzes the formation of hypochlorous acid (HClO) from the H_2O_2-mediated oxidation of halide ions. It has been reported that under various pathological situations, degranulation of cells leads to a release of MPO into the extracellular space, where it can oxidize other substrates also, besides halide ions. By the same mechanism, MPO is responsible for tissue damage in atherosclerosis as it causes oxidative modification of LDL particles. Oxidized LDL molecules are the main culprit molecules in the formation of atheromatous plaque, thus suggesting a link between MPO and coronary artery disease.

15.5.5 Xanthine Oxidase

The enzyme xanthine oxidoreductase is present as two interconvertible forms, i.e., as xanthine dehydrogenase (XDH) and xanthine oxidase (XO), though expressed by a single gene. Both XDH and XO catalyze the terminal two steps in purine degradation in humans, i.e., conversion of hypoxanthine to xanthine and further conversion to uric acid. For these reactions, XDH uses hypoxanthine or xanthine as a substrate and NAD^+ as a cofactor to produce uric acid and NADH. But under some pathological circumstances, especially involving inflammatory conditions as in CKD, XDH gets converted to XO by posttranslational modification involving oxidation of the cysteine residues in the protein molecule. XO has a greater affinity for oxygen as a cofactor, leading to the formation of uric acid, along with $O_2^{\bullet -}$ or H_2O_2, thus adding up to the existing oxidative stress.

15.5.6 Paraoxonase-1

The enzyme paraoxonase-1 (PON1) belongs to the paraoxonase family, which helps in protecting the body against oxidation of molecules, including lipoproteins; thus, it may be used as a marker of antioxidant status. The activity of PON1 has been found to be decreased in patients with CKD. The importance of this marker may be understood from the fact that the R allele of the Q192R variant of the PON1 gene is directly related to the severity of LVH and cardiovascular dysfunction in patients with CKD. It has also been observed that patients of CKD who were homozygous for the R allele of this gene showed significantly increased plasma levels of the lipid peroxidation marker, 8-isoprostane. Thus, PON-1 has the potential to be used as a marker of antioxidant status in patients with CKD.

15.6 Treatments to Target Oxidative Stress in CKD Patients

As oxidative stress has an important pathophysiological role in patients with chronic renal disease, different treatments to counter this have been suggested [14].

15.6.1 Antioxidant Vitamins (Vitamins C and E)

Patients of CKD are known to suffer from anemia and are commonly administered intravenous iron for treatment. Parenteral iron is a potential source for generating oxidants in the body. Supplementation of vitamin E by oral route in these patients has been observed to be associated with a decreased oxidative stress status and lesser oxidative susceptibility of LDL particles, conferring upon it a cardioprotective role. In this regard, administration of both vitamin E and vitamin C, both by oral and parenteral routes, has been found helpful, as assessed by an improvement in hematocrit and a lesser requirement of erythropoietin in these patients. Hemodialysis is an additional source for increasing oxidative stress in patients of CKD. Use of dialysis membranes that are antioxidant based has been found effective in decreasing endothelial dysfunction and the oxidation of LDL particles. The introduction of hemolipodialysis using vitamin-E-containing liposomes and vitamin C in the dialysate to reduce dialysis-induced oxidative stress also appears promising.

15.6.2 Angiotensin-Converting Enzyme (ACE) Inhibitors

Angiotensin-converting enzyme (ACE) inhibitors and angiotensin receptor blockers have been observed to be effective in preventing or slowing the process of development of nephropathy in patients with diabetes. These act by interfering with the renin–angiotensin system and also inhibiting angiotensin-II-dependent NADPH oxidase activation quite effectively and have potential to be successful in limiting oxidative stress in patients with CKD/ESRD.

15.6.3 Statins

These are the drugs of choice to treat dyslipidemia, which may be associated with CKD. Besides inhibiting the enzyme 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG CoA) reductase competitively, these produce an anti-inflammatory effect also. Reports from nonrenal patients put forward their potential as antioxidants also as these inhibit the activation of small GTPases such as Rac1-inhibiting NADPH oxidase, independent of its lipid-lowering effect. This effect of statins might be linked to their anti-inflammatory action.

15.6.4 Allopurinol

Allopurinol is the structural analog of purines and is known to inhibit the enzyme XO competitively. It has also been suggested to be a free radical scavenger, an antioxidant, and a "scavenger" of hypochlorous acid. It has been found to be cardioprotective too because of its capacity to improve endothelial dysfunction and oxidative stress in patients of CKD, and this action is much more prominent than that of exogenous antioxidant vitamins. This may possibly be because allopurinol prevents the formation of superoxide anions by inhibiting their significant source, i.e., the enzyme xanthine oxidase, while antioxidant vitamins try to scavenge the excess free radicals that have already been generated. Large doses of antioxidant vitamins are needed to produce the desired effect, giving allopurinol an upper hand in this regard.

15.6.5 Other Measures

Recent measures mainly target the uncoupling of eNOS to prevent the generation of excessive oxidative stress. Therefore, BH4 and its precursor, sepiapterin, a potential recoupler of eNOS, could prove to be beneficial in CKD.

The rationale behind the use of a glutathione peroxidase mimetic may be its ability to reconvert BH4 to a nonoxidized state, which helps in the normalization or recoupling of eNOS.

In an attempt to increase the production of NO, administering high doses of L-arginine has also been suggested. But this poses a challenge as L-arginine is, generally, given as a chloride salt, which, being an acidifying agent, is not advisable in renal patients, especially with ESRD.

15.7 Hemodialysis and Oxidative Stress

Patients with CKD on hemodialysis (HD) are subjected to increased oxidative stress. In these patients, altered dietary pattern may cause the depletion of antioxidants like vitamins C and E, mainly because of lesser intake of vegetables and fruits because of the risk of hyperkalemia. Besides this, malnutrition, loss of vitamins during HD procedure, reduced selenium levels, and reduced function of antioxidant enzymes are some of the other reasons. Further, additional factors responsible for increasing oxidative stress in these patients include chronic inflammatory state associated with CKD, uremia, comorbidities (e.g., hypertension, diabetes, obesity, dyslipidemia, advanced age, and vascular calcification), and other factors related to the HD procedure per se.

Chen et al. [4] suggested that HD procedure promotes the formation of $O2^{\bullet -}$, a powerful prooxidant reactive oxygen molecule, and that there is a direct increase in ROS levels in plasma after each HD session. It has been observed that within minutes after the initiation of an HD session, exposure to dialyzer membranes and

dialysate triggers the activation of complement factors, platelets, and PMNLs, followed by ROS production. PMNL stimulation is a significant biomarker for oxidant stress, which gets progressively enhanced with stages of CKD and is more pronounced in patients undergoing HD. Then within 30 min of HD initiation, lipid peroxidation products start increasing. The activation of complement or the production of free fatty acids induced by heparin might be the pathophysiologic mechanisms leading to these effects. The duration of HD treatment is also a significant independent factor of oxidative stress as prolonged dialysis sessions are characterized by increased inflammation and lipid peroxidation effects.

15.7.1 Antioxidants and Hemodialysis

The HD procedure *per se* is characterized by a significant depletion of antioxidants, and patients undergoing chronic hemodiafiltration have been observed to possess significantly lower plasma levels of vitamins C and E and lower activity of antioxidant enzymes, along with increased levels of several oxidants, as compared to their healthy counterparts. Further, it has been speculated that the administration of antioxidants such as vitamins E and C might be of benefit in HD patients. *In vitro*, vitamin E is the most powerful lipid-soluble antioxidant molecule in cell membranes. It not only preserves the stability of biological membranes and protects them from injury induced by ROS and lipid peroxides, but it also modifies cell reaction to oxidants via the regulation of signal transmission molecular pathways. It was observed in a study on salt-sensitive hypertensive rodents that supplementation of vitamins E and C, in combination, ameliorated the accumulation of oxidative products, improved kidney hemodynamics, delayed disease progression, and subsequently protected the kidney from further damage. However, there is accumulating data suggesting that supplementation of various antioxidants such as vitamins C, E, and N acetyl cysteine (NAC) might reduce oxidative stress in HD; the studies available in this regard are not consistent. This may be due to several factors [4]: oxidative status assessed by numerous different biomarkers in different time lines and in heterogeneous and cohorts of patients [13], dosage and route of administration of antioxidants differing between the trials [6], small sample size and nonclarity of pathophysiologic mechanism, and [15] the degree of OS abrogation by antioxidants. Therefore, antioxidant intake has not yet been adopted in guidelines or everyday clinical practice [16].

15.8 Conclusion

Thus, it may be concluded that oxidative stress plays a major role in CKD and its consequent complications. Furthermore, patients on hemodialysis for treatment are subjected to an augmented oxidative stress. Therapeutic strategies incorporating the measures to tackle oxidative stress at different levels may prove to be promising in this regard.

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