Oxidative Medicine and Cellular Longevity

Reactive Oxygen Species as a Link between Uraemia and Atherosclerosis

Lead Guest Editor: Kamil Karolczak
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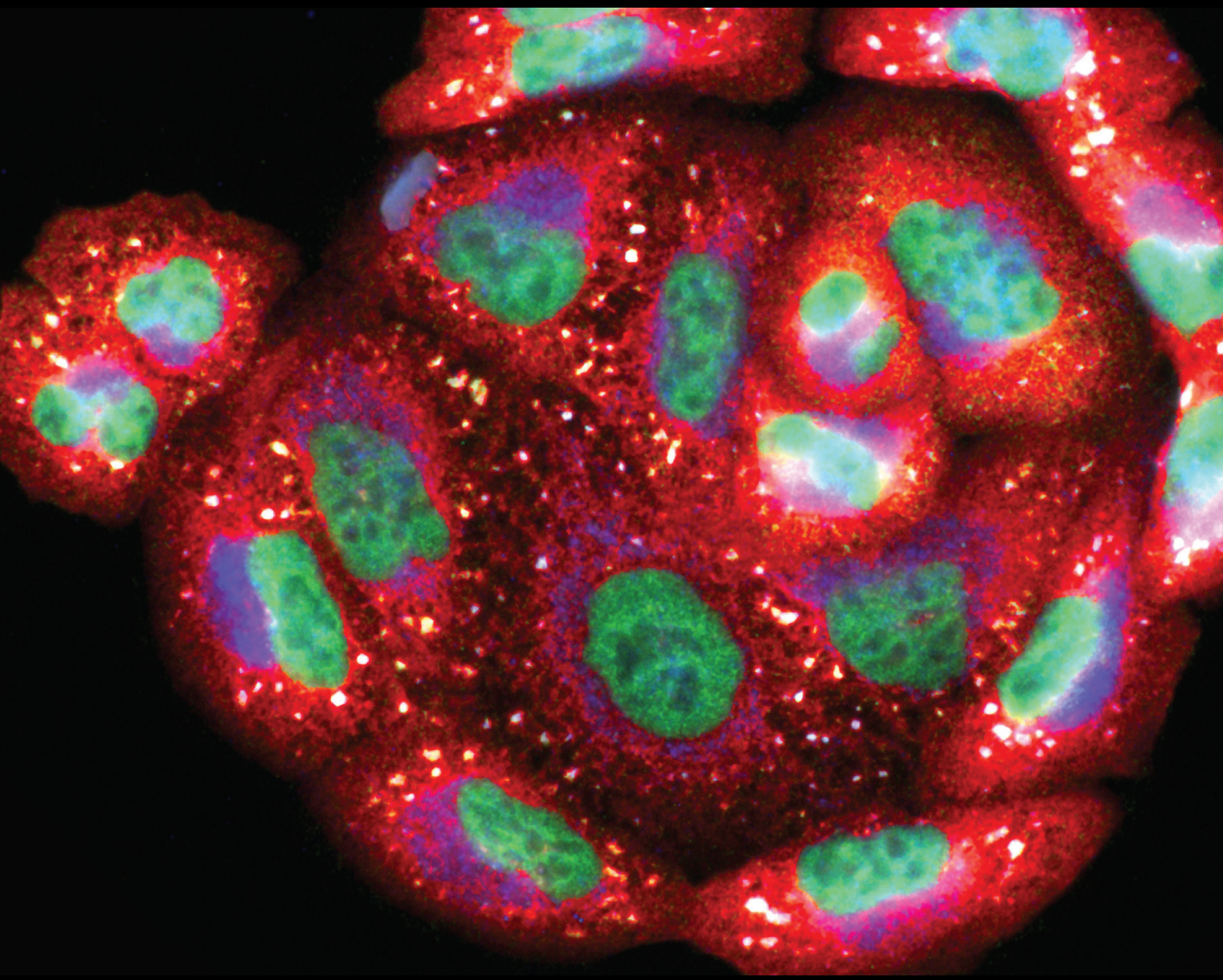

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Review Article

Renal Replacement Modality Affects Uremic Toxins and Oxidative Stress

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Nowadays, the high prevalence of kidney diseases and their related complications, including endothelial dysfunction and cardiovascular disease, represents one of the leading causes of death in patients with chronic kidney diseases. Renal failure leads to accumulation of uremic toxins, which are the main cause of oxidative stress development. The renal replacement therapy appears to be the best way to lower uremic toxin levels in patients with end-stage renal disease and reduce oxidative stress. At this moment, despite the increasing number of recognized toxins and their mechanisms of action, it is impossible to determine which of them are the most important and which cause the greatest complications. There are many different types of renal replacement therapy, but the best treatment has not been identified yet. Patients treated with diffusion methods have satisfactory clearance of small molecules, but the clearance of medium molecules appears to be insufficient, but treatment with convection methods cleans medium molecules better than small molecules. Hence, there is an urgent need of new more validated, appropriate, and reliable information not only on toxins and their role in metabolic disorders, including oxidative stress, but also on the best artificial renal replacement therapy to reduce complications and prolong the life of patients with chronic kidney disease.

1. Introduction

As a result of the deterioration of kidney function, patients with chronic kidney disease develop conduction, accumulation of toxic substances called uremic toxins and related symptoms. In dialyzed patients, the development of sarcopenia and deterioration in nutritional status may be associated with increased mortality [1–3].

The development of chronic inflammation is associated, among other things, with the accumulation of uremic toxins and activation of neutrophils and monocytes, and the production of proinflammatory cytokines and reactive oxygen species increases oxidative stress [3, 4]. In patients with chronic kidney disease, IL-6 and CRP may play a major role in the pathophysiology of inflammation, and in dialyzed patients also, IL-1, 2, 4, 5, 6, 8, 12, and 13 and tumor necrosis factor-alpha (TNF-alpha) seem to be important [3–5].

The emerging chronic inflammation (increase in CRP and IL-6 concentration) is proportional to the severity of chronic kidney disease and may cause the development of cardiovascular diseases and may affect renal function, because oxidative stress damages the endothelium and develops atherosclerotic lesions in the blood vessels [4–6].

2. Uremic Toxins and CKD

Among uremic toxins, there are 3 main groups that differ in size, protein-binding ability, and hydro- and lipophilicity. For this reason, these substances have different importance, and the possibility of their elimination from the patient's body depends on the physicochemical characteristics of these substances [7, 8]. In their work, La Manna and Ronco paid attention not only to the size but also to the structure of the particles. The virtual molecular radius (Einstein-Stokes radius) or the radius of the ball describing the molecule may be important for the rate of substance removal due to changes in diffusion coefficient and screening values [8].

Small hydrophilic particles with a molecular weight of up to 500 Da, not bonded to proteins, form a seemingly homogeneous group of substances. The best known example of this group is urea; the high concentration of which leads to an increase of osmotic pressure, impaired nitric oxide synthesis, proinflammatory endothelial dysfunction and apoptosis, and death of smooth muscle cells [9–11]. Other substances, such as guanidine, may competitively inhibit NO synthase and contribute to the development of hypertension, progression of renal failure and renal fibrosis, and to adverse cardiovascular events and increased mortality in patients with renal failure [12–14].

The adverse clinical effects of protein-related toxins, including indoxyl sulfate (IS) and p-cresol sulfate (p-CS), mostly concern glomerulosclerosis, interstitial fibrosis, and deposits in the extracellular renal matrix [15, 16]. Atherosclerotic lesions are also developed [17]. These toxins activate the transforming growth factor-β1 (TGF-β1) and kappa B nuclear factor (NF-κB), which reduce Klotho protein expression, as well as the formation of free oxygen radicals (e.g., by increasing the production of NAD(P)H oxidase and reducing glutathione) [16, 18]. Additionally, epithelial cells are inducted into mesenchymal cells and renin-angiotensin-aldosterone system is activated [19].

The last group of toxins consists of middle molecules. Similar to the previous groups, they may cause various biochemical and metabolic disorders, which will intensify the inflammation and damage the blood vessel wall [20]. Glorieux et al. [21] investigated the serum beta2-microglobulin (B2MG) and advanced oxidation protein products (AOPP) as middle molecule uremic toxins and protein carbonyl (PCO) as oxidative stress marker in uremic patients undergoing high-flux versus low-flux hemodialysis (HD). They showed that high-flux HD results in reduction of some of the middle molecule toxins and protein carbonyl levels better than low-flux HD, and was associated with a better response to erythropoietin. The HEMO study confirmed the beneficial effect of removing mid-sized uremic toxins on patient survival, and beta2-microglobulin concentrations had a significant impact on patient mortality [22, 23]. In patients undergoing long-term dialysis, beta2-microglobulin deposits can be found in tissues, which some researchers associate with the development of vascular disease and activation of inflammatory markers such as TNF-alpha and IL-6 [24, 25]. Other important mid-sized toxins include advanced glycosylation end products (AGEs), which in patients with chronic kidney disease are formed as a result of carbonyl stress [26]. An increase in their concentration results in an increased inflammatory reaction and inactivation of nitric oxide and tissue damage [7]. In patients with diabetes, the formation of glycosylation end products amplifies tissue damage and impairs their functions [27].

3. Oxidative Stress and Chronic Kidney Disease (CKD)

Excessive production of reactive oxygen species (ROS) in cell mitochondria by cytochrome oxidase enzymes or failure of antioxidant mechanisms leads to oxidative stress, resulting in changes in the structure and function of various biomolecules which may aggravate atherosclerotic lesions and accelerate organ damage, including kidney damage [28, 29].

Due to the increase in nicotinamide adenine dinucleotide phosphate oxidase (NADPH) activity and decrease in superoxide dismutase (SOD) activity already in stage 3 chronic kidney disease (CKD), there may be an increase in superoxides (O_2^-), which are the cause of peroxynitrite ($ONOO^-$) and hypochlorous acid (HOCl) formation, and carbonyl stress is the cause of inflammation [28].

In addition, elevated levels of endogenous nitric oxide inhibitors (NOS), including asymmetric dimethylarginine (ADMA), in the endothelium of patients with chronic kidney disease decrease the bioavailability of nitric oxide (NO). The abovementioned pathways may cause vasoconstriction, hypertension, development of end-stage renal disease (ESRD), cardiovascular events, and neurological and immunological complications [30, 31]. Reduced ADMA level can delay kidney function loss in CKD patients [32].

As mentioned above, 4 major oxidative stress pathways are known: the classical pathway, associated with an imbalance between NADPH and SOD, the nitrosative and chloride pathways, associated with the synthesis of $ONOO^-$ and HOCl, respectively, and the carbonyl pathway, associated with increased production of AGEs [28, 33] (Figure 1).

In elderly patients, as well as those with chronic renal failure, diabetes, or chronic inflammation, neutrophils and phagocytes are activated and ROS are increased. Similarly, HD and PD treatments may increase O_2^-, which is associated with the use of bioincompatible accesses, membranes, and dialysis solutions. Furthermore, uremic toxin accumulation can simultaneously activate the prooxidant system and inhibit the antioxidant system [28, 34–37] (Figure 1).

ROS activation results in the activation of oxidative stress pathways, and the development of chronic kidney disease due to glomerular damage and renal parenchymal fibrosis, and various comorbidities such as atherosclerosis [32].

4. Endothelial Damage in Chronic Kidney Disease (CKD)

The endothelium is a physical barrier, which affords movement of small solutes in preference to large molecules through vessel wall; therefore, it is involved in tissue autoregulation, regulating cellular and nutrient trafficking. The endothelial cells mediate vasoactivity. Normally, the endothelium maintains the vessel in a relatively dilated state. Secretion of nitric oxide, and in minor extent of prostacyclin, C-type natriuretic peptide, and different endothelial-derived hyperpolarizing factors by endothelial cells gives the vasodilation effect; however, the endothelium can secrete also several vasoconstrictor substances including thromboxane A2, endothelins, angiotensin II, and reactive oxygen species [38–40]. The endothelium maintains the local balance between pro- and anti-inflammatory mediators, i.e., ICAM-1 (intercellular adhesion molecule-1), VCAM-1 (vascular adhesion molecule-1), E-selectin, nitric oxide, and nuclear factor κB (NF-κB), and interacts with circulating blood cells, i.e., mediates adherence of leukocytes and platelets to the

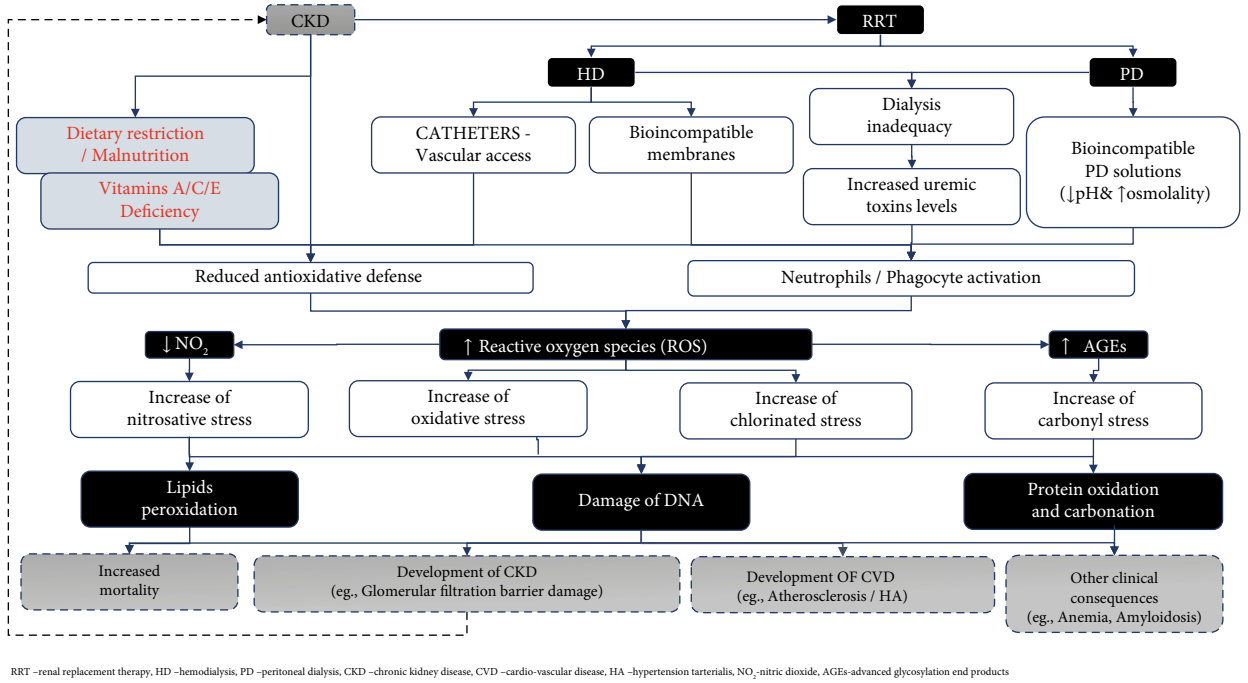

Figure 1: Endothelial dysfunction in patients with chronic kidney disease with its complications. RRT: renal replacement therapy; HD: hemodialysis; PD: peritoneal dialysis; CKD: chronic kidney disease; CVD: cardiovascular disease; HA: arterial hypertension; NO_2: nitric dioxide; AGEs: advanced glycosylation end products.

vessel wall during injury and inflammation [38–41]. The endothelial cells also maintain the local balance between procoagulant and anticoagulant factor activities, i.e., nitric oxide and prostacyclin which inhibit platelet aggregation, thrombomodulin which inactivates thrombin, and others: plasminogen activator (t-PA), its inhibitor (PAI-1), von Willebrand factor (vWF), thromboxane A2, tissue factor pathway inhibitor (TFPI), and fibrinogen [38–41]. The endothelium is also involved in new blood vessel generation—angiogenesis. Endothelial cells are heterogeneous, which allow regulation of their activity and functions in specific places, i.e., in the glomeruli [42]. Generally, the steady-state endothelial cells have a vasodilatory, antiadhesive, and anticoagulant phenotype, whereas the activated endothelium has vasoconstricting proadhesive and procoagulant character [38, 43]. In patients with CKD, endothelial cell damage is connected to disturbances in vasorelaxant, anti-inflammatory, and antithrombotic activities due to reduced level of nitric oxide [44].

In clinical practice, there are no reliable markers of endothelial dysfunction, so confirmation of its disorders seems to be difficult. On the other hand, the intact endothelium might initiate or progress the disease. Progressive endothelial damage in the renal medullary capillary system may be the cause of progressive renal injury, and chronic renal failure may develop endothelial dysfunction and atherosclerosis and can lead to higher cardiovascular mortality and development of microalbuminuria and renal failure in CKD patients [45, 46].

5. Oxidative Stress Induction by Uremic Toxins

Decreased renal function leads to development of inflammation due to longer half-lives of proinflammatory markers and biochemical markers of endothelial dysfunction, e.g., IL-6, CRP, and TNF-α [5, 47, 48]. Together with progression of renal failure, higher activity of soluble adhesion molecules (ICAM-1, VCAM-1, and vWF) and matrix metalloproteinases can be observed due to activation of NF-κB pathway and decrease of Klotho protein [49–51]. In the majority of patients with CKD, alterations in calcium-phosphate balance are present. High phosphate levels can suppress endothelial NO synthase and increase ROS formation [52]. Endothelial cell dysfunction in uremia can also be associated with low triiodothyronine level which can change ADMA effect on endothelial function [53, 54]. Increased AGE and decreased soluble AGE receptor levels in patients with renal failure can change glycation processes and increase atherosclerotic formation [55, 56].

6. Uremic Toxins and Renal Replacement Therapy

In patients with end-stage renal failure, the initiation of renal replacement therapy and reduction of uremic toxin concentration as a result of such treatment may be important to reduce inflammation, although on the other hand, it may also exacerbate inflammation, e.g., due to infections [34]. Renal replacement therapy may also intensify the oxidative stress generated by urea, but the mechanism depends on the type of dialysis: hemodialysis (HD) increases lipid and protein peroxidation, while peritoneal dialysis (PD) increases protein oxidation [57].

Oxidative stress may not only increase due to the bioincompatible catheters, membranes, and dialysis fluids in HD patients but also due to the low pH and high osmolarity of

fluids in peritoneal dialysis patients [28, 36, 37, 58]. In addition, the loss of vitamins and/or trace elements during HD using high-permeable membranes may lead to disruption of the antioxidant system [59].

Another problem is the formation of toxins in the gastrointestinal tract, which worsens the effects of dialysis treatment [60, 61]. Therefore, some authors propose the use of symbiotics that reduce the production and absorption of p-cresol sulfate and indoxyl sulfate [62].

The low, 50% 3-5-year survival rate of dialysis patients may also result from the fact that dialysis is currently mainly focused on urea elimination, although its toxicity is controversial [22, 63]. It seems that using different renal replacement therapies (intermittent hemodialysis or peritoneal dialysis), it is possible to obtain urea and other small particles' clearance comparable to 15% of normal kidney function, and only in the case of home night hemodialysis performed 7 times a week or in renal transplant recipients, urea clearance may reach 50% of normal kidney function. Despite these results, patients with chronic kidney disease, with urea clearance as high as 20-25 ml/min, comparable to patients on dialysis, have fewer clinical signs of uremia. This may be related to a nonproportional clearance of other substances, especially medium molecules [8, 64]. Improved clearance of larger molecules can be achieved with the prolonged duration of treatments [64]. It has been shown that lower concentrations of beta2-microglobulin in dialyzed patients can also be observed in patients with preserved renal residual function, as demonstrated in a study comparing peritoneal dialysis patients with those treated with hemodialysis [65].

6.1. Hemodialysis. The purification efficiency for different substances varies depending on the type and parameters of hemodialysis and the class of filtration membranes, which differ in terms of biocompatibility, medium-molecular-weight screening factor, start of molecular weight retention and molecular weight cutoff for dissolved substances with different molecular weight, presence of electric charges and Z potential, thickness, and diffusion coefficient (K_o) for different substances [28, 66]. Cuprophan membranes used in the past triggered inflammatory reactions and intensified amyloidosis development [67].

Better biocompatibility of hemodialysis, even compared to hemodiafiltration, can now be achieved by use of dialyzers with vitamin E in low-flow bicarbonate hemodialysis, which reduces inflammation associated with the activity of indoleamine 2,3-dioxygenase 1 and nitric oxide synthesis [68]. Despite the fact that during longer procedures, the clearance for beta2-microglobulin and phosphates is greater, the removal of protein-related toxin has not changed significantly [69, 70].

Standard hemodialysis is the most commonly used renal replacement therapy, which purifies the blood of toxins in the diffusion mechanism, i.e., the passage of <500 Dalton particles from the blood to the dialysis fluid according to the concentration difference [71]. The removal of medium and large particles is impossible during standard hemodialysis, although the use of high-flux membranes during hemodialysis only slightly improves the elimination of medium particles [72].

Another solution is the use of MCO dialysis membranes with medium cutoff values. The clearance of mean particles for these membranes is much higher than for standard membranes used in classical hemodialysis treatments, both low flux HD and high flux HD, and comparable with membranes used in hemodiafiltration [73–75]. Extended hemodialysis using MCO membranes may lead to reduction in mortality comparable to hemodiafiltration treatment, which can be combined with similar efficacy in removing both small, such as urea and creatinine, and medium, such as beta2-microglobulin, particles [73, 75].

The hemodialysis procedure can also remove protein-bound toxins, but only from the free fraction, and the effectiveness of this process is only about 30%. On the other hand, the use of MCO filters may lead to moderate hypoalbuminemia, which may improve the removal of protein-bound toxins [17, 76–80].

6.2. Hemodiafiltration. In contrast to the diffusion mechanism, in which substances pass through the membrane at different speeds depending on the blood flow and dialysis fluid, the type of dialysis membrane, and the size of toxins, the convection mechanism, i.e., removal of toxins together with the solvent through the highly permeable membrane, prevails in hemofiltration and not so much in hemodiafiltration. The implementation of such a method allows to purify the body form substances of different sizes, even middle molecules, and their removal is directly proportional to their concentration in plasma. Unfortunately, it requires a return administration of ultrapure fluids, which can be administered both before and after the filter (Figure 2).

Hemodiafiltration is more expensive than hemodialysis because it involves better membrane biocompatibility and the use of ultrapure dialysate. Hemodiafiltration better than hemodialysis removes uremic toxins, both small and medium, because diffusion and convection are responsible for removing the toxins (Figure 2). Hemodiafiltration, compared to hemodialysis, improves parathormone clearance and proinflammatory cytokines (e.g., IL-6, IL-8, and IL-12) and reduces the concentration of β2-microglobulin, ADMA, SDMA, and appetite suppressants such as leptin, cholecystokinin, tryptophan, and albumin [81, 82]. Patients treated with HDF also have better clearance of homocysteine, guanidine, and polyamines, which reduce nitric oxide production and promote AGE formation. Therefore, patients treated with HDF have lower inflammation and cardiovascular risk [83]. The removal of p-CS during hemodiafiltration may, according to some authors, be comparable to the use of low-flux hemodialysis and high-flux hemodialysis, which emphasizes the low importance of convection in the removal of these dissolved substances [84]. The removal of medium-sized toxins is slightly greater because convection is responsible for the removal of medium-sized toxins during HDF. However, Gomółka et al. [85] found that there are no major differences in the serum clearance of IS and p-CS depending on the dialysis modality (low-flux hemodialysis, high-flux hemodialysis, and postdilution hemodiafiltration). They concluded that

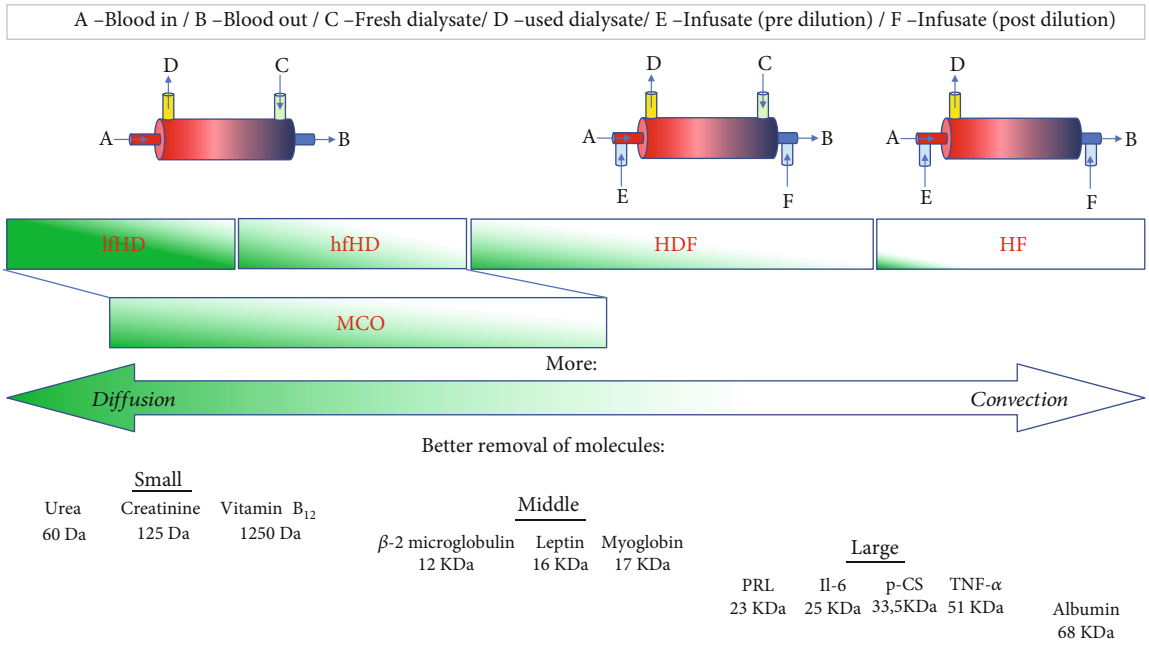

Figure 2: Uremic toxin removal by different modalities.

these protein-bound toxins were significantly cleared from the serum already during the first dialysis session, but their level tended to revert during weeks' long dialysis sessions.

Moreover, the method of plasma fluid replenishment is also important—postdilution is more effective than predilution [86–90], and mixed, pre-, and postdilution supplementation is the most effective in purification [91–94]. It has been noted that in people treated with hemodiafiltration, there is less risk of amyloidosis and carpal tunnel syndrome episodes, and that with large exchange volumes, purification is more efficient, which may further reduce mortality from general and cardiovascular causes [95–100].

Studies comparing different types of renal replacement therapy have shown that hemofiltration reduces mortality among patients undergoing renal replacement therapy. The clearance of small and medium molecules during online hemofiltration is similar to that of extended hemodialysis, but may be higher for larger medium molecules [101]. Therefore, the use of hemofiltration improves the removal of medium molecules, including beta2-microglobulin, compared to classical hemodialysis, but also with peritoneal dialysis [90, 102].

7. Summary

Elimination of the waste products and toxins generated from a variety of metabolic processes is one of the major kidney functions [103]. Efficient elimination of these solutes is provided by normal kidney function; thus, their blood and tissue concentrations are kept at relatively low levels. On the contrary, these toxin retentions appear to be a major contributor to the development of uremia in patients with advanced chronic kidney disease (CKD) and end-stage renal disease (ESRD) [104]. In addition, progression of CKD contributes to the oxidative stress produced by intracellular uremic toxins, leading to inflammation and tissue destruction [105]. Uremic toxins, found in high concentrations in the circulation in patients with ESRD, play an important role in endothelial dysfunction/damage, which in turn contributes to the pathogenesis of cardiovascular diseases, such as atherosclerosis and thrombotic events [106–110]. In CKD, and in particular in dialyzed population, endothelial dysfunction and atherosclerosis are almost universal, as well as cardiovascular complications. Lindner et al. [111] were the first to draw attention to the excessive incidence of atherosclerotic cardiovascular mortality in hemodialyzed patients. Implications of uremic toxins and oxidative stress to atherosclerosis were recently presented in the elegant review by Wojtaszek et al. [112]. Hypoalbuminemia is a frequent finding in CKD, and multiple factors may be contributory, including inflammation, malnutrition, and dialytic losses [113]. Structure of albumin as well as uremia-induced changes in the albumin concentration also may influence protein-bound uremic toxins binding from both a quantitative and qualitative perspective. It was demonstrated that protein-bound compounds, including drugs and endogenous toxins, are secreted by renal proximal tubule cells [114, 115]. Unbound solutes (drugs, uremic toxins, etc.) are transported by specific organic anion transporters; thus, the equilibrium established between bound and unbound solute forms is critical. On the one hand, dialysis increases the state of oxidative stress, and the involved mechanisms include use of bioincompatible membranes and fluids and contamination of dialysate with bacterial endotoxins and occult infections [116–118]. On the other hand, renal replacement therapy, in particular hemodiafiltration, by lower concentration of uremic toxins diminishes oxidative stress leading to reduced cardiovascular and thromboembolic risk. Moreover, successful kidney

transplantation leads not only to the at least partial restoration of kidney function with amelioration of metabolic abnormalities but also to the significant improvement in OS-related markers. Sufficient graft function seems to be a key factor in the restoration to near normal levels of OS biomarkers.

At this moment, despite the increasing number of recognized toxins and their mechanisms of action, it is impossible to determine which of them are the most important and which cause the greatest complications. According to many authors, further studies are needed to assess the clinical consequences of different types of renal replacement therapy as so far large prospective trials have not addressed the effect of various renal replacement modalities on uremic toxin removal with respect to patient outcomes.

Abbreviations

ADMA: Asymmetric dimethylarginine
AGEs: Advanced glycation end products
CKD: Chronic kidney disease
CRP: C-reactive protein
CVD: Cardiovascular disease
eGFR: Estimated glomerular filtration rate
ESRD: End-stage renal disease
HD: Hemodialysis
HDF: Hemodiafiltration
HOCl: Hypochlorous acid
ICAM-1: Intercellular adhesion molecule-1
IL-1: Interleukin 1
IL-2: Interleukin 2
IL-4: Interleukin 4
IL-5: Interleukin 5
IL-6: Interleukin 6
IL-8: Interleukin 8
IL-12: Interleukin 12
IL-13: Interleukin 13
IL-18: Interleukin 18
IS: Indoxyl sulfate
K_o: Diffusion coefficient
MCO: Medium cutoff
NADPH: Nicotinamide adenine dinucleotide phosphate
NF-κB: Nuclear factor kappa-light-chain-enhancer of activated B cells
NO: Nitric oxide
O_2: Superoxides
ONOO: Peroxynitrite
PAI-1: Inhibitor of tissue plasminogen activator
p-CS: p-Cresyl sulfate
PD: Peritoneal dialysis
ROS: Reactive oxygen species
SDMA: Symmetric dimethylarginine
SOD: Superoxide dismutase
TGF β1: Transforming growth factor β1
TFPI: Tissue factor pathway inhibitor
TNF-α: Tumor necrosis factor-α
t-PA: Tissue plasminogen activator
VCAM-1: Vascular adhesion molecule-1
vWF: von Willebrand factor.

Conflicts of Interest

There is no conflict of interests to disclose.

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Review Article

Reactive Oxygen Species and Their Involvement in Red Blood Cell Damage in Chronic Kidney Disease

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Reactive oxygen species (ROS) released in cells are signaling molecules but can also modify signaling proteins. Red blood cells perform a major role in maintaining the balance of the redox in the blood. The main cytosolic protein of RBC is hemoglobin (Hb), which accounts for 95-97%. Most other proteins are involved in protecting the blood cell from oxidative stress. Hemoglobin is a major factor in initiating oxidative stress within the erythrocyte. RBCs can also be damaged by exogenous oxidants. Hb autoxidation leads to the generation of a superoxide radical, of which the catalyzed or spontaneous dismutation produces hydrogen peroxide. Both oxidants induce hemichrome formation, heme degradation, and release of free iron which is a catalyst for free radical reactions. To maintain the redox balance, appropriate antioxidants are present in the cytosol, such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and peroxiredoxin 2 (PRDX2), as well as low molecular weight antioxidants: glutathione, ascorbic acid, lipoic acid, α-tocopherol, β-carotene, and others. Redox imbalance leads to oxidative stress and may be associated with overproduction of ROS and/or insufficient capacity of the antioxidant system. Oxidative stress performs a key role in CKD as evidenced by the high level of markers associated with oxidative damage to proteins, lipids, and DNA *in vivo*. In addition to the overproduction of ROS, a reduced antioxidant capacity is observed, associated with a decrease in the activity of SOD, GPx, PRDX2, and low molecular weight antioxidants. In addition, hemodialysis is accompanied by oxidative stress in which low-biocompatibility dialysis membranes activate phagocytic cells, especially neutrophils and monocytes, leading to a respiratory burst. This review shows the production of ROS under normal conditions and CKD and its impact on disease progression. Oxidative damage to red blood cells (RBCs) in CKD and their contribution to cardiovascular disease are also discussed.

1. Introduction

Chronic kidney disease (CKD) is a pathological condition in which, as a result of impaired excretory function, associated with a decrease in the number of nephrons, toxic substances accumulate in the body. Waste products that are normally excreted by the kidneys in the urine accumulate in amounts that are toxic to the body and are referred to as uremic toxins. Many of the uremic toxins retained in the body exhibit biological/biochemical activity contributing to the development of the uremic syndrome and endogenous poisoning of the body [1]. Many of them cause chronic inflammation and oxidative stress. Both inflammation and oxidative stress can contribute to the development of chronic kidney disease and its many complications. The release of ROS in the body is related to their physiological role as signaling molecules. However, their increased production and/or insufficient performance of antioxidant systems can lead to oxidative stress which is associated with the damage or/and oxidative modification of vital molecules such as nucleic acid, proteins (enzymes), and lipids [2].

In chronic kidney disease, oxidative stress performs a key role in disease progression as evidenced by the high level of markers associated with oxidative damage to proteins, lipids, and DNA *in vivo*. Oxidative stress is related to the overproduction of reactive oxygen species (ROS) and reduced

antioxidant capacity in which there is a decrease in the activity of SOD, glutathione peroxidase, peroxiredoxin 2, and antioxidants with low molecular weight, such as glutathione and vitamins C, A, and E. An additional factor increasing oxidative stress is low-biocompatibility dialysis membrane treatments used in hemodialysis, which lead to the activation of phagocytic cells, especially neutrophils and monocytes [3]. This review shows the production of ROS in normal conditions and in chronic kidney disease and their impact on disease development.

Maintaining the balance between ROS production and utilization has an important role in cell signaling and hemostasis. This balance is also important for the functioning of blood vessels. Its disturbance related to the excessive production of ROS due to acute infection or inflammation may lead to the damage of biological material [4]. ROS is involved in the pathogenesis of many diseases, including cardiovascular diseases such as hypertension, ischemic heart disease, and thrombosis [5].

In this review, the oxidative damage of red blood cells in CKD, which affects the rheological properties of the blood but is also associated with the development of cardiovascular diseases, was also taken into consideration. We also emphasize the role of oxidative stress, which disrupts redox homeostasis, exacerbates the disease in patients with chronic kidney disease, and is a major contributor to the cardiovascular disease that accompanies chronic kidney disease.

2. ROS Production and Oxidative Stress

Oxidative stress is a consequence of living under aerobic conditions. ROS are released in organisms under normal physiological conditions and act as signal molecules. However, their overproduction and/or antioxidant system failure can lead to oxidative stress. RFT as strong oxidizing agents lead to the damage/modification of life-important molecules such as DNA, proteins, and lipids. The precursor of ROS is the superoxide anion radical ($O_2^{\bullet-}$), which is the product of one-electron reduction of molecular oxygen. Superoxide is usually formed in the body by catalyzed reactions and/or as a result of nonenzymatic electron transfer, when the electron is converted to molecular oxygen [6]. The main source of reactive oxygen species is mitochondria, where there is a leakage of electrons in the respiratory chain and reduction of molecular oxygen. There are 11 sites in the mitochondria that generate the superoxide radical [7]. Large amounts of superoxide are produced by NADPH oxidase (NOX) from the cytoplasmic membrane and from the enzyme complex of the mitochondrial electron transport chain, but also from sources such as xanthine oxidase (XO), lipoxygenase (LOX), cyclooxygenase (COX), and cytochrome P450 (endoplasmic reticulum) from peroxisomes of other organelles [8–10]. Other sources of ROS are the endoplasmic reticulum, nuclear envelope, cytoplasm, and endosomal and plasma membranes [9, 10]. ROS are also produced by cytoplasmic membranes, lysosomes, mitochondria, and peroxisomes [11, 12]. NADPH oxidase catalyzes the one-electron reduction of molecular oxygen, thus producing $O_2^{\bullet-}$. Supposedly 1% to 3% of oxygen running through the mitochondria is reduced to $O_2^{\bullet-}$ [13].

Xanthine oxidase which is an important enzyme that contributes significantly to the production of superoxide in ischemia-reperfusion can also reduce nitrite to nitric oxide and may be a potential source of peroxynitrite ($ONOO^-$) [14]. Generally, superoxide, which is a precursor to other reactive oxygen species, shows low reactivity with few exceptions. One of them is the reaction with nitric oxide, and the other is the Haber-Weiss reaction catalyzed by transition metal ions (Fe, Cu, Ti, Ni, etc.) and the spontaneous dismutation reaction ($O_2^{\bullet-}/HO_2^{\bullet}$) and by superoxide dismutase with the constant rate of 10^5 to 10^9 $mol^{-1} \times s^{-1}$ at pH 7, respectively [15]. However, this reactivity increases with its protonated form, i.e., hydroperoxide radical (perhydroxyl radical), which can initiate lipid peroxidation and thiol oxidation [16].

Disproportionation reaction of superoxide leads to hydrogen peroxide, which is a signaling molecule but also a strong oxidant. However, it does not react with most biological molecules due to its high activation energy barrier, but it can oxidize thiols [17]. An important reaction, also *in vivo*, is its reduction catalyzed by transition metals and superoxide. To maintain the conditions of proper homeostasis, cells have at their disposal antioxidant enzymes such as superoxide dismutase [18], catalase [19], glutathione peroxidases [20], heme oxygenase-1 (HO-1) [21], the thioredoxin system [22], and low molecular weight antioxidants soluble in water (glutathione [23], ascorbic acid) and soluble in lipid (α-tocopherol, ubiquinol, and β-carotene) [24]. Despite the well-developed antioxidant system, vital particles and macromolecules are damaged. The Fenton reaction in which hydrogen peroxide is reduced to a hydroxyl radical by Fe(II) is of key importance here. The $^{\bullet}OH$ radical is the most reactive form of oxygen and is one of the strongest oxidants. Most reactions of the $^{\bullet}OH$ radical with biological molecules, such as proteins (e.g., albumin and hemoglobin), aromatic amino acids, unsaturated fatty acids, DNA bases, or ascorbic acid, occur with constant rates of $>10^{10}$ ($mol^{-1} \times s^{-1}$) and are diffusion-controlled reactions [15]. Hydroxyl radical has a very short lifetime, and its radius of action is 10^{-8} m [15]. Interestingly, Fe(II) ions on the water surface react with H_2O_2 more than 100 times faster than those in water [25]. Another radical is nitric oxide released by most of our body's cells, and it is a vasodilator and, therefore, leads to lowering of blood pressure and increased blood flow. $NO^{\bullet}$ is synthesized from L-arginine by oxidation of the guanidine group in the presence of stereospecific enzyme $NO^{\bullet}$ synthase and NADPH and tetrahydrobiopterin as cofactors [26].

Hemoglobin is not only a protein that supplies nonoxygen to tissues but also a nitric oxide transporter. By supplying $NO^{\bullet}$, it regulates the tension of blood vessels. The autooxidation of hemoglobin causes the formation of methemoglobin (MetHb), which leads to inflammation associated with the release of heme from MetHb. In normally functioning erythrocytes, the redox state is maintained due to the presence of methemoglobin reductase. This enzyme with the participation of NADPH reduces Fe(III) in MetHb to Fe(II) present in Hb. Nitric oxide is released into the lumen of the vessel and is captured by red blood cells (RBCs) [27, 28]. Inside,

the $NO^{\bullet}$ is bound by a hemoglobin molecule to form S-nitrosohemoglobin (HbFe(II)SNO) [27, 29, 30]. Under anaerobic conditions, hemoglobin (deoxyhemoglobin) may bind to nitric oxide to form nitrosyl-hemoglobin (HbFe(II)NO) (reaction (1)):

$$\text{Hb Fe (II)} + NO^{\cdot} \rightarrow \text{Hb Fe (II)NO} \quad (1)$$

The concentration of HbFe(II)NO in venous blood is approx. 30-fold higher than that of S-nitrosohemoglobin, while in arterial blood, it is only approx. 2-fold [31]. However, the reaction of oxyHb with $NO^{\bullet}$ leads to oxidation of oxyHb to metHb and nitrate [32]. This reaction (reaction (2)) is irreversible and causes a decrease in the bioavailability of nitric oxide, thereby interfering with the vasodilator dependent on the blood vessels [33].

$$\text{Hb Fe (II)}O_2 + NO^{\cdot} \rightarrow \text{Hb Fe (III)}NO_{3^-} \quad (2)$$

This mechanism is crucial in the expansion of blood vessels with the participation of $NO^{\bullet}$. Even a small degree of hemolysis can lead to $NO^{\bullet}$ binding and inhibit endothelium-dependent vasodilation [32]. In turn, oxyHb released in plasma can react with $NO^{\bullet}$ and produce $ONOO^{-}$/ONOOH and metHb [34]. It was shown that the treatment of red blood cells with nitric oxide led to metHb formation and oxidative damage of lipids and proteins in these cells [35]. An important group of compounds are quinones, of which the reduction leads to the formation of reactive semiquinones ($Q^{\bullet -}$). An example would be reduction by xanthine oxidase in a nitrogen atmosphere, which is a method that ensures a continuous production of semiquinone. Semiquinones can also be formed by the autooxidation of hydroquinones. Semiquinones may react with hydrogen peroxide generating the hydroxyl radical (reaction (3)) [36]:

$$Q^{\cdot -} + H_2O_2 \rightarrow Q + HO^{\cdot} + HO^{-} \quad (3)$$

Some xenobiotics and drugs, e.g., adriamycin (doxorubicin) with xanthine oxidase and xanthine in an oxygen-free atmosphere in the presence of H_2O_2, resulted in the production of hydroxyl radicals in a similar manner [37]. Similar to other heme-containing proteins such as cytochrome c (associated with electron transport) and catalase or cytochrome oxidase, which are proteins involved in the breakdown of peroxides, Hb and Mb may also exhibit similar properties to other heme proteins. As a result of the oxidation of both proteins, toxic derivatives are formed, such as ferryl forms, ferrylmyoglobin Mb(FeIV=O), and ferrylhemoglobin Hb(FeIV=O), respectively, as well as radical ferryl forms, formed as a result of the oxidation of metmyoglobin and methemoglobin: Mb(FeIV=O···$Tyr^{\bullet}$) and Hb(FeIV=O···$Tyr^{\bullet}$), respectively, with the location of the unpaired electron on the rest of Tyr(β145) of the globin chain [38, 39]. It has been shown that both hemoproteins Hb and Mb in an oxidized state, for example, in ferryl and ferryl radical forms, can induce lipid peroxidation by abstraction of a hydrogen atom in the hydrocarbon chain [39, 40]. Relations (4) and (5) show ferryl and ferryl radical formation from the porphyrin (Por) compound including myoglobin and hemoglobin.

$$\text{Por-Fe(II)} + H_2O_2 \rightarrow \text{Por-Fe(IV)} = O + H_2O \quad (4)$$

$$\text{Por-Fe(III)} + H_2O_2 \rightarrow \text{Por}^{\cdot} + \text{Fe(IV)} = O + H_2O \quad (5)$$

Ferryl forms can be reduced by myoglobin or hemoglobin to metMb and metHb, respectively (reaction (6)).

$$\text{Por-Fe(IV)} = O + \text{Por-Fe(II)} + 2H^{+} \rightarrow 2\text{Por-Fe(III)} + H_2O \quad (6)$$

The ferryl form and the ferryl radical form were first discovered in horseradish peroxidase, but it is now known that these forms are found throughout the heme enzyme family, which includes all peroxidases, heme catalases, P450, cytochrome oxidase, and NO synthase [41]. The ferryl form of myoglobin initiated the process of lipid peroxidation in the membranes to form isoprostane, as well as the reduction of ascorbates or urates [39].

In inflammation, neutrophil accumulation occurs, which as a result of activation, in addition to superoxide and hydrogen peroxide, produces hypochlorous acid (HClO), which is produced in the oxidation reaction of chlorides by hydrogen peroxide catalyzed by myeloperoxidase (MPO) [42]. HClO is a strong oxidant capable of oxidative modification of molecules and macromolecules. Hypochlorous acid shows a strong affinity for low molecular weight thiols and protein thiols but also to methionine. It leads to the oxidation of glutathione (GSH) to oxidized glutathione (GSSG) [43]. HClO causes chlorination of tyrosine to form two derivatives 3-chlorotyrosine and 3,5-dichlorotyrosine in proteins and peptides [44]. HClO reacts with amino groups to form chloramines. In reaction with proteins and peptides, carbonyl compounds are formed [45]. Another way of forming aldehydes is through the breakdown of chloramines. In addition, hypochlorous acid reacts with compounds that contain a double bond to form chlorohydrins. In the case of biological material, it reacts with unsaturated fatty acids and cholesterol to form the corresponding chlorohydrins [43]. The reaction of hypochlorous acid with reducing agents such as Fe(II) and superoxide, which is another source of the hydroxyl radical, is also important (reactions (7) and (8)) [46]:

$$\text{Fe(II)} + \text{HC1O} \rightarrow \text{Fe(III)} + HO^{\cdot} + C1^{-} \quad (7)$$

$$O_2^{\cdot -} + \text{HC1O} \rightarrow O_2 + HO^{\cdot} + C1^{-} \quad (8)$$

Myeloperoxidase (MPO) can also directly convert superoxide to singlet oxygen ($^1O_2$). $^1O_2$ can also be produced in a reaction of HClO with H_2O_2 [47]. Singlet oxygen is also produced in many enzymatic reactions in which heme proteins, lipoxygenases, and activated leukocytes participate, as well as in nonenzymatic reactions involving free radicals. $^1O_2$ is involved in the oxidation of proteins, leading to changes in both the side chains and the main backbone of amino acids, peptides, and proteins. It also forms reactive peroxides with

Tyr, His, and Trp residues, which may further be involved in protein oxidation [48].

3. Protection of Cells and Tissues against Oxidative Stress

The role of antioxidants is to inactivate ROS which initiate oxidative damage. The imbalance between oxidants and antioxidant systems causes oxidative damage in the cell, which leads to overexpression of oncogene genes, generation of mutagenic compounds, and promotion of atherosclerotic activity and in consequence to cancer, neurodegenerative diseases, cardiovascular diseases, diabetes, and kidney diseases.

Antioxidants act to directly scavenge oxygen free radicals, and other oxidizing molecules, and regenerate damaged biomolecules. Typically, antioxidants are classified into two groups. The first line of defense includes antioxidant enzymes, which include superoxide dismutase, catalase, and glutathione peroxidase. The second group of nonenzymatic antioxidant consists of low molecular weight antioxidants that can be divided into antioxidants soluble in the water environment and in the lipid environment [49, 50]. The additional group consists of repair systems that regenerate oxidatively damaged biopolymers, remove oxidized proteins by proteolytic enzymes, and repair oxidized lipids with the participation of phospholipases, peroxidases, or acyl transferases [51, 52]. Another group is represented by enzymatic systems that repair nucleic acids damaged by oxidation [53].

Primary antioxidants react directly with free radicals (hydroxyl $HO^{\bullet}$, alkoxyl $RO^{\bullet}$/lipoxyl $LO^{\bullet}$, or peroxyl $ROO^{\bullet}$) through the donation of a hydrogen atom, interrupting chain reactions. Secondary antioxidants include, for example, singlet oxygen quenchers, metal chelators, and inhibitors of oxidizing enzymes such as COX, LOX, and NADH oxidase [50].

The maintenance of redox homeostasis involves antioxidant enzymes such as superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, and nonenzymatic systems such as proteins (ferritin, transferrin, ceruloplasmin, and albumin) and low molecular weight antioxidants, like glutathione, ascorbic acid, uric acid, coenzyme Q, and lipoic acid [52].

The superoxide anion radical, a precursor to other ROS, is transformed by superoxide dismutase to H_2O_2. In mammals, there are three types of superoxide dismutase: zinc-copper dismutase (SOD1) which is found in the cytosol, manganese superoxide dismutase (SOD2) which is found in the mitochondria, and extracellular superoxide dismutase (SOD3).

The hydrogen peroxide produced during dismutation of superoxide is reduced to water by catalase. This enzyme is present in most cells, organs, and tissues and at elevated concentrations in the liver and erythrocytes [54]. Other enzymes that remove hydrogen peroxide are peroxiredoxin (Prx), thioredoxin reductase (TrxR), and glutathione peroxidase (GPx) [55]. GPx, in addition to the decomposition of H_2O_2, also breaks down organic peroxides into alcohols and oxygen. A similar function is also performed by glutathione S-transferases (GST), which can reduce lipid hydroperoxides. Thioredoxins (Trxs) and glutaredoxins (Grxs) repair oxidized cysteine residues. Thioredoxin reductase catalyzes the reduction of the disulfide at the Trx active site [56]. TrxR also participates in the regeneration of other antioxidant molecules, such as dehydroascorbate, lipoic acid, and ubiquinone [57].

The "second line of defense" consists mainly of reduced thiols and low molecular weight (LMW) antioxidants, both water- and fat-soluble, reduced glutathione, ascorbate, tocopherols, retinols, and others. LMW antioxidant can move to specific places in cells in which oxidative damage occurs [58, 59].

Another important group of antioxidants is thiols, which react with most of the physiological oxidants. They are important in maintaining the homeostatic intracellular and tissue redox status based on the redox pair. Multiple studies show that the redox state in cells is important for ROS-mediated signaling and mitochondrial function [60]. Thiols are sensitive to oxidation which leads to the formation of dithiol/disulfide. This reaction occurs in the case of glutathione, thioredoxins (with -SH groups in the active center), and other proteins containing cysteine [61]. Glutathione (GSH) is one of the most important intracellular antioxidants because its concentration is high and ranges from 5 to 10 mM. Multiple studies show that the redox state in cells is important for ROS-mediated signaling and mitochondrial function [60]. The decrease in GSH concentration in the cytosol leads to an increase in the production of mitochondrial ROS and depolarization of the mitochondrial membrane [62]. Glutathione, as a water-soluble antioxidant, primarily protects the proteins present in the cytosol. As an antioxidant, it reacts with $O_2^{\bullet-}$ and $HO^{\bullet}$ radicals, hydrogen peroxide, and chlorinated oxidants [61].

Cysteine-rich proteins and peptides can bind to heavy metals due to the presence of thiol groups. A special group here is metallothioneins (MT), peptides, and proteins with a molecular mass ranging from 500 to 14000 g/mol located in the membrane of the Golgi apparatus, which bind to both physiological metals such as zinc, copper, and selenium and toxic heavy metals including cadmium, mercury, silver, lead, arsenic, manganese, cobalt, and nickel. MT regulate zinc levels and the distribution in the intracellular space. In addition to zinc-metallothionein interactions, MT is an important regulator of glutathione synthesis [63]. In the Zn-MT complex, a cysteine residue may induce redox properties to participate in the MT redox cycle. Moreover, MT has an antioxidant effect, taking part in the inactivation of reactive oxygen and nitrogen species, including free radicals, which has been proven in many *in vivo* and *in vitro* studies [64, 65].

Ascorbic acid/ascorbate (vitamin C), soluble in water, is an important and ubiquitous antioxidant that is easily oxidized to dehydroascorbic acid (Figure 1). Ascorbic acid assists in the maintenance of the integrity of blood vessels and connective tissue, takes part in iron absorption, and participates in neuroprotection and hematopoiesis [66, 67]. It also protects membrane lipids from peroxidation and is an important antioxidant that protects the brain tissue and is involved in the biosynthesis of catecholamines [68]. Ascorbic acid protects membranes and other hydrophobic compartments from oxidative damage by regenerating the

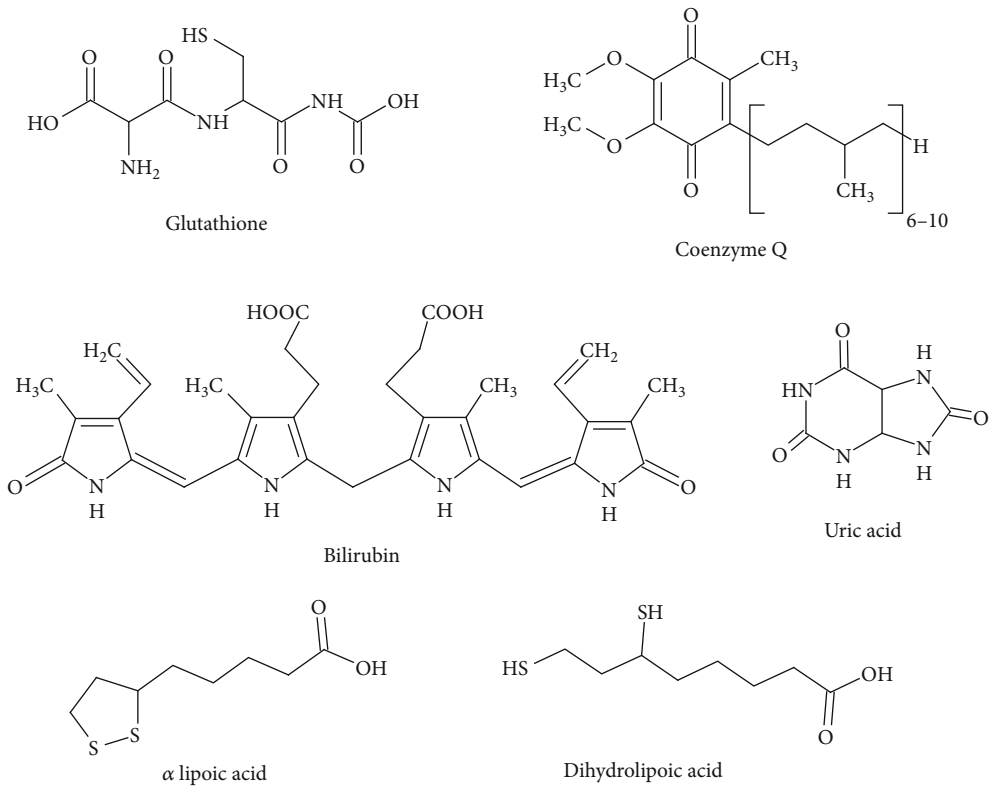

Figure 1: Endogenous low molecular weight antioxidants.

antioxidant form of vitamin E. In addition, ascorbic acid effectively reacts directly with $HO^{\bullet}$ radicals and peroxide radicals with rate constants from 10^6 to $10^8\,M^{-1}\,s^{-1}$. It is also a singlet oxygen scavenger [69]. Although ascorbic acid does not directly remove lipophilic radicals, it acts synergistically in conjunction with tocopherol to remove lipid peroxide radicals. Moreover, it reacts with the membrane-bound tocopheroxyl radical regenerating it to the active tocopherol [70].

Another antioxidant with a low molecular weight is the fat-soluble α-tocopherol (vitamin E) containing in the structure a chroman ring with a branched saturated side chain (Figure 1). Natural vitamin E consists of α-, β-, and γ-tocopherols, but the greatest share is held by α-tocopherol. Vitamin E protects lipids against peroxidation by interrupting free radical chain reactions by providing a hydrogen atom with reactive lipid ($L^{\bullet}$), lipoxyl ($LO^{\bullet}$), and peroxyl ($LOO^{\bullet}$) radicals, forming lipids, alcohols, and hydroperoxides, respectively. The resulting tocopheryloxyl radical is regenerated by ascorbic acid and/or glutathione [71]. Vitamin E also protects low-density lipoproteins against free radical damage. α-Tocopherol inhibits proatherogenic processes through the proliferation of smooth muscle cells in vivo and adhesion of monocytes to the endothelium [72]. α-Tocopherol also contributes to the stabilization of atherosclerosis [72, 73]. Vitamin E performs a protective role in the formation of cancer, the aging process, arthritis, and cataracts. It can also prevent excessive platelet aggregation that can lead to atherosclerosis; in addition, it also helps to reduce the production of prostaglandins such as thromboxane, which cause platelets to stick together [74].

β-Carotene (β-Car) is an antioxidant that is soluble in the lipid environment. It belongs to the carotenes, which are terpenoids (isoprenoids) (Figure 1). β-Carotene, unlike lycopene, has beta-cyclohexene rings at both ends of the molecule. β-Car is a highly effective physical singlet oxygen quencher, which is formed in the skin with the help of endogenous photosensitizers under the influence of sunlight [75]. It also participates in quenching singlet oxygen, which contributes to cataract formation and macular degeneration in the eye [76]. The action of β-Car is here supported by another carotenoid, lycopene, and α-tocopherol. The antioxidant activity of β-carotene was comparable to that of α-tocopherol [77].

Coenzyme Q10 (CoQ, ubiquinone) is a benzoquinone with 10 isoprenyl units in its fat-soluble side chain (Figure 1). CoQ is crucial in the mitochondrial electron transport chain [78]. It occurs especially in the heart, skeletal muscle, liver, kidney, and brain [79]. Its low concentration in plasma can lead to cardiovascular disorders. Coenzyme Q10 is an intracellular antioxidant, but it is also present in plasma to protect LDL lipoproteins and cell membranes from oxidative damage [80]. Coenzyme Q10 reduces the oxidized form of vitamin E, restoring its antioxidant properties [69]. The reduced form of coenzyme Q10 inactivates carbon-centered lipid radicals and lipid peroxyl radicals [81]. On the other hand, CoQ may exhibit prooxidative properties, as its single-electron reduction leads to a semiquinone which,

when reacting with hydrogen peroxide, generates a highly reactive hydroxyl radical [82].

α-Lipoic acid (LA) is a short-chain fatty acid containing a five-membered ring (dithiolane ring) with two sulfur atoms. Lipoic acid is unique in its solubility as it is soluble in both water and lipids. However, its reduced form contains two groups -SH (dihydrolipoic acid (DHLA)) (Figure 1). It has been shown that both the oxidized and reduced forms have antioxidant properties in the inactivation of free radicals and other reactive oxygen species such as hydrogen peroxide and hypochlorous acid, as well as the ability to chelate transition metals [83, 84]. LA and DHLA effectively chelate directly toxic metals such as manganese, zinc, cadmium, lead, cobalt, nickel, iron, copper, cadmium, arsenic, and mercury. Moreover, they show the properties of regeneration of endogenous antioxidants such as glutathione, vitamin C, and vitamin E, metal chelating activity, and repair of oxidized proteins [85]. Lipoic acid and dihydrolipoic acid are involved in the prevention of cardiovascular diseases, and they also have anti-inflammatory, anticancer, antiaging, and neuroprotective properties [86].

One of the low molecular weight antioxidants is uric acid (UA), present in plasma. Uric acid inactivates the hydroxyl and peroxyl radicals and is an effective singlet oxygen scavenger (Figure 1). It has been shown to protect the erythrocyte membrane against lipid peroxidation. It has also been reported that uric acid is a unique scavenger of peroxynitrite in the extracellular space [87]. In experimental allergic encephalomyelitis (EAE), uric acid inhibited the nitration of neuronal proteins via peroxynitrite and inhibited the growth of the blood-brain barrier, resulting in less leukocyte infiltration [88]. However, the protective effect of UA may not be related to direct inactivation of peroxynitrite in neurons but may be due to a reduction in endothelial nitric oxide levels. Uric acid has also been shown to reduce the bioavailability of nitric oxide in endothelial cells [89]. On the other hand, the prooxidative effect of UA, which appears in cardiovascular diseases and may perform a role in the pathogenesis of these diseases, is also shown [90].

Bilirubin (BIL) belongs to amphiphilic antioxidants and has effective cytoprotective activity in relation to lipids (Figure 1). Acting as an antioxidant, it is oxidized to biliverdin. In turn, biliverdin is reduced to bilirubin by biliverdin reductase. BIL inactivates the hydroxyl, superoxide anion, and nitric oxide radicals and shows excellent protective activity against mitochondrial oxidative stress [91]. However, the nanomolar concentrations of bilirubin in tissues (about 20-50 nM) are far too low to counteract the activities of the reactive oxygen species found in millimolar concentrations [92].

The nonenzymatic antioxidants also include metal-binding proteins, which include transferrin, ferritin, lactoferrin, and ceruloplasmin. Their mechanism of action is related to the sequestration of transition metal ions that catalyze the reactions in which most of the oxygen-derivative radicals are formed, including the Fenton and Haber-Weiss reactions. Transferrin is the main iron-binding protein in the blood. Its low iron saturation, about 15%, indicates anemia, while high saturation, over 60%, indicates iron overload or hemochromatosis [93]. Another iron-binding protein is ferritin, found in cells in the cytosol, but small amounts are found in plasma where it acts as a carrier for iron. The protein not only binds to iron but also releases it in a controlled manner. Plasma ferritin is also a marker of the total amount of iron stored in the body [94]. Lactoferrin found in the milk of mammals is a protein that is also found in saliva, tears, and nasal secretions. This protein binds to iron and is carried through various receptors to and between cells, serum, bile, and cerebrospinal fluid. Lactoferrin is one of the components of the body's immune system, is part of the innate defense, and has antibacterial and antiviral properties [95].

Ceruloplasmin is an enzymatic glycoprotein containing 6 copper ions. It is the major copper-carrying protein in the blood. Ceruloplasmin has a ferroxidase activity that is important in iron homeostasis and defense mechanisms in oxidative stress. Its main role is related to the oxidation of Fe(II) to Fe(III), which in the oxidized form can be transported by transferrin and bind to ferritin [96]. Certain mutations in the ceruloplasmin gene lead to disturbances in iron metabolism and distribution, leading to massive Fe accumulation in the liver, brain, and pancreas, as well as problems with the retina and diabetes [96]. Ceruloplasmin is an important antioxidant that protects biomolecules from damage induced by free oxygen radicals. Ceruloplasmin was shown to be a much more effective scavenger of peroxide radicals than SOD, deferoxamine, and albumin, but slightly less effective than catalase [97]. Ceruloplasmin, regardless of its catalytic activity of peroxidase, is an effective antioxidant that breaks the chains of free radical reactions.

In addition to endogenous antioxidants such as GSH, UA, BIL, CoQ, LA, DHLA, and polyamines such as spermine, spermidine, and putrescine [98], the remaining antioxidants enter the body through food, mainly from vegetables and fruits. Antioxidant properties characterize also monophenol, diphenol, and polyphenol derivatives. The monophenols include derivatives of benzoic and cinnamic acid and their esters, most often methyl, propyl, and lauryl (Figure 2). Derivatives of benzoic acid include protocatechuic acid and gallic acid, and derivatives of cinnamic acid include coumaric acid, caffeic acid, ferulic acid, and chlorogenic acid effective free radical scavengers [99, 100]. The next group is diphenol derivatives (stilbene derivatives), resveratrol, and picetannol (Figure 2) [101]. Flavonoids are a large group of compounds with antioxidant properties, inactivating free radicals and other ROS, inhibiting prooxidative enzymes such as cyclooxygenases, lipoxygenases, and oxidase, chelating heavy metals, and modulating antioxidant enzymes [102, 103]. In addition, the flavonoids have antioxidant, immunomodulatory, anti-inflammatory, and anticancer properties, as well as potential antiviral effects [104, 105]. Currently, there are over 8,000 flavonoids known, and their structure is based on the chroman ring with a phenyl substituent, which is present in the 2, 3, or 4 positions (Figure 2). Depending on the position of the phenyl substituent, we have flavonoids, isoflavonoids, or neoflavonoids. Their antioxidant properties are determined by the number of hydroxyl groups present in the phenyl substituent and associated with the chroman ring, as well as the presence of a carbonyl group and a double bond in the ring. Additionally, hydroxyl groups

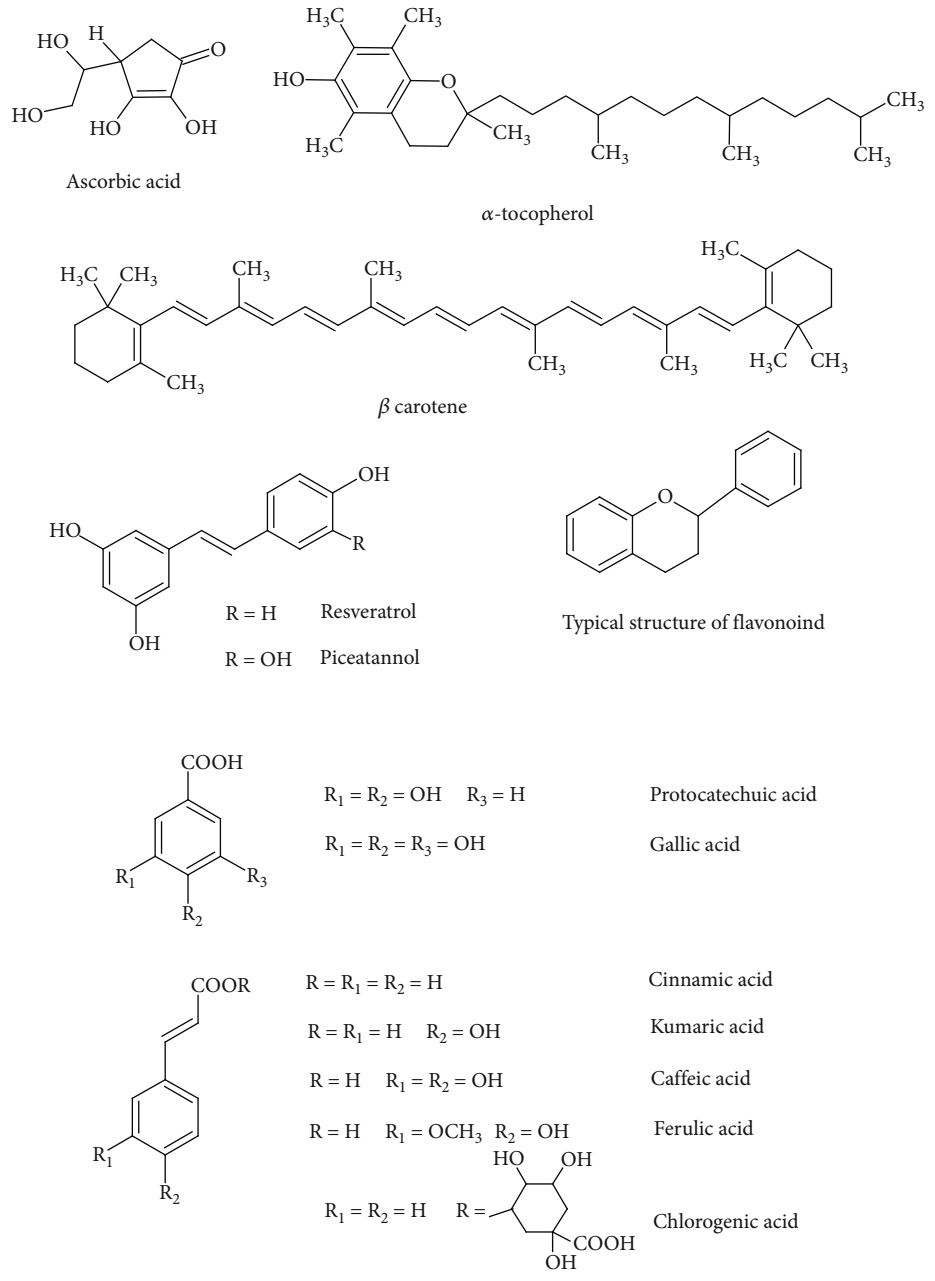

Figure 2: Exogenous low molecular weight antioxidants.

can be ester bound to organic acids such as gallic, malonic, malic, ferulic, and others or/and form O- or C-glycosidic bonds with sugar residues [104, 106]. Flavonoids can condense to form tannins, oligomers, dimers to pentamers, and sometimes polymers composed of 14-15 monomer molecules [107]. The introduction of two double bonds into the chroman ring leads to the formation of anthocyanins and anthocyanidins of colored pigments, characterized by the presence of a positive charge (flavylium ion) [103].

4. Oxidative Stress in CKD Patients

Permanent oxidative stress occurs in patients with chronic kidney disease. Markers of oxidative stress associated with the progression of CKD can be measured in body fluids such as plasma, red blood cells, and urine (Table 1). In plasma and saliva, antioxidant enzymes (SOD, Cat, GPx, and Trx) and low molecular weight antioxidants (GSH, Vit. C and E, and β-carotene), protein oxidation products (SOPPs, AGEs, and protein carbonyls), and lipids (MDA, 4-HNE, and F2 isoprostanes) are determined, while in the urine, the oxidation products of nucleic acids 8-OHG (8-hydroxyguanosine) and 8-OHdG (8-hydroxy-2′-deoxyguanosine) are determined [108, 109]. Another proposed biomarker that can be measured in the urine is neutrophil gelatinase-associated lipocalin (NGAL) resistant to degradation and rapidly excreted in the urine. NGAL is now an approved biomarker of CKD progression [110, 111].

Table 1: Markers of oxidative stress determined in saliva, plasma, and red blood cells in CKD.

Patients	Marker	Increase ↑/decrease ↓	Reference
Saliva (NWS)			
CRF (not requiring dialysis) vs. ESRD (peritoneal dialysis)	UA	↓	[117]
	TAS	↓	
	GPx	↑	
	SOD	↑	
Pediatric patients with CKD vs. healthy controls	UA	↑	[118]
	GSH	↓	
	CAT	—	
	GPx	—	
	SOD	↑	
	TAS	—	
	AGE	↑	
	AOPP	↑	
	MDA	↑	
Plasma			
CRF (not requiring dialysis) vs. ESRD (peritoneal dialysis)	UA	↓	[117]
	TAS	↑	
	UA	↑	
	GSH	↓	
Pediatric patients with CKD vs. healthy controls	CAT	—	[118]
	GPx	—	
	SOD	↑	
	TAS	↑	
	AGE	↑	
	AOPP	↑	
	MDA	↑	
CRF (treated by hemodialysis) vs. healthy controls	GPx	↓	[119]
	GR	↓	
	TBARS	↑	
	AOPP	↑	
	Carbonyl	↑	
CRF (treated by hemodialysis) vs. healthy controls	TAS	↑	[120]
Red blood cells			
ESRD (treated by hemodialysis) vs. healthy controls	SOD	↓	[121]
	CAT	↑	
	GPx	—	
CRF (treated by hemodialysis) vs. healthy controls	SOD	↓	[119]
	GSH	↓	
	GPx	—	
ESRD (treated by hemodialysis) vs. healthy controls	SOD	↓	[122]
	CAT	↓	
	GPx	↓	
	TBARS	↑	

NWS: nonstimulated saliva; AGE: advanced glycation end products; AOPP: advanced oxidation protein products; carbonyl: carbonyl group; CAT: catalase; GPx: glutathione peroxidase; GR: glutathione reductase; GSH: reduced glutathione; MDA: malondialdehyde; SOD: superoxide dismutase; TAS: total oxidant status; TBARS: thiobarbituric acid reactive substances; UA: uric acid.

Less commonly used markers include thiols and oxLDL. It has been observed that patients with chronic CKD have increased levels of plasma thiol oxidation even contributing to progressive renal dysfunction [112]. In turn, OxLDL has recently been reported to predict the development of renal dysfunction [113]. Another marker of oxidative stress in

inflammatory diseases is 3-nitrotyrosine ($TyrNO_2$) associated with the overproduction of NO [114].

Kidney disease is associated with permanent inflammation accompanied by oxidative stress [115]. Markers of inflammation include C-reactive protein, interleukins (IL-1, IL-6), tumor necrosis factor alpha (TNF-α), and fibrinogen, among others. Another marker is MPO, which has been found in serum and is associated with inflammation in CKD patients [116].

On the one hand, there is an overproduction of ROS; on the other hand, the activity of antioxidant enzymes is reduced and the level of antioxidants with low molecular weight is lowered. The decrease in the activity of SOD, decrease in the level of GSH, and higher GSSG/GSH ratio were described in RBC from hemodialyzed patients [119, 121, 123]. Additionally, chronic kidney disease is associated with inflammation and sometimes acute infections. Contact of dialysis membranes with blood causes the activation of phagocytic cells, which in turn leads to respiratory burst in 15-20 min of hemodialysis [3]. A respiratory burst is characterized by a decrease in the level of neutrophils by about 80% and of monocytes by about 60% and the release of large amounts of ROS. Neutrophils belong to cells that are crucial in the innate immune response against pathogens. These cells, which release superoxide ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2), can be generated with xanthine oxidase [124] and $NO^{\bullet}$ by neutrophilic nitric oxide synthase (NOS) [125]. Nitric oxide synthesized by NOS affects various physiological functions but is also involved in pathology. Nitric oxide is characterized by low reactivity; as an inert molecule, it easily penetrates plasma membranes. However, in the presence of oxygen, it forms highly toxic nitrogen dioxide ($NO_2^{\bullet}$), a strong oxidizing and nitrating agent [126]. Neutrophils are cells that actively participate in inflammatory and cardiovascular diseases. Neutrophils can also produce a strong oxidant peroxynitrite in the reaction of superoxide and nitric oxide [127]. Increased production of $O_2^{\bullet-}$ and H_2O_2, catalyzed by xanthine oxidase, is accompanied by an increase in the synthesis of peroxynitrite, the factor responsible for damaging the biological material (Figure 3) [126, 128]. Moreover, neutrophils produce hypochlorous acid in the presence of myeloperoxidase located in neutrophil granules, where hydrogen peroxide oxidizes chlorides to HClO, a strong oxidizing and bacteria killing agent. Using appropriate antibodies, it was shown that hypochlorous acid was responsible for inducing atherosclerotic lesion damage to macromolecules [129, 130]. Inflammation contributes to oxidative changes in proteins caused by reactive oxygen species. ROS and modified proteins may contribute to the development of changes in blood vessels that lead to atherosclerosis. In addition to the development of atherosclerosis, oxidation of low-density lipoprotein (LDL) also leads to glomerular sclerosis. It has been shown that HClO can be an important modifier of proteins and lipids and is involved in atherosclerotic changes and inflammation [130]. Using spin trap DMPO (5,5-dimethyl-1-pyrroline N-oxide) in EPR spectroscopy, hydroxyl radical generation in the blood at 20 min of hemodialysis of CKD patients was found [131]. Additionally, using another spin trap, N-tert-butyl-alpha phenylnitrone and also DMPO superoxide anion radical production during hemodialysis were detected [132]. These conditions further increase the release of ROS and the associated damage to the biological material of the host. This is the case with CKD patients. Additionally, oxidative stress is increased due to the presence of uremic toxins. Moreover, these patients experience oxidative stress during hemodialysis, as the contact of blood with artificial dialysis membranes leads to a respiratory burst of neutrophils and the associated release of high amounts of ROS including oxygen free radicals [3]. Oxidative stress appears to be the leading cause of mortality in patients with chronic kidney disease (CKD) due to the high risk of cardiovascular disease.

As a result of oxidative stress, oxidation of amino acid side chains, oxidation of peptide backbones, cross-linking of proteins, and advanced oxidation protein products (AOPP), carbonyl compounds are also released and a decrease in plasma thiol group concentration was observed [133, 134]. Free thiol groups (-SH) are of key importance in protection against oxidative stress because they are very sensitive to oxidation by ROS *in vivo* [135]. Using the spin labelling technique in EPR spectroscopy, we showed changes in the structure of hemoglobin HbA1c and HbA in patients with CKD both before and after hemodialysis. Conformational changes also concerned the pool of nonheme proteins present inside the erythrocytes [136]. These changes were caused by oxidative stress. It was also shown that mild oxidative stress caused hemoglobin to bind to the plasma membrane [137]. Hemoglobin conformational changes in CKD patients were accompanied by a decrease in total thiols in hemolysate before and after hemodialysis. The conducted studies showed the loss of the -SH groups in HbA1c and HbA hemoglobin as well as in nonheme proteins [136].

The disturbance of the redox balance is associated with the increase in ROS production and a decrease in antioxidant capacity. In turn, irreversible oxidation of the residue of the cysteine βCys93 in the globin chain may lead to disintegration of the structure of Hb and, consequently, to the release of heme, which also catalyzes free radical reactions [138]. There is a decrease in the activity of antioxidant enzymes [119, 121, 123]. Moreover, the amount of superoxide can be increased by activating the NADPH oxidase. Generally, it leads to oxidative stress, which causes disturbances in the structure and functioning of these cells, disintegration of the membrane, changes in its permeability, and hemoglobin leakage. The release of Hb from red blood cells can damage proteins, lipids, and other important molecules and macromolecules. Oxidative stress in CKD patients leads to the peroxidation of lipids and proteins. In the case of lipids, the end products of oxidation are malondialdehyde (MDA), isoprostanes, oxysterols, and 4-hydroxynonenal (HNE). For example, oxysterols initiate and worsen atherosclerosis [139]. In addition to lipid peroxidation, ROS leads to the oxidation of proteins, carbohydrates, glycoproteins, and others, of which the products are advanced glycation end products (AGE), carbonyls, and advanced protein oxidation products (AOPP). These products are also biologically active [140].

It has been shown that after hemodialysis, a decrease in the total antioxidant capacity and glutathione (GSH) in the

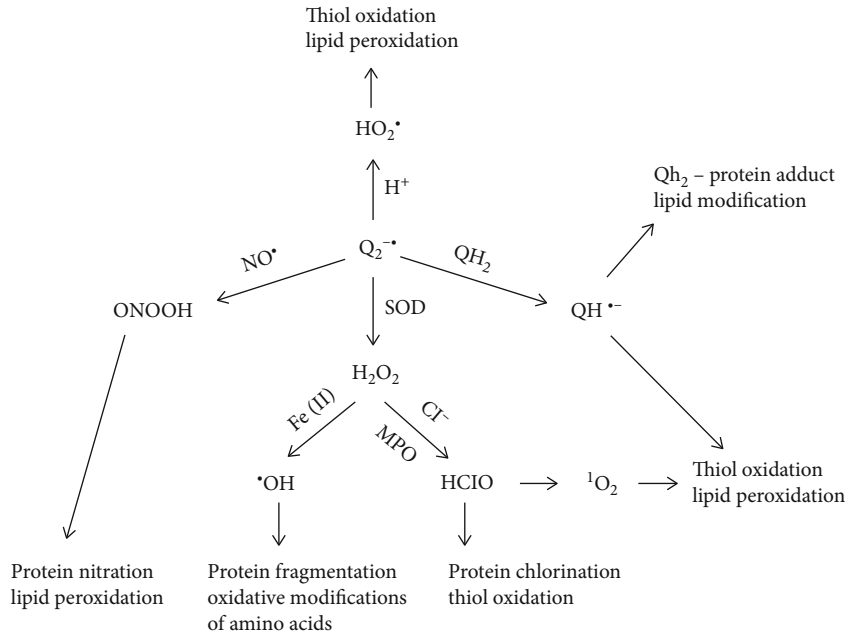

Figure 3: Reactive oxygen species production from superoxide anion radical and biological material damage by ROS. $O_2^{\bullet-}$, a precursor of other ROS, such as H_2O_2, which can oxidize chloride to HClO in the presence of MPO, $^1O_2$ from HClO, and NO which produce $ONOO^-$ and $HO^{\bullet}$ release in the Fenton reaction.

blood was found, while a much higher level of MDA was noted. In addition, a decrease in the activity of glutathione peroxidase and superoxide dismutase was observed in erythrocytes before and after hemodialysis, while the activity of catalase increased [141]. The range of changes of these parameters was influenced by the dialysis membrane. Polysulfone membranes were characterized by greater biocompatibility than cellulose membranes, and the observed decreases in antioxidants were lower than those for cellulose membranes. Also, a smaller increase in the level of MDA was recorded for the polysulfone membrane than for the cellulose dialyzer [141].

Advanced glycation end products (AGEs) are produced in patients with chronic kidney disease [133]. High levels of these substances are due to decreased renal clearance. AGEs are produced in the Maillard reaction, a series of chemical reactions that occur between amino acids, lipids, nucleic acids, and reducing sugars. AGEs are also produced in diseases with high levels of oxidative stress. Interactions between AGEs and their receptors (RAGE- (receptor for advanced glycation end products-) transmembrane, immunoglobulin-like receptor) can initiate oxidative stress and inflammation, leading to cardiovascular complications. It has been shown that neutrophils can generate more ROS by responding to AGEs via the NADPH oxidase complex [142]. Additionally, ample evidence suggests that the interaction between AGE and RAGE has a significant effect on inducing vascular damage, including endothelial dysfunction and arterial stiffness [143].

5. Red Blood Cells in CKD

Red blood cells (RBC) are permanently exposed to high oxygen concentration, which promotes the production of ROS. Within 24 hours, 3% of hemoglobin is oxidized and a superoxide radical is generated. In addition, hemoglobin itself is a catalyst for free radical reactions. Redox balance is maintained due to the presence of antioxidant enzymes and reducing agents with low molecular weight (Figure 4). Oxidative changes in erythrocyte components may lead to their deformation, which is influenced by the fluidity of the plasma membrane and the internal viscosity of RBC. In turn, the deformability of red blood cells is of key importance in microcirculation, because their diameter is larger than the diameter of the capillaries through which they flow. Oxidative damage to the RBC membrane has been reported to have a significant effect on the viscoelastic properties of the membrane [144]. In addition, the fluidity of the membrane is also important in the function of the RBC and the removal of toxic metabolites from the cell. Oxidative damage to the erythrocyte plasma membrane leads to impaired oxygen supply and leads to accelerated aging of red blood cells [145].

The red blood cells of patients with CKD had a greater fluidity of plasma membranes measured at different depths of the lipid monolayer than the RBC of healthy volunteers. The fluidity of the membranes increased with the time of hemodialysis. In the conducted experiment, we showed that RBCs from CKD patients were significantly more sensitive to oxidative stress induced by hydrogen peroxide than erythrocytes from healthy subjects [146]. The increase in membrane fluidity was accompanied by deepening changes in the membrane cytoskeleton [132, 146]. Moreover, plasma membranes of CKD erythrocytes were characterized by higher osmotic fragility compared to RBC of healthy individuals (Figure 2). Additionally, the fragility increased significantly after treatment with hydrogen peroxide [146]. It can, therefore, be assumed that each subsequent hemodialysis

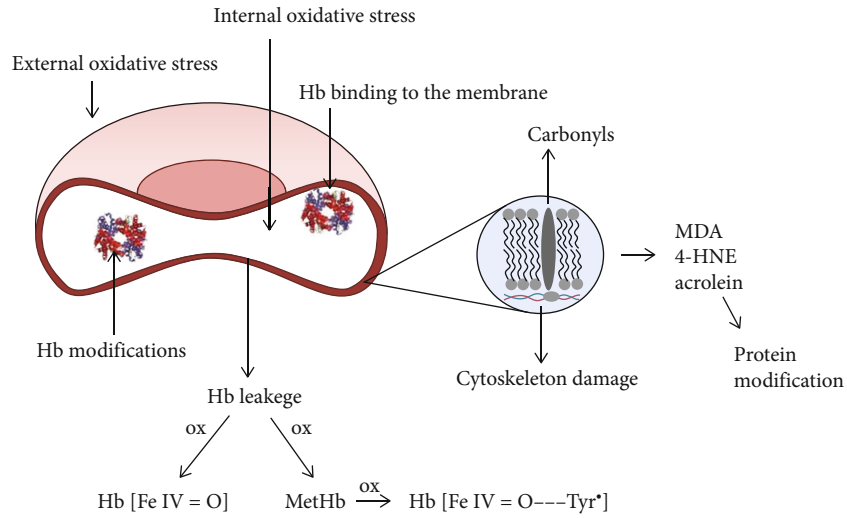

Figure 4: Red blood cell membrane damage from internal and external sources and hemoglobin (Hb) release. Ferryl (Hb[FeIV=O]) and ferryl radical form of hemoglobin (Hb[FeIV=O···Tyr•]). MDA: malondialdehyde; 4-HNE: 4-hydroxynonenal.

treatment can deepen the oxidative damage in these cells. Oxidative stress in the red blood cell leads to aging erythrocytes and phosphatidylserine exposure. The lifespan of a normal erythrocyte is 120 days. On the other hand, the lifetime of erythrocytes in chronic kidney disease is much shorter by up to 70% [147, 148]. Toxic uremic environment such as uremic toxins and oxidative stress shorten the survival time of red blood cells, which leads to anemia in chronic kidney disease. In the blood of patients, younger blood cells dominate, which are more susceptible to oxidative stress, and that may additionally contribute to the shortening of the survival time of young erythrocytes in patients with CKD.

There is a decrease in the activity of antioxidant enzymes [119, 121, 123]. In addition, the superoxide pool can be increased by activation of NADPH oxidase. In general, such a situation leads to oxidative stress, which causes disturbances in the structure and function of these cells. As a result of the oxidation of the components of the cell membrane, i.e. proteins and lipids, changes in its permeability occur, which leads to hemoglobin leakage.

Red blood cells can be damaged from both internal and external sources. The dominant factor of oxidative stress within the RBC is Hb. Oxygen derivative free radicals are generated as a result of autooxidation of Hb associated with the inner surface of the membrane, mainly with cytoskeleton proteins [149]. The released superoxide anion and the product of its dismutation, hydrogen peroxide, lead to the formation of hemichromes and degradation of heme, releasing free iron that catalyzes the Fenton and Haber-Weiss reactions. Additionally, hydrogen peroxide oxidizes the corresponding Hb and Met Hb to the ferryl form and the radical ferryl form.

Nitrite ions can diffuse into the interior of the erythrocyte, which are the source of NO or NO coming from the endothelial cells. However, most of the NO that the RBC is exposed to originate from endothelial e-NOS [150]. In turn, RBC can release the superoxide anion radical out through band 3. Thus, NO can also react with the superoxide in the cell and plasma to produce peroxynitrite, a powerful oxidant that can damage RBC inside and out [149].

Hemoglobin released from erythrocytes is dangerous because it is toxic and may initiate oxidation reactions in the biological material. To prevent damage to proteins, the lipid and other molecules are Hb bound by haptoglobin (Hp), which is an acute-phase protein that reduces oxidative damage. However, binding of haptoglobin to hemoglobin increases the level of ferryl formation during Hb-catalyzed lipid peroxidation. The increased stability of the Hp-Hb complex was also observed in the absence of lipids with the presence of external reducing agents [38]. The release of free Hb from red blood cells occurs in hemodialysis as a result of mechanical damage to red blood cells by dialysis pumps [151, 152]. We have repeatedly observed the presence of hemoglobin in the plasma of patients who have completed hemodialysis.

The free Hb released in plasma is bound by haptoglobin; however, with a high degree of intravascular hemolysis, the level of haptoglobin is too low for all of the released Hb to be bound. Hb dimers are then filtered by the glomeruli and reabsorbed through the proximal tubule. When the reabsorption capacity is exceeded, hemoglobin appears in the urine [153]. Both hemoglobin and myoglobin are prooxidants; if the released heme is not bound by hemopexin, then the iron redox cycle in the heme leads to globin radicals that induce lipid peroxidation [39]. Kidney damage by free Hb is similar to that by Mb in rhabdomyolysis, where Mb accumulates in the renal tubules, heme is released, and oxidative stress damages the renal parenchyma.

The products of lipid peroxidation are malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE). MDA is more mutagenic compared to 4-HNE, which in turn is the most toxic product of lipid peroxidation [154]. Its high toxicity is caused by reactions with thiols and amino groups, which lead to stable adduct proteins [155]. 4-HNE is also a signaling molecule and second only to the lipid peroxide toxic messengers of free radicals. 4-HNE not only is a signaling molecule

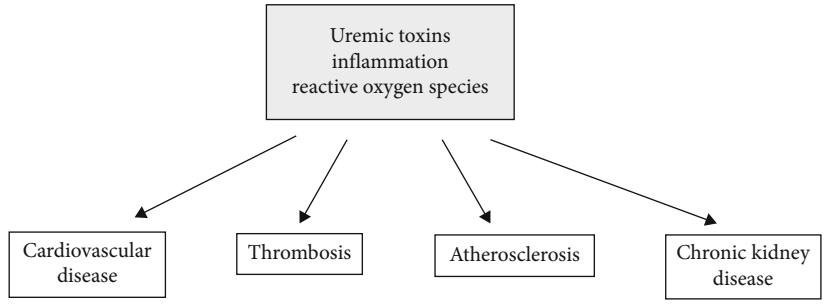

Figure 5: Uremic toxins, inflammation, and reactive oxygen species lead to chronic kidney disease, cardiovascular disease, thrombosis, and atherosclerosis.

but also is involved in the regulation of several transcription factors such as factor 2-bound nuclear factor 2 (Nrf2), activating protein-1 (AP-1), NF-κB, and receptors activated by peroxisome proliferators (PPARs). Furthermore, it performs roles in cell proliferation and/or differentiation, cell survival, autophagy, aging, apoptosis, and necrosis [156].

In chronic renal failure, many oxidized lipids have a toxic effect on cells and tissues, having a proapoptotic and proinflammatory effect, especially in the cardiovascular system. They include isoprostanes, especially isoprostane F2, of which the concentration increases with the development of the disease. Their accumulation and harmful effects meant that they were classified as uremic toxins. In CKD, oxidatively damaged lipoproteins are also observed, which lead to impaired HDL activity and may be strongly involved in accelerated atherosclerosis in patients with end-stage renal disease [157]. The risk of death in CKD patients is high due to cardiovascular complications [158]. Undoubtedly, premature death cannot be explained solely by the classic cardiovascular risk factors, such as hypertension, diabetes, and obesity. Recent studies show that the cause is uremic toxins, which are responsible for the increase in cardiovascular mortality in patients with CKD [159]. One of the toxins is spermine, a tetramine, but recent research has challenged this. It turned out that acrolein is the product of spermine oxidation with the participation of amine oxidase serum. Increased activity of serum amine oxidase leading to increased degradation of spermine was observed in CKD patients [160]. Acrolein is a very strong lacrimator, causing severe irritation to mucous membranes, eyes, and the upper respiratory tract. Already at a concentration of 2 ppm in the air, it can cause death. It was used for a period of time during World War I as a war gas. Acrolein is also released during lipid peroxidation, a process that is intensified in CKD patients.

6. Role of RBC in Cardiovascular Disease

The first cause of death in patients with end-stage renal disease on hemodialysis is a cardiovascular disease (CVD), which occurs in most patients. Mortality in this group is 20 times higher than that in the general population [161]. The short lifetime of RBCs in CKD causes anemia, a pathological condition characterized by a reduced number of circulating RBCs and a consequent low blood hemoglobin concentration compared to normal. Anemia can lead to serious complications of a cardiovascular disease (CVD), such as venous thrombosis, which can lead to stroke [162]. To increase the number of circulating erythrocytes, blood is transfused or erythropoietin is administered, which stimulates erythropoiesis. However, these actions do not always bring the expected results. Anemia leads to an increase in morbidity and mortality in cardiovascular diseases, which is associated with hypoxia. The consequence of hypoxia is an increased likelihood of thromboembolism, but also a hyperdynamic state associated with increased cardiac output, left ventricular hypertrophy and progressive enlargement of the heart, and possibly a proatherogenic role (Figure 5) [163].

The elderly are much more likely to develop venous thrombosis (VT)/thromboembolism (VT/E) due to the aging process characterized by an overproduction of reactive oxygen species (ROS). Red blood cells may perform a key role in initiating venous thrombosis during aging, according to recent studies (Figure 5). During RBC aging, RBC redox homeostasis is generally disrupted due to the imbalance between ROS production and the performance of antioxidant systems [164]. The main source of ROS is the autoxidation of hemoglobin and the activation of NADPH oxidase. RBCs can also be damaged by ROS from external sources and by other cells in the circulation. It has recently been shown that certain molecules produced during the blood clotting process can stimulate PMNs to produce ROS. ROS released by PMNs may damage RBCs, endothelium, and platelets and affect the coagulation process [165]. The consequence of the overproduction of ROS is oxidative damage to proteins and membrane lipids, which leads to a loss of membrane integrity and reduced deformability. These changes disrupt RBC functions in hemostasis and thrombosis, leading to hypercoagulability through increased RBC aggregation and RBC binding to endothelial cells, which may limit the availability of nitric oxide. In addition, RBC can activate platelets, modulating their activity. An important factor in hematology is the coagulation system and the activation of platelets, which not only contributes to hemostasis but also accelerates the coagulation system [166]. The interactions of RBCs with coagulation factors by influencing and activating them are also important.

During aging, the amount of ROS released increases, which disturbs the balance between thrombosis and hemorrhage. Emerging pathophysiological changes include disturbances in blood coagulation and related vascular function, blood flow, and coagulation pathways [167, 168]. Venous

thrombus is characterized by a high content of RBC and fibrin; therefore, it is believed that red blood cells (RBCs) are now a critical mediator of venous thrombosis. Tissue factor (TF) is involved in the clotting pathway in the clotting process. Recently, the presence of TF has been demonstrated in neutrophils. It turned out that the interaction of neutrophils with endothelial cells is a critical stage, taking place earlier than the accumulation of platelets in the initiation of arterial thrombosis in damaged vessels [169].

Although RBCs are the major cellular component of venous clots, they are not the primary active causes of DVT. However, they influence the formation of blood clots through oxidative mechanisms. RBCs contain a large amount of hemoglobin which can autooxidize with the release of superoxide to form MetHb. Hb and MetHb can be, respectively, oxidized to the ferryl form and/or the radical ferryl form. Both forms initiate oxidative stress. The release of larger amounts of Hb from RBCs, which in the later stages intensifies the oxidative stress, leads to the activation of blood platelets, endothelial cells, and the formation of a thrombus [170]. These changes can be counteracted by haemoxygenase-1. HO-1 is an enzyme that catalyzes heme degradation and performs a key role in defending the body against oxidant-induced damage in inflammation. The role of HO-1 in the protection of the renal tubules against oxidative damage has been demonstrated. This enzyme is important as these cells are constantly exposed to oxidative stress. In an HO-1 deficiency, the renal tubular epithelium is more prone to oxidative stress [171]. Moreover, hemoglobin also increases the expression of functional TF in macrophages and reduces the sensitivity of TF to antioxidants [172]. Free hemoglobin generated during RBC hemolysis as a result of degradation releases heme that may initiate NETosis [173]. It has been shown *in vivo* that hemolysis associated with heme release activates inflammasome 3 (NLRP3) in macrophages. Macrophages, inflammasome, and IL-1R components have also been shown to contribute to hemolysis-induced mortality [174].

Systemic hypoxia has been shown to accelerate thromboembolic events through an inflammasome complex containing 3 (NLRP3) and increased IL-1β secretion. NLRP3 has also been shown to be mediated by inducible factor 1-alpha hypoxia (HIF-1α) [175]. It can be assumed that the abovementioned factors associated with the pathology of venous thrombosis are more severe in patients with chronic kidney disease. This would partly explain the high mortality of CKD patients from cardiovascular diseases.

7. Conclusion

ROS are involved in inducing oxidative stress in the blood of patients and oxidative damage to the kidneys and in the development of chronic kidney disease. ROS are responsible for RBC damage, anemia and, consequently, general hypoxia of the body. Overgeneration of ROS leads to stimulation of the blood coagulation, activation of platelets, endothelial cells, neutrophils, and other processes that promote the formation of venous clots leading to thromboembolism and associated complications.

Conflicts of Interest

The authors declare that they have no conflict of interest.

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Review Article

Potential Effects of Immunosuppression on Oxidative Stress and Atherosclerosis in Kidney Transplant Recipients

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Chronic kidney disease is a public health problem that, depending on the country, affects approximately 8–13% of the population, involving both males and females of all ages. Renal replacement therapy remains one of the most costly procedures. It is assumed that one of the factors influencing the course of chronic kidney disease might be oxidative stress. It is believed that the main mediators of oxidative stress are reactive oxygen species (ROS). Transiently increased concentrations of ROS play a significant role in maintaining an organism's homeostasis, as they are part of the redox-related signaling, and in the immune defense system, as they are produced in high amounts in inflammation. Systemic oxidative stress can significantly contribute to endothelial dysfunction along with exaggeration of atherosclerosis and development of cardiovascular disease, the leading cause of mortality in patients with kidney disease. Moreover, the progression of chronic kidney disease is strictly associated with the atherosclerotic process. Transplantation is the optimal method for renal replacement therapy. It improves better quality of life and prolongs survival compared with hemodialysis and peritoneal dialysis; however, even a successful transplantation does not correct the abnormalities found in chronic kidney disease. As transplantation reduces the concentration of uremic toxins, which are a factor of inflammation per se, both the procedure itself and the subsequent immunosuppressive treatment may be a factor that increases oxidative stress and hence vascular sclerosis and atherosclerotic cardiovascular disease. In the current work, we review the effect of several risk factors in kidney transplant recipients as well as immunosuppressive therapy on oxidative stress.

1. Introduction

Kidney transplantation (KTX) has evolved over the years to become the preferred means of renal replacement therapy for patients with end-stage renal disease, improving overall life expectancy and quality of life in these patients. Patient and graft survival rates are spectacular and usually provide excellent short-term and medium-term results. Despite this progress, there has been little improvement in the long-term renal graft and patient survival in a sense of various clinical complications that can develop due to the high complexity of this procedure [1–4]. It is well known that renal transplantation confers a survival advantage over dialysis treatment for patients with end-stage renal disease (ESRD) [1, 2]. However, the survival of transplant recipients is significantly lower than age-matched controls in the general population. The higher mortality in renal transplant recipients is, in part, due to comorbid medical illness, pretransplant dialysis treatment, and factors related to transplantation, including immunosuppression and other drug effects [3, 4]. Despite successful kidney transplantation, cardiovascular disease (CVD) remains the leading cause of morbidity and mortality in patients with chronic kidney disease (CKD) including predialysis, dialysis, and after renal transplantation subjects. Besides traditional risk factors, oxidative and nitrosative stress as well may contribute to the progress of CVD through the formation of atherosclerotic plaque [3, 4]. Oxidative stress, an imbalance between generation of oxidants and antioxidant defense system, is one of the major events which affects not only early posttransplantation phase but also graft and patient's long-term outcomes [5, 6]. This imbalance contributes to the elevated CVD morbidity and

mortality as well as to the development of chronic allograft nephropathy, which is characterized by gradual decline in kidney function [7].

Kidney transplantation is aimed at restoring kidney function, but it incompletely mitigates pathological pathways and mechanisms of disease, such as chronic low-grade inflammation with persistent redox imbalance [8]. Among the other factors that can be involved in long-term kidney transplant complications as well as in elevated oxidative and nitrosative stress, immunosuppressive treatment has its role. After renal transplantation, there is an increase in oxidative phenomena related to endothelial dysfunction, inflammation, and atherosclerosis, which are responsible for both damage to the graft and cardiovascular complications, one of the major causes of patient death [9]. A number of studies demonstrate the prooxidant effects of both calcineurin inhibitors [9–11]; however, CsA has been described as a more potent oxidative stress inducer than TAC [12].

As we well know, the imbalance in the oxidant/antioxidant mechanisms leads to oxidative stress which plays a crucial role in vascular injury. The major mechanism leading to oxidative stress is the overproduction of ROS (reactive oxygen species). Disease entities such as hypertension and diabetes—the most common causes of ESRD—are characterized by high ROS production in the arterial walls [13, 14]. This underlies arterial remodeling and atherogenesis due to endothelial dysfunction and vascular inflammation. If we consider kidney failure as a consequence of these diseases, the farther kidney failure goes, the more pronounced the process becomes. Additional factors influencing the quality of the vessels will be the process of hemodialysis or aging in the pretransplant period itself. Detailed qualification of kidney transplant recipients and donors reduces the risk of failure, but there is no chance of organs deprived of this process. Surely, the transplant reduces the concentration of uremic toxins, which are a factor of inflammation per se, but both the procedure itself and the subsequent immunosuppressive treatment may be a factor that increases oxidative stress and hence vascular sclerosis and atherosclerotic cardiovascular disease (ASCVD).

2. Donor/Recipient Selection

2.1. Live > Death, Female > Male. A death donor kidney transplant is the most common organ donation procedure. Brain death, however, is associated with severe homodynamic disturbances [15], e.g., increasing blood pressure, decreasing cardiac output, and hormonal disturbances [16] which alter in tissue perfusion and activate the inflammatory process. The disturbances of hemodynamics and metabolism lead to ROS formation in the donor and correlate with ROS-mediated posttransplant kidney function. The significance of free radicals, measured by a quantitative evaluation of malondialdehyde (MDA), a stable product of lipid membrane peroxidation and total antioxidant status (TAS), was shown, i.e., by Kosieradzki et al. [17] in 2003. The gender of the recipients could be also important: some animal studies have shown an increased superoxide radical production and (in consequence) renal injury risk secondary to 17b-estradiol level, which may suggest greater oxidative stress in male recipients [18].

2.2. Less > More Risk Factors (including Age). When performing a kidney transplant procedure in a patient with numerous risk factors, such as advanced pretransplant atherosclerosis, poorly controlled arterial hypertension, and especially advanced age of the recipient, it should be taken into account that vascular sclerosis could accelerate. The most commonly known age-associated changes in the endothelium are decreased activity (but not expression) of eNOS, increased arginase activity (decreased production and/or availability of NO), increased expression and activity of cyclooxygenases (COX) and their vasoconstrictors, and increased ROS production [19]. All this inevitably leads to an intensification of the existing oxidative stress and, consequently, to accelerated atherosclerosis, including vessel occlusion and graft ischemia; even if these changes were not significantly macroscopically expressed before transplantation [20], considering that atherosclerotic cardiovascular disease (ASCVD) is the leading cause of mortality [21], there is substantial risk of death-censored graft loss.

3. Transplant-Related Immune Activation

3.1. Role of CD8+ T Cell Activation. In human atherosclerosis lesions, we can find an increasing presence of CD8+ and CD4+ T cells. Some studies show that it may have an impact on the development of these lesions. Kyaw et al. [22] in their experiment proved that CD8+ T lymphocyte depletion has an intermediary influence on inflammatory cytokine TNF alpha and reduced atherosclerosis. Cochain et al. [23] drew a conclusion that CD8+ T cell promotes atherosclerosis because of controlling monopoiesis.

3.2. CMV Infection. Some factors during the posttransplant period, such as delayed graft function, cytomegalovirus infection, and microalbuminuria, which may damage renal function, produce a decreased antioxidant capacity (lower glutathione peroxidase (GPx)) [24]. Exposure of CMV before transplantation and posttransplant replication may have proatherogenic effects in relationship to the cellular immune response against CMV antigens. Ducloux et al. reported that CMV infection is associated with an accumulation of CD57+CD28-CD8+ T cells and divided patients into 3 groups: CMV negative, CMV positive without replication after Tx, and those with presented CMV replication after transplantation. The frequency of the presence of CD57+CD28-CD8+ T cells was highly related with the incidence of atherosclerotic events [25]. Interestingly, these terminally differentiated T cells are increased also in patients with ESRD (pretransplantation period) and IV stage CKD and they also correlate with CMV seropositivity. This might be the premature T cell aging effect, and it seems to be an unmodifiable factor even after successful kidney transplantation [25, 26].

3.3. Role of Allogenic Stimulation. In reference to the expected proatherogenic correlation between circulating CD57+CD28-CD8+ T cells and repeated stimulation of viral

antibodies, there might be a relationship with HLA mismatch number and atherogenic-related events. In the cohort study analysis, Ducloux et al. observed higher risk in groups with increased points of HLA noncompliance. They assessed it as an independent risk factor of atherosclerosis [27]. As discussed above, atherosclerosis and cardiovascular diseases are associated with both the so-called traditional risk factors such as diabetes, hypertension, broadly understood endothelial dysfunction, and nontraditional risk factors such as oxidative stress (OS). We have increasing possibilities of biochemical, physical, and cellular evaluation of the impact of individual procedures, including immunosuppressive treatment, on the development of both oxidative stress itself and atherosclerosis—a consequence of OS and other components. In the case of oxidative stress, the most frequent biochemical factors assessed are TAC (total antioxidant capacity), MDA (malondialdehyde), GSH (glutathione), SOD (superoxide dismutase) activity, GPx (glutathione peroxidase) activity, CAT (catalase) activity, or oxLDL (oxidized LDL). SOD, GPx, and CAT are antioxidant enzymes; the concentration of which in patients with CKD is reduced and stage dependent. The improvement of glomerular filtration, a reduction in the concentration of uremic toxins, and other positive effects of KTX are followed by an increase in antioxidant factors; however, they do not reach the values observed in healthy individuals [28]. To evaluate atherosclerosis in patients after kidney transplantation, we can measure carotid intima media thickness (IMT) with ultrasound. There is a positive correlation between IMT and increased cardiovascular risk. An independent predictor of cardiovascular disease is also arterial stiffness—correlated with increased intravascular thrombosis due to some drug toxicity. This factor can be evaluated by measuring augmentation index and pulse wave velocity (PWV) [29].

3.4. Overview of Drugs Currently Used in Renal Transplantation. The current management of the renal transplant recipients using maintenance immunosuppression therapy is multimodal where most immunosuppressive regimens generally include a calcineurin inhibitor plus an adjunctive antiproliferative agent and steroids. The addition of induction therapy with a variety of monoclonal or polyclonal antibodies provides a more potent immunosuppression, and its use is more relevant in patients with a high immunological risk. More recently, mammalian target of rapamycin inhibitors has been incorporated in different protocols.

Immunosuppressive agents used in immunosuppressive therapy can be classified into three categories: induction therapy, maintenance therapy, and treatment for rejection.

Immunosuppressive medication can be divided into several subgroups; the most common are as follows:

(1) Drugs that inhibit the production of cytokines involved in the activation of cells and their clonal expansion.

  (i) Calcineurin inhibitors (CNI): cyclosporine A (CsA) and tacrolimus (TAC)

(2) Inhibitors of the proliferation signal

  (i) Mammalian target of rapamycin inhibitors (mTORi): sirolimus (SIR) and everolimus (EVERL)

(3) Drugs that inhibit cell division

  (i) Nonselective: azathioprine (AZA)

  (ii) Selective: mycophenolate mofetil (MMF)

(4) Other drugs

  (i) Costimulant inhibitor: belatacept

  (ii) Lymphodepletive therapy: ATG (antithymocyte globulin)

  (iii) Anti-CD20 chimeric human and mouse monoclonal antibody: rituximab

4. Influence of Selected Groups of Drugs on Oxidative Stress and Atherosclerosis

4.1. Calcineurin Inhibitors (CsA, TAC). Calcineurin inhibitors (CNIs) such as CsA and TAC are the main immunosuppressive drugs used to prevent the rejection in solid organ transplant recipients. Long-term treatment with CNIs increases the risk of adverse effects such as malignancy, chronic allograft dysfunction, and cardiovascular risk factors in this clinical population. In patients after transplantation treated with CNI, the most common complications are arterial hypertension secondary to endothelial damage and dysfunction causing vasoconstriction. They are also promoted intravascular fibrosis leading to increased arterial stiffness (chronic toxicity). In addition, there is evidence that CNI causes direct vascular toxicity by damaging vascular smooth muscle cells (VSMCs) [20–22]. Vascular damage leads to the decrease of renal function, which means CNIs have a potential nephrotoxicity effect [30, 31].

Also, they may lead to free radical overproduction [9–12]. Some authors confirmed that TAC patients have lower production of free radicals than patients on CsA-based regimen [32]. In spite of that, others conclude that there is no difference in oxidative stress parameters between the two immunosuppressive treatments [33].

Tacrolimus has a better cardiac-lipid profile than cyclosporine A. Some reports about the beneficial effect of tacrolimus on the level of oxidative stress in the organism have appeared. In particular, in vitro studies and animal tests indicate antioxidative properties for tacrolimus. Decreases in parameters of oxidative stress, such as the concentration of malondialdehyde (MDA), the activity of myeloperoxidase (MPO), and neutrophilic infiltration, have been observed after treatment. In in vitro studies on endotheliocytes, tacrolimus induced oxidative stress more weakly than other medications and was the only one that did not increase the production of nitric oxide (NO). The protective effect of tacrolimus on inflammatory response in rat liver during

ischemia-reperfusion injury was also described. Findings in renal transplant recipients are not so clear and even indicate that the influence of tacrolimus on the activity of antioxidative enzymes in the kidneys may be involved in side effects of tacrolimus.

Moreno et al. studied 67 stable kidney transplant patients treated with calcineurin inhibitors who were not receiving cholesterol-lowering therapy and 14 healthy subjects. They demonstrated that the oxidative status did not differ between the cyclosporine and tacrolimus cohorts. Furthermore, transplanted patients showed a higher oxidative status (MDA increase and GPx decrease) than healthy subjects [24].

Recent studies have suggested that increased plasma malondialdehyde (MDA) levels are a consequence of specific immunosuppressive therapies. The study of Perrea et al. showed that immunosuppressive combined therapy with CyA was associated with the high values of MDA that were measured posttransplant. Moreover, this study provided strong evidence that tacrolimus is significantly associated with improved free radical metabolism [32].

4.2. Mechanisms. The research group Rodriguez-Diez et al. assessed the effects of CNI on murine endothelial cells. They observed dose-dependent upregulation of the synthesis nuclear factor kappa-light-chain enhancer of activated B cell- (NF-κB-) dependent chemokines such as IL-6 and TNF-alpha. Moreover, both substances CsA and TAC induced the synthesis of important vascular proinflammatory cytokines IL-6 and TNF-alpha which in turn cause inflammation and endothelial damage [34].

The impact of NFK on heart disease has been shown, among others, in Van der Heiden et al.'s study [35].

The key events mediating between CNI and inflammation on endothelial cells are Toll-like receptor signaling (TLRs). The vascular response to injury develops through signaling mediated by TLRs and is a key component of innate immunity.

To assess the effect of TLRs on NF-κB, the effects of CNI in mice with the MyD88 adapter protein gene silenced were studied, which prevented the synthesis of agonists in the TLR activation pathway. Administration of TAC to such a modified organism resulted in the much lower activity of the NF-κB-dependent pathway.

As TLR4 is particularly important in the development of vascular diseases, in the next step, pharmacological signal transmission, specifically from the intracellular part of TLR4, was blocked pharmacologically. After analysis, a decreased expression of genes leading to the synthesis of proinflammatory cytokines was found [34].

In addition, decreased ROS production was also noted in VSCM cells and endothelial cells, which means reducing the oxidative stress and its consequences described in the previous paragraphs.

Data on whether any of the CNIs have a lower proinflammatory effect are inconclusive; in some, there are data that CsA increases the risk of OS [36]; in others, the impact of both CsA and TAC on OS is assessed as similar [28, 37].

4.3. CNI (CsA) vs. Belatacept. Due to the CNI side effects, including nephrotoxicity, some analyses are trying to bring new, alternative solutions to immunosuppression—with a lower intensity of vascular (and as a consequence renal) side effects. Costimulation inhibitor belatacept (BELA), one of the promising ones, although not yet registered in all countries (e.g., not available in Poland), is registered in Europe and the USA in 2011 (Nulojix BMS). Pooled analysis of the BENEFIT study and BENEFIT-EXT showed, among others, belatacept (costimulant inhibitor) as an alternative which is associated with less hypertension, hyperlipidemia, and NODAT (new-onset diabetes) [37–41]. In a 46-patient study organized by Seibert et al. [38], PWV was assessed in two groups, with a similar profile of comorbidities—23 participants treated with CsA and 23 with belatacept. In the measurement of brachial blood pressure, serum lipid level was also used for the assessment. Statistically, significantly higher systolic blood pressure and faster heart rate were observed in the group treated with CsA, as is the rate of NODAT and level of serum lipids. PWV and augmentation pressure were lower in patients receiving belatacept, but this did not show a statistically significant difference.

The authors believe that the lack of unequivocal benefit associated with the use of belatacept, despite its lower vasoconstriction potential, thus a lower incidence of HT and other complications, may be associated with a too small control group and too short observation time. Therefore, it seems justified to extend the study to new participants with an extension of the study duration. Looking at the limited data, it seems that it has the potential to reduce atherosclerosis and the incidence and death of cardiovascular diseases.

4.4. Mammalian Target of Rapamycin (mTOR). mTOR is a subunit of 2 distinct multicomplexes (mTORC1 and mTORC2), which play a crucial role in various processes, e.g., cell proliferation, protein synthesis, and glycolysis. Activation of mTORC1 is triggered by several stimuli, such as availability of nutrients and ATP, growth factors, and oxidative stress [42]. Systemic lupus erythematosus (SLE) patients exhibit various disturbances in the immune system that can be linked to, among other things, mitochondrial dysfunction of T cells, which results in increased generation of ROS and glutathione (GSH) depletion. Subsequent oxidative stress-related mTORC1 activation leads to dysregulation of various T cell subpopulations [43]. Interestingly, according to a study conducted by Lai et al., treatment with NAC increases levels of GSH and reduces mTOR activation in peripheral blood lymphocytes, which leads to improvement in disease activity scores in SLE patients [44]. In kidney transplant recipients, ischemia-reperfusion injury (IRI) is a vital problem that is responsible for delayed graft function as well as immune activation, which in turn results in acute rejection and chronic graft nephropathy. Initial consequence of IRI is associated with oxygen depletion and production of ROS in mitochondria of kidney tubular cells. The resulting oxidative stress has a damaging effect on kidney tissue and creates a proinflammatory environment, which even after restoration of sufficient blood flow continues to exert detrimental influence, promoting apoptosis, inflammation, and fibrosis [45]. Therapeutic strategies of targeting mTOR in order to ameliorate IRI have been evaluated in various animal models. Kezić

Table 1: Overview of published data of the studies designed to assess the oxidative state of renal transplant patients.

Study	Objective	Results/conclusions
Moreno et al. [24]	The study was designed to assess the oxidative state of transplant patients with stable renal function; 67 stable kidney transplant patients treated with calcineurin inhibitors were studied.	Transplanted patients showed a higher oxidative status (MDA increase and GPx decrease) than healthy subjects. The oxidative status did not differ between the cyclosporine and tacrolimus cohorts.
Ruiz et al. [6]	The study was designed to determine the relationship between the presence of carotid artery lesions and oxidative parameters in 50 renal transplanted patients with stable renal function.	The serum GPx level among patients without atheroma plaques, calcification, or stenosis was higher than in those with ultrasound signs.
Perrea et al. [32]	The study included 26 renal transplant patients, treated with a different combination of immunosuppressive agents: CyA-MMF-PRED-basiliximab and TAC-MMF-PRED-daclizumab. Plasma MDA levels were measured before transplantation and 1 and 6 months after TX.	Levels of MDA were increased before the transplantation in all renal patients. Immunosuppressive combined therapy with CyA was associated with the high values of MDA posttransplant. This study provides strong evidence that TAC is significantly associated with improved free radical metabolism.
Zadrazil et al. [33]	AOPP and TAS were evaluated in transplanted patients on different calcineurin inhibitors. 35 patients were treated with CsA and 33 with TAC.	No significant differences in AOPP and TAS were found with respect to treatment. The only exception was the higher mean concentration of AOPP at month 1 in recipients treated with CsA.
Szymczak et al. [53]	The aim of this study was to compare the effect of immunosuppressive regimens using either mTORi or CNI on the risk of atherosclerosis in RARs. The study involved 24 RARs treated with mTORi and 20 RARs treated with immunosuppressive regimen based on CNI. Carotid atherosclerosis was evaluated by measurement IMT of the common and internal carotid artery walls and detection of carotid plaques by high-resolution ultrasonography. The study was performed 3-24 years after TX.	The mTORi group showed higher level of TC, LDL-C, and TG. Posttransplant diabetes developed in 34% of the mTORi group compared with 25% in the CNI group. There was no beneficial effect of immunosuppressive treatment with mTORi on carotid atherosclerosis in RARs.
Joannidès et al. [58]	The study was designed to evaluate whether or not CsA-free immunosuppressive regimen based on SRL prevents aortic stiffening and improves central hemodynamics in RARs. 44 patients enrolled in the trial were randomized at week 12 to continue CsA or switch to SRL, both associated with MMF. cSBP, cPP, AIx, and PWV: aortic stiffness was blindly assessed at W12, W26, and W52 together with ET-1, TBARS, and SOD and CT erythrocyte activities.	At W12, there was no difference between groups. At follow-up, PWV, cSBP, cPP, and AIx were lower in the SRL group. In parallel, ET-1 decreased in the SRL group, while TBARS, SOD, and CT erythrocyte activities increased in both groups but to a lesser extent in the SRL group. These results demonstrate that a CsA-free regimen based on SRL reduces aortic stiffness, ET-1, and oxidative stress in RARs suggesting a protective effect on the arterial wall that may be translated into cardiovascular risk reduction.
Juskowa et al. [5]	The study was designed to examine markers of lipid peroxidation and antioxidant potential in the blood (serum, plasma, and RBC) of 51 RARs and sex-matched volunteers as a control group (C). RARs were divided into two subgroups: RARs-A ($n = 28$) were treated with triple-drug therapy including CsA and RARs-Z ($n = 23$) were on double-drug regimen: PRED and AZA. We used several automated assays to estimate MDA, TRAP, GPx, GSH, SOD, CAT, vit. E, and lipid profiles.	Patients of RARs-A were found to have significantly elevated triglycerides, cholesterol-LDL, MDA, and TRAP and decreased activity of RBC glutathione peroxidase as compared with those of RARs-Z and group C. In conclusion, our data show that oxidative stress (with prooxidant effect of CsA partly at least), with reduced antioxidant potential of defense system, is associated with KTX.
Chrzanowska et al. [10]	The aim of the study was to analyze the relation between total antioxidant capacity and immunosuppressive therapies, renal function, and hematocrit in kidney transplant patients. The study included 46 adult patients following renal transplantation, treated with different combinations of immunosuppressive agents: with CsA ($n = 23$) or TAC ($n = 15$).	There was a significantly negative correlation between TAOC and plasma creatinine and a positive correlation between TAOC and creatinine clearance or hematocrit in patients treated with TAC but not with CsA. Immunosuppressive therapy with CsA was associated with higher TAOC. Anemia can be an independent risk factor for an increase of oxidative stress. TAOC was positively associated with renal function in patients treated with TAC.
Vural et al. [11]	23 KTX patients were included in the study. MDA, plasma selenium (se), GSH-Px, SOD, EZn, and ECu levels were	The GSH-Px, SOD, ECu, EZn, and selenium levels were lower and MDA levels were higher in patients than controls

Table 1: Continued.

Study	Objective	Results/conclusions
	studied before and in the 1st, 3rd, 7th, 14th, and 28th days after TX. 11 recipients were treated with CsA whereas 12 patients were treated with TAC.	before TX. MDA levels decreased and SOD, GSH-Px, ECu, and EZn levels increased in parallel to the decrement of serum creatinine levels following KTX. No difference was found among the patients regarding the treatment regime. The study data suggest that the improvement in oxidative state parameters begins at the first day of KTX and continues at the 28th posttransplant day in living donor TX.
Cofan et al. [12]	The objective of this study was to analyze the effect of converting from cyclosporine to tacrolimus on lipoprotein oxidation in renal transplant recipients. 12 recipients were studied treated with a CsA-MMF-PRED combination that was converted to TAC-MMF-PRED.	The conversion to TAC resulted in significant decrease in TC levels and produced a nonsignificant decrease in Ab-oxLDL. In renal TX, TAC therapy was associated with a better lipid profile and lower in vivo LDL oxidation when compared with CsA treatment.

Ab-oxLDL: oxidized LDL autoantibodies; AOPP: advanced oxidation protein products; AIx: augmentation index; AZA: azathioprine; CAT: catalase; CNI: calcineurin inhibitor; Cr: creatinine; CsA: cyclosporine A; ECu: erythrocyte Cu; ET-1: endothelin-1; EZn: erythrocyte Zn; GPx: glutathione peroxidase; GR: glutathione reductase; GSH: glutathione; GSH-Px: erythrocyte glutathione peroxidase; IMT: intimal media thickness; KTX: kidney transplant patients; LDL: low-density lipoprotein cholesterol; MDA: malondialdehyde; mTORi: mammalian target of rapamycin inhibitors; MMF: mycophenolate mofetil; OS: oxidative stress; PRED: prednisone; SOD: superoxide dismutase; cPP: pulse pressure; PWV: aortic stiffness carotid-to-femoral pulse-wave velocity; RARs: renal allograft recipients; cSBP: carotid systolic blood pressure; SIR: sirolimus; TAC: tacrolimus; TAOC: total antioxidant capacity; TAS: total antioxidant status; TC: total cholesterol; TG: triglycerides; TBARS: thiobarbituric acid-reactive substances; TRAP: total radical-trapping antioxidant potential; TX: transplantation.

et al. evaluated the effect of everolimus on IRI-associated NF-kappa B activity, production of IL-1-beta, TNF-alpha, and IL-10. It turned out that everolimus-treated animals displayed higher concentration of proinflammatory cytokines in the early phase of IRI [46]. An earlier study conducted by Suyani et al. compared, among other things, the effect of everolimus on levels of malondialdehyde (MDA), superoxide dismutase (SOD), and myeloperoxidase (MPO) in rats subjected to IRI. While levels of MDA and MPO were significantly lower in the everolimus group compared with the nontreated group, which indicated lower lipid peroxidation and decreased neutrophil and mononuclear infiltration, SOD activity remained low in both groups, corresponding with SOD depletion associated with oxidative stress [47]. The results proved to be inconclusive—on the one hand, mTOR inhibition immediately after transplantation may interfere with recovery of graft function likely as a result of antiproliferative and proapoptotic effect of mTORi, as well as overactivation of autophagy, while on the other hand long-term beneficial effect of mTORi on oxidative stress and immune activation improved outcomes during the recovery phase [48, 49].

The currently available studies assessing the impact of mTORis on cardiovascular diseases do not provide conclusive data. This drug group is associated with an increased risk of hyperlipidemia, endothelial dysfunction, and diabetes, which are known risk factors for both atherosclerosis and heart disease [50, 51]. On the other hand, part of the research on animals suggests the antiatherosclerotic effects of mTOR [52]. Taking into account these discrepancies in the data, e.g., in a small study by the team of Szymczak et al. [53], the effect of using sirolimus and tacrolimus versus CNI/MMF was assessed in a group of 44 patients after KT. Analysis included laboratory data such as serum lipid level (LDL, HDL, and TG), uric acid, and glycated hemoglobin. The severity of atherosclerosis was assessed by ultrasound IMT measurement—wall thickening > 14 mm over a length > 10 mm was treated as an atherosclerotic plaque. The results of this study revealed higher levels of total cholesterol and triglycerides in patients taking mTOR (statistically significant difference) compared to those on CNI. Both groups received statins. There were statistically more cases of NODAT in the mTORi group (34% vs. 25%). This translated into an increased risk of myocardial infarction per patient per 5 years. However, no significant difference was found in the mean IMT thickness [53]. Therefore, this was the opposite conclusion compared to the studies proving the prevention of coronary artery disease in patients after heart transplantation receiving mTOR, including antirestenotic activity of the stent achieved in the coronary arteries [54, 55]. It seems impossible to extrapolate these achievements in terms of the group of patients after KTX. The reasons for this include a greater decrease of glomerular filtration, disturbances in calcium-phosphate balance, and a more frequent tendency to hypertension occurring in kidney transplantation recipients.

Steroids and calcineurin inhibitors inhibit inducible nitric oxide, thus helping to determine endothelial dysfunction associated with onset and progression of atherosclerosis and vascular calcification. Much more complex are the vascular effects of mTOR inhibitors. Rapamycin inhibits smooth muscle cell proliferation, while everolimus impairs the vasoactive and antithrombotic function of endothelial cells [56]. Some studies suggest a relationship between vascular calcification and impaired bone metabolism as well as an involvement of immunosuppressive drugs on expression, regulation, and function of RANKL, RANK, and osteoprotegerin (OPG) system working in the skeletal and vascular systems. In particular, sirolimus inhibits osteoclast formation, unlike steroids and cyclosporine [57].

mTORis, in their pathomechanism of action, inhibit the formation of atherosclerotic plaques—i.e., they inhibit

macrophages and VSMC proliferation, but this is a beneficial effect of vessels with plaque forming, not existing ones. Despite reports on the beneficial effect of sirolimus in aortic stiffness [58] by reducing oxidative stress and plasma endothelin-1 concentration, the advantage over CNI in terms of atherosclerotic complications cannot be unequivocally recognized—similar to other groups of drugs, patients treated with mTOR have an increased cardiovascular risk and require intensive monitoring.

4.5. Lymphodepletive Therapy: ATG Treatment. Antithymocyte globulin (ATG) for many years was used as an immunosuppressive treatment in solid organ transplantation. These polyclonal antibodies lead to T cell depletion and induce wide and persistent changes in T cell subpopulations including CD8+ T cell expansion [25].

As previously described, the repopulation of T cells with a predominance of CD8+ T cells is clinically correlated with an increased risk of atherogenesis. Considering the additional effect of CMV infection in transplant patients, Havenith et al. [59] in their work noted a significant acceleration of atherosclerosis in CMV-positive patients taking ATG, with no significant difference in CMV I patients. Therefore, ATG should be taken into account in the mechanism of atherosclerotic lesion formation as a cofactor in combination with CMV infection, without a significant effect in patients without this burden.

4.6. Rituximab. Rituximab is a chimeric human and mouse monoclonal antibody that reacts with CD20 antigen presented on pre-B and mature B lymphocytes. Therefore, it is often used in transplantation for pretransplant desensitization in patients with HLA or ABO incompatibility and posttransplant treatment of acute antibody-related rejection or lymphoproliferative diseases, including posttransplant [60]. Thus far, the effect of rituximab on the formation of atherosclerotic plaques in patients with rheumatic diseases has been reported. There are also studies, mainly with small groups of subjects, assessing the same effect in transplant patients. They are based on the qualitative and quantitative evaluation of biomarkers related to the atherosclerosis process—e.g., a study by Aliyeva et al. [61] assessed the presence and abundance of factors such as Il-10, TNF-alpha, and CD56+ NK (natural killer) cells. What draws attention are two conflicting effects on vascular sclerosis. As in the case of ATG, there is a significant correlation with CMV infection—here, however, rituximab is not so much a cofactor as it increases the risk of CMV infection/reinfection and related vascular complications. At the same time, there are (limited) data that the use of rituximab has a positive effect on the concentration of IL-10 and anti-oxLDL, which reduces systemic inflammation. However, these are data for patients with rheumatoid arthritis. These data do not currently support patients receiving rituximab for kidney transplantation (higher baseline cardiovascular risk?). Due to the existing antiatherogenic potential, a positive effect of CMV prophylaxis combined with rituximab is possible, but it requires a further randomized and larger group of patient trials [61].

5. Final Considerations and Future Perspectives

Kidney transplantation is the treatment of choice for end-stage renal disease. Despite the fact that successful kidney transplant improves the quality of life and reduces mortality for most patients relative to those on maintenance dialysis, immunosuppressive therapy bears the risk of infection, malignancy, and cardiovascular disease. Immunosuppression maybe also a factor that increases oxidative stress and hence vascular sclerosis and atherosclerotic cardiovascular disease. Table 1 presents an overview of published data of the studies designed to assess the oxidative state of renal transplant patients. Oxidative stress, an imbalance between the generation of oxidants and antioxidant defense system, is one of the major events which affects not only early post-transplantation phase but also graft and patient's long-term outcomes. This imbalance contributes to the elevated cardiovascular morbidity and mortality as well as to the development of chronic allograft nephropathy, which is characterized by gradual decline in kidney function leading finally to graft loss. There is no ideal immunosuppressive regimen for kidney transplant recipients; all schemes have unwanted side effects. However, it is a price to pay to have a better and longer life. Reactive oxygen species can be removed by our intrinsic enzymatic system. In addition, a range of antioxidant chemical agents can be introduced to the organism, e.g., in a diet. Antioxidant therapies have not become a standard of care in renal patients up to date and more investigations are needed. It mainly remains unknown how antioxidant treatment can potentially alter the progression of chronic kidney disease itself. We also have to take into consideration not only kidney function but also the effects of immunosuppression on the biomarkers of oxidative stress. In clinical research, antioxidant therapies require more time to confirm the applicability of various antioxidant agents as effective treatment methods, in particular in heterogeneous vulnerable populations. The most important question of correlation between disturbance in the balance of pro- and antioxidant systems and its influence on the development and progression of chronic kidney disease still remains unanswered, so an era of tailored immunosuppressive therapy for kidney transplant recipients. Personalized medicine in the field of clinical transplantation is eagerly awaited; however, due to pandemic, it may be postponed due to many reasons (shortage of donors, shortage of financial resources, other priorities such as vaccines, new antiviral drugs, etc.).

Conflicts of Interest

There is no conflict of interest.

Authors' Contributions

Marlena Kwiatkowska and Urszula Oldakowska-Jedynak contributed equally to this work.

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Review Article

Uremic Toxins, Oxidative Stress, Atherosclerosis in Chronic Kidney Disease, and Kidney Transplantation

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Patients with chronic kidney disease (CKD) are at a high risk for cardiovascular disease (CVD), and approximately half of all deaths among patients with CKD are a direct result of CVD. The premature cardiovascular disease extends from mild to moderate CKD stages, and the severity of CVD and the risk of death increase with a decline in kidney function. Successful kidney transplantation significantly decreases the risk of death relative to long-term dialysis treatment; nevertheless, the prevalence of CVD remains high and is responsible for approximately 20-35% of mortality in renal transplant recipients. The prevalence of traditional and nontraditional risk factors for CVD is higher in patients with CKD and transplant recipients compared with the general population; however, it can only partly explain the highly increased cardiovascular burden in CKD patients. Nontraditional risk factors, unique to CKD patients, include proteinuria, disturbed calcium, and phosphate metabolism, anemia, fluid overload, and accumulation of uremic toxins. This accumulation of uremic toxins is associated with systemic alterations including inflammation and oxidative stress which are considered crucial in CKD progression and CKD-related CVD. Kidney transplantation can mitigate the impact of some of these nontraditional factors, but they typically persist to some degree following transplantation. Taking into consideration the scarcity of data on uremic waste products, oxidative stress, and their relation to atherosclerosis in renal transplantation, in the review, we discussed the impact of uremic toxins on vascular dysfunction in CKD patients and kidney transplant recipients. Special attention was paid to the role of native and transplanted kidney function.

1. Introduction

Patients with chronic kidney disease (CKD) are at a high risk for cardiovascular disease (CVD), and approximately half of all deaths among patients with CKD are a direct result of CVD. Premature cardiovascular disease extends from mild to moderate stages of CKD, and the severity of CVD and the risk of death increase with a decline in kidney function [1–3].

Moreover, the nature and spectrum of cardiovascular disease in CKD are recognized to be different from that in people without kidney disease including atherosclerosis, arteriosclerosis, calcific arterial and valve disease, left ventricular remodeling and dysfunction, arrhythmia, and sudden cardiac death.

Successful kidney transplantation significantly decreases the risk of death relative to long-term dialysis treatment [4]. Nevertheless, the prevalence of cardiovascular disease in this population is high and is responsible for approximately 20-35% of mortality in renal transplant recipients [5].

The prevalence of traditional and nontraditional risk-factors for CVD is higher in patients with CKD compared with the general population; however, it can only partly explain such sorely increased cardiovascular burden in CKD patients [2, 6]. Nontraditional risk factors, unique to CKD patients, include proteinuria, disturbed calcium and phosphate metabolism, anemia, fluid overload, and accumulation of uremic toxins. This accumulation of uremic toxins is associated with systemic alterations including inflammation and

oxidative stress which are considered crucial in the progression of CKD-related CVD.

Kidney transplantation can mitigate the impact of some of these nontraditional risk factors, but they typically persist to some degree following transplantation. The restoration of renal function favorably modifies cardiovascular risk in transplant recipients, and each 5 ml/min/1.73 m^2 increase in eGFR is associated with a 15% reduction in cardiovascular disease and mortality [7]. However, some specific for this population factors, such as immune activation and immunosuppressant agents, may be involved in the increased cardiovascular risk of cardiovascular disease [5].

2. Uremic Toxins

The progressive loss of kidney function is accompanied by the retention of plenty of metabolites, due to a decrease in their renal clearance and/or a rise in production. Many of these solutes have been shown to exert biological activity, thereby affecting the functioning of cells and affecting metabolic processes, resulting in the uremic syndrome. Generally, they may originate from endogenous metabolism, be produced by microbial metabolism, or be ingested from an endogenous source. According to the European Uremic Toxin Work Group (EUtox) organic uremic toxins are classified according to their physicochemical properties and possibilities of removal by dialysis [8]:

(1) Small, water-soluble molecules with a maximum molecular weight (MW) of 500 Da which can be easily removed by dialysis; molecules in this group include, i.a., guanidines (asymmetric dimethylarginine (ADMA) and symmetric dimethylarginine (SDMA)), oxalate, methylamines (trimethylamine-N-oxide (TMAO)), polyamines, urea, carbamylated compounds, and purines

(2) Middle molecules—small proteins or peptides with MW ≥ 500 Da, although most of them have MW > 10000 Da. They are often expressed in response to other toxins (e.g., cytokines), and their concentration depends both from retention and on endocrine and paracrine mechanisms. Dialytic removal of middle molecules is possible with membranes with a large enough pore size used in either diffusive or convective mode. Compounds in this group include angiogenin, atrial natriuretic peptide (ANP), β_2-microglobulin, complement factors D and Ba, cytokines (IL-6, IL-18, IL-1β, and TNFα), endothelin, fibroblast growth factor-23 (FGF-23), modified lipids and lipoproteins, pentraxin-3, VEGF, and parathyroid hormone

(3) Protein-bound molecules—the heterogeneous group of generally low MW solutes, which due to their protein binding are difficult to remove by dialysis; many of these molecules are generated by the intestine microbiota; the main compounds in this group are advanced glycation end products (AGEs), cresols (p-cresyl sulfate, p-cresyl glucuronide), hippurates, homocysteine, indoles (indoxyl sulfate, indole acetic acid), kynurenines, and phenols (phenylacetic acid) [8]

Accumulating data suggest that uremic toxins contribute substantially to the development and severity of cardiovascular disease in CKD patients. Table 1 summarizes the mechanisms of action of selected uremic toxin impact on cardiovascular damage.

3. Atherosclerosis in Chronic Kidney Disease

Accumulating data suggest that atherosclerosis starts from early stages of CKD and remaining high as CKD progresses [33]. CKD-related endothelial dysfunction plays an important role in the development of atherosclerosis [34, 35]. It is characterized by increased oxidative stress, expression of proinflammatory and prothrombotic molecules, and decreased capabilities of endothelial repair. Uremic toxins can contribute to these deleterious effects on the endothelium [36–38]. There is a correlation between inflammation, oxidative stress, endothelial dysfunction, and markers of vasculopathy and kidney function [39–41].

The vascular toxicity of uremic toxins has been demonstrated in clinical studies among chronic kidney disease, dialysis, and kidney transplant patients. Decreased kidney function impacts the levels of these solutes and may be a relevant confounder when the association between uremic toxins and hard cardiovascular outcomes is studied. The factors potentially contributing to atherosclerosis in CKD patients are presented in Figure 1.

4. Uremic Toxins and Kidney Function

4.1. Protein-Bound Uremic Toxins. Protein-bound uremic toxins (pbUTs)—p-cresyl sulfate (p-CS), p-cresyl glucuronide (p-CG), indoxyl sulfate (IxS), and indole-3-acetic acid (IAA)—originate from the metabolism of the intestinal microbiota of aromatic amino acids (tyrosine, phenylalanine, and tryptophan) [42–44]. In the distal colon segment, tryptophan is converted into indole and IAA, and tyrosine and phenylalanine into p-cresol. In the colon mucosa and liver, p-cresol is partly detoxified into p-CS and p-CG, and indole into IxS [42–44]. In blood, pbUTs bind on serum albumin [45] are removed by the kidneys—free fraction by glomerular filtration and protein-bound via tubular secretion [43, 44].

The serum levels of pbUTs are inversely related to renal function, and the serum concentrations increase progressively with the progression of CKD in adults and pediatric CKD patients [44, 46–51]. It was demonstrated that free and total fractions of toxins increase progressively from early stages of CKD with significantly higher concentrations in later stages [44, 46–48, 51]. Total and free fractions of p-CS and IxS correlate inversely with eGFR [46–48] and are comparable in patients on peritoneal dialysis and hemodialysis [48]. In dialyzed patients, residual renal function substantially contributes to uremic toxin levels both in patients on maintenance hemodialysis and peritoneal dialysis [52, 53]. Together with the loss of kidney function serum concentrations, there is a rise in uremic toxin levels [52, 53].

Table 1: The mechanisms of action of selected uremic toxin impact on cardiovascular damage.

Protein-bound uremic toxins (para-cresyl sulfate, indoxyl sulfate)	Impairment of vascular reactivity and induction of vascular remodeling; induction of oxidative stress; stimulation of proinflammatory responses in vascular cells and macrophages; promotion of adhesion molecule expression; stimulation of the cross-talk between macrophages and endothelial cells promoting vascular wall infiltration by inflammatory cells [9–15]
Phosphate	Increase in contraction and decrease in endothelium-dependent relaxation of the vessels; increased production of ROS in VSMC and in endothelial cells via NADPH oxidase activation; induction of EMP shedding resulting in the impairment of endothelial cells with thrombotic, inflammatory, and antiangiogenic properties [16–19]
Klotho and FGF23	Arterial stiffness via a downregulation of SIRT1 expression in endothelial and smooth muscle cells; induction of an increase in oxidative stress, reduced NO production, induced the expression of cell adhesion molecules [20–23]
ADMA	Reduction of NO production; induction of oxidative stress and acceleration of the senescence of endothelial cells [24–27]
AGEs	Osteogenic-like differentiation of SMCs and subsequent calcification; promotion of inflammation and oxidative stress via activation of NADPH oxidase, upregulation of adhesion molecule expression; induction of vascular contraction by modulating ET-1 expression; induction of endothelial cell apoptosis and impairment of endothelial progenitor cell survival, differentiation, and function [28–32]

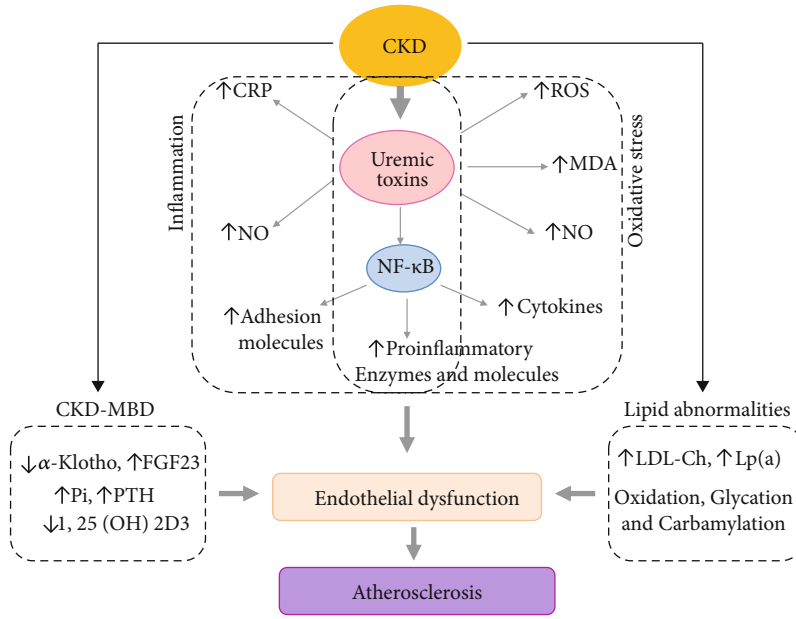

Figure 1: Factors potentially contributing to atherosclerosis in CKD. CRP: C-reactive protein; NO: nitric oxide; ROS: reactive oxygen species; MDA: malondialdehyde; NF-κB: nuclear factor kappa-light-chain-enhancer of activated B cells; FGF23: fibroblast growth factor 23; Pi: phosphates; PTH: parathyroid hormone; 1,25(OH)2D3: 1,25-dihydroxyvitamin D3; LDL: low-density lipoprotein; Lp(a): lipoprotein a; CKD: chronic kidney disease; CKD-MBD: chronic kidney disease-mineral bone disorder.

Few studies evaluated the levels of pbUTs in transplanted patients [51, 54–56]. In prospective studies by Liaeuf et al. [51, 55] and Poesen et al. [54], it was demonstrated that serum levels of IxS, IAA, and p-CS decreased significantly within a few days and then remained stable during 12 months after transplantation. Moreover, the levels of pbUTs in transplanted subjects were even lower than in controls with comparable kidney function. The cause of this phenomenon remains unclear. The possible explanations of these findings are the changes in gut microbiota after transplantation and the impact of immunosuppressant agents and antibiotics [57].

4.2. Asymmetric Dimethylarginine (ADMA) and Symmetric Dimethylarginine (SDMA). Serum levels of ADMA and SDMA are elevated in patients with CKD [58, 59]. For SDMA, renal excretion is the major pathway of elimination, and SDMA levels are closely related to eGFR. The kidneys also play a central role in the elimination of ADMA; however, the removal of ADMA takes place both by excretion in the urine and by degradation by dimethylarginine dimethylaminohydrolase (DDAH) and transamination by alanine glyoxylate aminotransferase 2 (AGXT2), enzymes primarily expressed in the kidneys. This may explain why in patients with

autosomal dominant polycystic kidney disease or kidney diseases with proteinuria, ADMA levels arise earlier and are highly independent on eGFR [60].

The data on levels of ADMA and SDMA in renal transplant patients are scarce and somewhat inconsistent. Most often, plasma ADMA levels demonstrated a biphasic course after successful kidney transplantation with a transient rise in the immediate postoperative period followed by a subtle decline in the weeks; however, the change did not correlate with improvement of graft function. ADMA levels remained elevated compared with CKD patients, matched for age and comorbidities [61–64]. A potential explanation of the increase of ADMA levels in the posttransplant period may be the effect of methylarginine release triggered by surgery, ischemia/reperfusion injury, and the catabolic effect of corticosteroids [61, 64, 65]. The persistence of increased levels may be related to activation of the immune system [61, 66] and metabolic side effects of immunosuppressive agents (calcineurin inhibitors and corticosteroids) [67, 68].

4.3. Advanced Glycation End Products (AGEs). Advanced glycation end products (AGEs) are a heterogeneous group of compounds derived from the nonenzymatic glycation of proteins, lipids, and nuclear acids through a complex sequence of reactions referred to as the Maillard reaction [69]. N-Carboxymethyllysine (CML), pentosidine, and hydroimidazolone are among the best characterized of at least 20 different types of AGEs and serve as markers of AGE accumulation in tissues [70, 71]. Interactions between AGEs, their receptors, and advanced glycation end product receptors (RAGE) trigger a cascade of various events leading to endothelial dysfunction, arterial stiffness, immune system dysregulation, and atherosclerosis progression [72].

Accumulation of AGEs in CKD patients is a result of oxidative stress and inflammation and comes from external sources such as diet and cigarette smoke [72, 73]. AGEs are metabolized and removed by the kidneys. They are filtered through the glomerulus and reabsorbed by renal proximal tubules, and both processes are complex and variable [74, 75]. The kidneys are also a place of accumulation and AGE-related organ damage [76], and progressive retention of AGEs occurring with declining renal function creates a vicious cycle of kidney damage and accelerated decline in renal function. Therefore, in CKD, increased levels of AGEs may be seen as a result of impaired clearance and enhanced formation in response to oxidative stress and/or carbonyl stress. Serum AGE levels correlate inversely with eGFR, and they appear to be predictive for the development of reduced glomerular filtration rate [77–79]. In Semba et al.'s [79] study, the increase in AGE levels was evident from CKD stage 3.

Kidney transplantation is the most effective therapy to reduce elevated levels of AGEs. Nevertheless, in renal transplant recipients, AGEs remain higher than in normal subjects and disproportionally higher than the GFR alone would imply [80, 81]. It suggests that other factors may influence the formation of AGEs. Factors contributing to increased accumulation of AGEs, and at the same time, the risk of chronic graft dysfunction, include the dialysis vintage before transplantation, donor age, and primary graft function. Closely related to the formation of AGEs is the state of increased oxidative stress typical of kidney transplant recipients, the determinants of which may be diabetes mellitus, ischemic/reperfusion injury, immunosuppressants, and renal failure [81–83]. In Shahbazian et al.'s [83] study, the levels of AGEs were significantly increased in renal transplant patients with measured GFR below 30 ml/min, and a significant association between the levels of AGEs and measured GFR was found.

4.4. Phosphate, Klotho, and FGF23. Abnormalities of mineral metabolism are universal complications of CKD associated with accelerated atherosclerosis and vascular calcification and correlated with increased mortality across all stages of CKD, independent of traditional risk factors [84–86]. The levels of serum phosphate, calcium, and parathyroid hormone are influenced by α-Klotho, FGF23, 1,25-dihydroxyvitamin D, diet, and medications, interacting with each other in complicated ways.

α-Klotho not only functions as one of the regulators of mineral homeostasis but also exerts pleiotropic biological effects including antioxidative stress, antiapoptosis, and antiaging [87, 88]. α-Klotho is expressed in multiple tissues; however, the strongest expression is in the kidney [89]. Kidney injury and subsequent renal impairment will result in the decrease of α-Klotho production. It has been shown that serum α-Klotho starts to decline in stage 2 CKD, and urinary α-Klotho even earlier, in stage 1 CKD [90], and for each 1 ml/min/1.73m^2 eGFR decrease, an adjusted mean decrease of 3.2 pg/ml of serum α-Klotho was revealed [91]. Furthermore, pbUTs inhibit α-Klotho expression [92]. Clinical and experimental studies have shown that the decrease of α-Klotho is positively associated with eGFR [87, 93, 94].

Fibroblast growth factor 23 (FGF23) is a bone-derived phosphatonin, which acts in the kidney to induce urinary phosphate excretion and suppress 1,25-dihydroxyvitamin D synthesis, in the presence of FGF receptor 1 (FGFR1) and its coreceptor α-Klotho [95, 96]. It has been also shown that FGF23 has a deleterious effect on vascular function—endothelial dysfunction, atherosclerosis, left ventricular hypertrophy, and increased risk of major cardiovascular events [97–99].

The increase in FGF23 is a compensatory reaction in response to decreased expression of transmembrane α-Klotho to maintain mineral homeostasis, so in early stages of CKD, serum phosphates are not elevated. In turn, increased levels of FGF23 decrease α-Klotho expression, and finally, dietary phosphorus overload cannot be compensated and contributes to overt hyperphosphatemia in advanced stages of CKD [96]. FGF23 levels increase progressively in early stages of CKD. It is suggested that renal injury itself may be an initial stimulus for FGF23 secretion [100]. In Isakova et al.'s [101] study, 33% of participants with eGFR $\geq$ 70 ml/min and 85% with eGFR 30-60 ml/min had elevated levels of FGF23, and in a dialyzed patient, serum levels of FGF23 are extremely high reaching levels that can be 1000-fold above the normal range [101]. Moreover, a strong correlation between eGFR and FGF23 was revealed [91, 101].

Close to 90% of patients with 3-4 CKD stage have normal phosphate levels, and with the progressive loss of functional nephrons, the compensatory mechanism is overwhelmed, and most patients with ESRD have overt hyperphosphatemia. Hyperphosphatemia is considered to be a risk factor for cardiovascular and all-cause mortality, and for each 1 mg/dl increase in serum phosphate, the risk of death is increased by 18-20% [102, 103].

The data on levels of α-Klotho and FGF23 in transplant recipients are scarce, and sometimes inconsistent. During the first week after kidney transplantation, the decrease in serum levels of α-Klotho was noted [104, 105]. This initial decline is probably multifactorial and may be a response to trauma and tissue injury, transient kidney tubular dysfunction, and the impact of immunosuppression therapy [104, 106]. In the consecutive weeks, the gradual increase of α-Klotho was observed with the highest levels exhibited at 52 weeks posttransplantation and compared with pretransplant levels [104]. However, no association between serum α-Klotho levels and kidney function has not been demonstrated in Tan et al.'s study, as well as in three other cross-sectional studies [107–109].

FGF23 levels decline in the postrenal transplantation period; however, they remain higher than in CKD patients matched for eGFR [104, 110–113]. Further reductions in FGF23 levels are observed over longer follow-up, approximating normal levels 1–3 years after transplantation [110].

In up to 90% of transplant recipients, mild to moderate hypophosphatemia is present. Phosphate levels remain low for longer than in patients with CKD matched for the eGFR [114]. Kidney function does not play a crucial role in posttransplant hypophosphatemia but persistently high levels of FGF23 and PTH [113, 115].

4.5. Oxidative Stress: The Impact of Kidney Function. Oxidative stress (OS) is defined as a state of imbalance between excessive prooxidant activities relative to antioxidant defense mechanisms. Oxidative stress leads to metabolic dysregulation and oxidation of lipids, proteins, and nucleic acids and oxidative damage in cells, tissues, and organs caused by ROS and reactive nitrogen species (RNS) [116, 117]. OS is frequently observed in CKD patients; contributes to inflammation, endothelial dysfunction, risk of atherosclerosis, and progression of CKD [118]; and is considered one of the nontraditional risk factors for cardiovascular and all-cause mortality [119, 120]. OS through generation of uremic toxins enhanced intestinal permeability to endotoxins and alteration in nitrogen handling [121–123]:

(i) Accumulation of AGEs activating transcription factors (NF-κB, AP1, and SP1) executed via RAGE, and activation of NADPH oxidases (NOXs) which directly generate free radicals [124, 125]

(ii) Inflammation, which is spliced with OS—inflammatory cells stimulate the release of reactive species, and oxidized end products stimulate phagocytic cells to release inflammatory cytokines and ROS creating a positive feedback loop; the leading feature is the two-way interplay between NOX, NF-κB, inflammasomes, and phagocytic cells [126, 127]

(iii) Dialysis increases the state of oxidative stress, and the involved mechanisms include the use of bioincompatible membranes and fluids, contamination of dialysate with bacterial endotoxins, and occult infections [128–130]

The imbalance in oxidant-antioxidant status begins early in the course of CKD. It was shown that increased levels of NADPH-generated ROS and lower levels of the antioxidant enzymes can be revealed in patients with 1 and 2 CKD stage [124, 131–133]. Progressive loss of renal function results in increased oxidative stress and inflammation, and a positive correlation between advancing stage of CKD and increasing oxidative stress has been demonstrated [134–137]. The inverse relationship between eGFR and markers of oxidative stress was revealed in several studies [136–138], but in some, the correlation was at least weak [139]. It is possible that this difference may be a result of biomarkers used and studied populations.

Successful kidney transplantation leads to a reduction in metabolic abnormalities and significant improvement in OS-related markers. Normalization of graft function seems to be a key factor in the restoration to near-normal levels of OS biomarkers. Despite the fact that surgical procedure of kidney transplantation and ischemic injury during the procurement and organ transfer cause an oxidative burst, the improvement of OS can start immediately after transplantation [140]. Sudden cessation of blood flow during organ donation cause ischemic/hypoxic injury [141, 142]. Cold storage promotes ROS production via mitochondrial dysfunction. ROS react with other molecules, leading to oxidative damage of proteins, nucleic acids, and lipid peroxidation and contribute to cell apoptosis [143–145]. The reperfusion stage, during which blood flow is restored, leads to a burst of ROS and is regarded as the final stage of ischemic injury [141–146]. OS in kidney transplant recipients may be, at least in part, caused by the immunosuppressive therapy. Most of the currently used immunosuppressive medications, such as corticosteroids and calcineurin inhibitors (cyclosporine A and tacrolimus), may contribute to the increased OS. The prooxidant activities of tacrolimus and cyclosporine A, the indispensable parts of immunosuppressive, have been studied. Some studies reported that increased levels of malondialdehyde are a consequence of immunosuppressive therapy and that OS is induced mostly by cyclosporine A [147, 148]. Other studies, however, have not confirmed these findings [140, 149, 150]. Other factors, such as opportunistic infection or immune response to allograft, may also trigger OS in kidney transplant recipients [151].

CKD-associated OS in pretransplant phase, reperfusion injury, and increased immunosuppression are considered the key factors of continual OS during the early phase of transplantation [151–153]. Over the next days, the improvement of antioxidant status is observed along with the restoration of kidney function, reduction in metabolic abnormalities, and decrease in OS [152, 154–157]. Some controversies regarding changes in enzymatic and nonenzymatic antioxidants as well as OS

biomarkers may probably arise from the study design and different observation periods. In some studies, the increase in antioxidant systems and decrease in OS were observed already in the early posttransplant period [154–157]. In other studies, during the first 2 weeks, a significant increase in lipid peroxidation [140, 151, 158] and decrease in erythrocyte glutathione or superoxide dismutase activities were observed [159, 160]; however, in longer observation (28-day posttransplantation), the decrease in lipid peroxidation along with antioxidant system activities was revealed [140, 151, 158]. The levels of advanced oxidation protein products (AOPPs) decrease immediately after transplantation. As long as reduction in the first day may be explained by blood loss during surgery, the decrease in subsequent days confirms that successful kidney transplantation provides efficient elimination of generated ROS [154–157, 161, 162].

Most studies have shown that reestablishment of kidney function improves the OS over few weeks after transplantation [140, 154–162]. Time-dependent changes in OS biomarkers are associated with improvement in kidney function, and the levels of AOPPs and low molecular AGEs correlate inversely with creatinine clearance [140, 151, 154, 155, 157]. Normalization of graft function may restore to near-normal levels of OS biomarkers, regardless of immunosuppression used; however, achieving any level of kidney function will decrease OS level [150, 163, 164]. The reduction in OS after transplantation may be also a prognostic factor of short- and long-term graft function and CVD in this patient population [163, 165].

4.6. Implications of Uremic Toxins and Oxidative Stress to Atherosclerosis. In CKD, endothelial dysfunction and atherosclerosis are almost universal, as well as cardiovascular complications as first reported by Lindner et al. [166], who drew attention to the excessive incidence of atherosclerotic cardiovascular mortality in dialyzed patients. Various CKD-specific factors and processes are involved in endothelial dysfunction in CKD as presented in Figure 1. It is characterized by proinflammatory and prothrombotic endothelial phenotype, structural damage, impaired capabilities of protective and repair mechanisms, and increased oxidative stress. Uremic toxins, when in high concentrations in the bloodstream, play an important role in endothelial dysfunction, which in turn contributes to the pathogenesis of cardiovascular diseases, such as atherosclerosis and thrombotic events [35–39]. Each toxin can play its own role in vascular dysfunction, as presented in Table 1; however, its accumulation and coexistence potentiate the deleterious effects.

Inflammation is considered one of the main mechanisms of atherosclerosis, and CKD is a state of systemic inflammation [34, 167, 168]. It depends both on the increased synthesis and decreased elimination of mediators of inflammation, and multiple cytokines are involved in the genesis of this proinflammatory milieu in CKD [169]. Uremic toxins induce inflammation in endothelial cells (ECs) and stimulate the cross-talk between ECs and macrophages [14, 35–37]. In the response to the injury, the concentration of cytokines is increased leading to the activation of endothelial, resident vascular cells, and circulating monocytes [8, 11, 36–38]. Uremic toxins (pbUTs, phosphates, and FGF23) increase the expression of adhesion molecules (E-selectin, P-selectin, ICAM-1, and VCAM-1) promoting the infiltration of monocytes and macrophages in the activated endothelium [11, 13, 15, 16, 20, 35, 37].

Uremic toxins promote the production of ROS and decrease antioxidant defenses, resulting in oxidative stress [10, 21, 27, 118, 119, 127]. ROS activate transcription factors leading to the expression of inflammatory cytokines, as well as causing mitochondrial dysfunction, inducing cell death [117, 126, 170]. At the same time, uremic toxins inhibit late-stage autophagy, making cells more sensitive to oxidative stress and contributing to endothelial dysfunction. It may lead to atherosclerosis and arterial aging [171, 172].

Uremic toxins contribute to structural damage of ECs resulting in increased endothelial permeability. In vitro studies demonstrated that uremic toxins (pbUTs and phosphate) induce cytoskeletal remodeling, resulting in the changes in EC morphology, and lead to the rupture of cell-cell junctions damaging endothelial barrier and contributing to increased permeability [173–175]. Endothelial damage results in a release of microparticles and specific miRNAs that may further promote vascular damage. Endothelial microparticles (EMPs) are important in intracellular communication. Uremic toxins (pbUTs and phosphate) induce the formation of EMPs from endothelial cells [19, 176–178]. Uremic toxins induced EMPs show different activities: they have an antiangiogenic effect on endothelial progenitor cells impairing endothelium repair process [179], have procoagulant activity due to the production of factor Xa and tissue factor (TF) [179], enhance the proliferation of VSMC contributing to neointimal hyperplasia [180], and finally increase osteocalcin expression in ECs, VSMC, and fibroblast, which indicates vascular calcification [181]. MicroRNAs participate in the regulation of EC function modulating angiogenesis and immune response [182]. Uremic toxins upregulate miRNAs causing suppression of expression of genes responsible for endothelial homeostasis and thus contributing to EC dysfunction and apoptosis [182, 183].

Uremic toxins also cause a reduction in the number and function of endothelial progenitor cells. Protein-bound UTs and AGEs suppress the expression of transcription factors, SIRT1 and KLF2, responsible for the maintenance of endothelial homeostasis, inhibiting oxidative stress and cell senescence [182, 184, 185].

Uremic toxins contribute to the prothrombotic state of endothelium leading to an increased risk of thrombotic events, such as thromboembolism and ischemia. Furthermore, in CKD, the processes of coagulation and fibrinolysis are impaired with increased levels of tissue factor (TF), von Willebrand factor (vWF), thrombomodulin, factor VIII, and D-dimer [186]. In vitro studies demonstrated that uremic toxins (IxS and IAA) increase the expression of TF and production of factor Xa indicating endothelial activation and procoagulant activity [179]. Uremic toxins (phosphate, IxS, and ADMA) also decrease the production and/or bioavailability of NO which acts as an inhibitor of platelet adhesion and aggregation [187–189].

Endothelial cell integrity and function are critical to the prevention of atherosclerosis; therefore, dysfunction of endothelium is critical in the development of vascular dysfunction and progression of CVD. Nevertheless, uremic toxins participate in atherosclerosis development in many steps. They influence proliferation, migration, calcification, and senescence of VSMC [9–11, 16, 20, 23, 26, 34, 35]. They also induce chronic activation of leukocytes (monocytes and neutrophils), stimulate the leukocyte-endothelial interactions, and promote vascular wall infiltration by inflammatory cells [12–15, 34, 37, 167–169]. And finally, uremic toxins participate in the formation of atherosclerotic plaque and its rupture [1, 33–35].

5. Final Considerations

It would be worth to mention that AKI contributes to the initiation and progression of CKD, and vice versa CKD predisposes to AKI [190–192]. AKI and CKD are interconnected syndromes. The accumulating data from basic and clinical research indicates that renal hypoxia is associated with CKD, AKI to CKD continuum, and AKI on top of CKD. Tubulointerstitial hypoxia is a key player in the pathophysiology of CKD and AKI to CKD transition [193–198]. Capillary rarefaction after AKI episode results in tubulointerstitial fibrosis, and damaged tubular epithelial cells that fail to redifferentiate may contribute to capillary rarefaction and thus aggravating hypoxia [193, 194, 199]. Moreover, hypoxia induces diverse epigenetic changes such as chromosome conformation, DNA methylation, or histone modification [199]. The mechanisms involved in the susceptibility of AKI and impairment of recovery from AKI in CKD patients remain largely unexplained. Multiple mechanisms at epigenetic, signaling, cellular, and tissue levels may be involved [200–202]. Briefly, oxidative stress is a key mechanism in the pathogenesis and progression of CKD and impaired renal regeneration after AKI episodes. Therapeutic strategies targeting hypoxia have been shown to be effective in blocking the progression to CKD and possibly AKI protection [192, 193, 199].

In CKD, the retention of a variety of metabolites, due to a decrease in their renal clearance and/or a rise in their synthesis, is found. These compounds could be small and water soluble, lipophilic and/or protein bound, or larger and in the middle-molecule range. Several solutes have been shown to exert biological activity, on cells and metabolic processes, leading to uremic syndrome. Moreover, dietary protein breakdown, alternative sources such as environmental contact, food additives, natural stimulants (coffee and tea), herbal medicines, or addiction to psychedelic drugs, may also play a role in uremic toxicity. Slowing of the progression of CKD thereby preservation of kidney function is crucial in the removal of uremic toxins. Successful kidney transplantation with good graft function offers the best possibility to lower the levels of uremic toxins. In addition, uptake of uremic toxins in the intestine could be decreased by influencing dietary uptake, oral administration of sorbents, or administration of prebiotics or probiotics influencing intestinal flora. Moreover, changing the source of protein intake from animal-based to plant-based diet may also reduce intestinal production of uremic toxins. Other therapeutic intervention includes administration of drugs countering the biological impact of uremic solutes such as angiotensin-converting enzyme inhibitors (ACEi) which neutralize Ca influx due to SDMA [203]. Moreover, the IxS level can be decreased by rising sulfotransferase activity, responsible for indole sulfation [204].

In addition, the development of therapeutic strategies to raise α-Klotho and lower phosphate, FGF23, and other uremic toxins is of great importance as they may contribute to the decline in cardiovascular morbidity and mortality in CKD and after kidney transplantation.

Abbreviations

ACEi: Angiotensin-converting enzyme inhibitor
ADMA: Asymmetric dimethylarginine
AGEs: Advanced glycation end products
AGTX2: Alanine glyoxylate aminotransferase 2
AKI: Acute kidney injury
ANP: Atrial natriuretic peptide
AP1: Activator protein 1
CKD: Chronic kidney disease
CKD-MBD: CKD-mineral bone disorder
CML: N-Carboxymethyllysine
CVD: Cardiovascular disease
DDAH: Dimethylarginine dimethylaminohydrolase
eGFR: Estimated glomerular filtration rate
ECs: Endothelial cells
EMP: Endothelial microparticles
ESRD: End-stage renal disease
ET-1: Endothelin 1
FGF23: Fibroblast growth factor 23
FGFR1: Fibroblast growth factor receptor 1
IAA: Indole-3-acetic acid
ICAM-1: Intercellular adhesion molecule-1
IL-6: Interleukin 6
IL-18: Interleukin 18
IL-1β: Interleukin 1β
IxS: Indoxyl sulfate
KLF2: Krüppel-like factor2
NADPH: Nicotinamide adenine dinucleotide phosphate
NF-κB: Nuclear factor kappa-light-chain-enhancer of activated B cells
NO: Nitric oxide
NOX: NADPH oxidase
PAI-1: Inhibitor of tissue plasminogen activator
p-CS: p-Cresyl sulfate
p-CG: p-Cresyl glucuronide
PTH: Parathyroid hormone
RAGE: Advanced glycation end product receptor
RNS: Reactive nitrogen species
ROS: Reactive oxygen species
SDMA: Symmetric dimethylarginine
SIRT1: Sirtuin 1
SMC: Smooth muscle cell
SP1: Specificity protein 1
TF: Tissue factor
TFPI: Tissue factor pathway inhibitor
TMAO: Trimethylamine-N-oxide

TNFα: Tumor necrosis factor α
t-PA: Tissue plasminogen activator
VCAM-1: Vascular adhesion molecule-1
VEGF: Vascular endothelial cell growth factor
vWF: von Willebrand factor
VSMC: Vascular smooth muscle cells.

Data Availability

There are no supporting data.

Conflicts of Interest

The authors declare no conflict of interest.

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Review Article

Oxidative Storm Induced by Tryptophan Metabolites: Missing Link between Atherosclerosis and Chronic Kidney Disease

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Chronic kidney disease (CKD) occurrence is rising all over the world. Its presence is associated with an increased risk of premature death from cardiovascular disease (CVD). Several explanations of this link have been put forward. It is known that in renal failure, an array of metabolites cannot be excreted, and they accumulate in the organism. Among them, some are metabolites of tryptophan (TRP), such as indoxyl sulfate and kynurenine. Scientists have become interested in them in the context of inducing vascular damage in the course of chronic kidney impairment. Experimental evidence suggests the involvement of TRP metabolites in the progression of chronic kidney disease and atherosclerosis separately and point to oxidative stress generation as one of the main mechanisms that is responsible for worsening those states. Since it is known that blood levels of those metabolites increase significantly in renal failure and that they generate reactive oxygen species (ROS), which lead to endothelial injury, it is reasonable to suspect that products of TRP metabolism are the missing link in frequently occurring atherosclerosis in CKD patients. This review focuses on reports that shed a light on TRP metabolites as contributing factors to vascular damage in the progression of impaired kidney function.

1. Introduction

Chronic kidney disease (CKD) is one of the most commonly occurring diseases in the world, with more than 850 million people afflicted [1]. As a consequence of impaired kidney filtration, metabolites, which are normally excreted from the body, accumulate and can lead to systemic damage. They are generally called uremic toxins and according to the European Uremic Toxins Work Group (EUTox) database, 146 compounds are classified to this category [2]. Because of their different physicochemical features, they have been divided into three groups: small solutes, middle molecules, and protein-bound toxins. The latter are extremely doubtful, because dialysis, commonly used as a treatment option in CKD patients, is inefficient in their elimination. Therefore, they may be responsible for systemic damage in CKD patients, even in those who receive dialysis.

Atherosclerosis, a chronic inflammatory disease, is characterized by the accumulation of inflammatory cells and lipids in the artery wall, intima thickening, and vessel calcification. Over decades, it has afflicted more and more people, with increasing number of patients suffering from the cardiovascular disease. One medical condition that increases the risk of atherosclerosis is CKD. There is growing evidence that vascular endothelium damage begins in an early stage of CKD. The consequence is higher mortality from CVD in CKD patients than in patients without kidney impairment [3]. Several uremic toxins are considered ROS generators,

and since it is known that their serum levels in CKD patients are elevated, efforts are being taken to establish their role in atherosclerosis development [4]. This review focuses on the link between renal impairment and atherosclerosis and on oxidative stress as a contributing factor to the development of these diseases separately and as a link between these two comorbid conditions. The main emphasis is on tryptophan (TRP) metabolites as potential therapeutic goals, the targeting of which could be beneficial in overcoming atherosclerosis incidence in CKD.

2. Oxidative Stress (OS) and Reactive Oxygen Species (ROS) in Chronic Kidney Disease and Atherosclerosis

Oxidative stress (OS) develops in conditions of imbalance between antioxidants and oxidants, with a predominance of the latter. Those highly reactive chemicals are represented by agents like superoxide (O_2-), alkoxyl radical (RO·), peroxyl radical (ROO·), hydroxyl radicals (OH·), peroxynitrate (ONOO−), hydrogen peroxide (H_2O_2), ozone (O_3), and hypochlorous acid (HOCl) [5]. Under physiological conditions, ROS play a role as second messengers and help to regulate processes like growth, signaling, apoptosis, and systemic functions, i.e., regulation of blood pressure or immune response [6]. Nevertheless, when produced in excess, they are harmful for cells, by causing lipid, protein, and DNA oxidation. That leads to systemic detrimental role of ROS, suggested in the pathogenesis of neurodegenerative disorders, cancer, diabetes, cardiovascular diseases, and kidney diseases [7–9]. On account of their physiological role in the body, there are multiple naturally occurring structures responsible for ROS generation. The main is the mitochondria, which, via electron chain transport, take part in ATP production. This process requires the company of nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide ($FADH_2$). However, during the electrons' migration between complexes, some of them can escape in a process called "electron leaking" [10]. This leads to the formation of reactive species, such as superoxide (O_2^-) and hydrogen peroxide (H_2O_2). Another broad source of ROS is the NADPH oxidases (NOX) family, which contains seven members [11]. These enzymes are responsible for electron transportation from the cytosol to the extracellular space. In this location, they are coupled with molecular oxygen. Moreover, NOX4 can directly produce H_2O_2 by itself, which enhances its role in oxidative stress generation [12]. Other enzymes that can directly produce ROS agents are those belonging to the nitric oxide synthase (NOS) family. From three isoforms, endothelial NOS (eNOS), responsible for maintaining proper vascular tone, is essential in the pathogenesis of cardiovascular disease. ROS production takes place in a process called eNOS uncoupling, where an enzyme produces superoxide instead of NO [13]. Xanthine oxidase (XO) takes part in the conversion of hypoxanthine to uric acid, with results in $O_2{\cdot}^-$ and H_2O_2 production [14]. It was proven that XO contributes to endothelial damage and the pathogenesis of CKD [15, 16]. Oxidative stress and ROS from different sources are suspected to be involved in the development of CKD and atherosclerosis. Moreover, they may be the missing link in the observed increased prevalence of atherosclerosis in CKD patients. Due to such a possibility, efforts are being made to discover in which mechanisms enhanced ROS production occurs in kidney impairment, which would explain a looping series of events: increased ROS level–kidney impairment–upregulated level of ROS–atherosclerosis. Thus far, accumulating tryptophan metabolites are the main suspects in this process.

During the development of CKD, several structural changes in the kidneys can be noticed. Increased apoptosis of podocytes, a reduction of functional nephrons, their sclerosis, and hypertrophy are hallmarks of kidney impairment [17]. Reactive oxygen species and oxidative stress are suspected to take part in this pathogenesis, and the results of experiments comparing ROS marker levels between healthy volunteers and CKD patients serve as support of this view. Elevated ROS levels are observed in the latter. Systemic ROS content needs to be evaluated by their markers, such as end products of lipid peroxidation, DNA damage, or the oxidation of proteins and amino acids, because of the unstable nature of ROS, which makes direct measurement impossible [18]. Aberration of ROS levels can be detected from the early stages of the disease and is noticeable in both adults and children [19, 20]. Also, experimental proofs of antioxidant intake in CKD patients suggest that molecules like vitamins C and E and omega-3 polyunsaturated fatty acids improve CKD patient conditions [21]. Reinforced efforts are made to explain how OS and ROS contribute to renal impairment. The kidneys, as large energy consumers, are rich in mitochondria, an organelle responsible for ATP production. As described above, mitochondria are one of the main sources of ROS, but they are also highly vulnerable to oxidative stress. Because of that, it is worth noting that mitochondria can be the cause and the victim of CKD, and a positive correlation between the stage of CKD and lowered mitochondrial DNA (mtDNA) was proven [22, 23]. It was shown that mitochondrial ROS can be generated after exposition to free fatty acids (FAAs), which have an influence on the loss of mitochondrial membrane potential [24]. This event leads to cytochrome c release, followed by the activation of the caspase cascade and finally podocyte apoptosis [25]. Palmitic acid was identified as one of the agents that cause the overproduction of mitochondrial superoxide in podocytes, followed by damage to those organelle and caspase-depended cell death in the final stage [26, 27]. In another study, podocyte injury was triggered by exposure to aldosterone, which enhances mitochondrial derived ROS production [28]. Podocyte apoptosis has been also induced by plasminogen (Plg) in the mechanism including ROS production by NADPH oxidase (NOX). Moreover, in the same experiment, the authors highlighted the crosstalk between Plg-activated NOX2 and the induction of mitochondrial NOX4 via the generation of O_2^- by NOX2 [29]. The gathered data show that mitochondria-derived ROS take part in podocyte injury, which then leads to the progression of CKD. Dysfunction and apoptosis of podocytes are one of the features occurring in renal fibrosis. Another event that led to this state, and then

to CKD progression, included increased fibroblast proliferation, the epithelial–mesenchymal transition (EMT), and the accumulation of the extracellular matrix (ECM) and mesangial cells as well as thickening of the tubular and glomerular membranes. Accumulation of proinflammatory cytokines and chemokines followed by chronic inflammation contributes to those negative changes [30]. Oxidative stress enhances fibrosis and CKD progression by making an impact on the aforementioned processes, and experimental studies indicate a significant decrease in renal fibrosis after OS decreasing [31]. Accumulation of ECM is induced by NOX-depended ROS generation, which promotes fibroblast transdifferentiation to myofibroblasts [32]. ROS-provoked upregulation of TGF-β1 activates the PI3K/Akt signaling pathway and thus epithelial to mesenchymal transition [33, 34]. All of these pathological changes lead to a worsening of kidney function and deterioration of filtration efficiency. As a result, metabolites accumulate and through different mechanisms affect the body's structures, causing further damage.

Atherosclerosis is characterized by endothelial dysfunctions, inflammatory processes, and enhanced lipoprotein storage, with consequent plaque formation. Oxidative stress and ROS are involved in atherosclerosis development. Recently, it was reported that monocytes express NOX5, which contribute to ROS formation, and oxLDL enhances its expression. Moreover, a comparison of an atherosclerotic and a nonatherosclerotic artery depicted significant upregulation of NOX5 in the first group [35]. Foam cell formation can also be enhanced by xanthine oxidase (XO), which as already mentioned is responsible for ROS generation. This naturally occurring protein in vascular smooth muscle cells stimulates the LOX-1 (lectin-like oxidized low-density lipoprotein receptor-1) expression, with subsequent oxLDL accumulation and proinflammatory mediator release. Moreover, by increasing arginase expression, oxLDL causes eNOS uncoupling, resulting in intensified ROS production and enhancing pathological process [36, 37]. ROS also contribute to cell apoptosis in a way dependent on the Fas ligand or by the activation of NF-κB via the p38MAPK or PI3K pathways [38, 39]. Moreover, oxLDL is a source of proinflammatory agents, i.e., IL-12 and IL-18, which act like chemoattractants for T cells. They in turn secrete TNF-α and IFN-γ, which increase endothelial cell inflammation and apoptosis [40]. Additionally, according to results obtained by Ng et al., INF-γ leads to endothelial cell hyperpermeability by the activation of the p38MAPK kinase and actin rearrangement, which loops events leading to atherosclerosis development [41]. The same cytokine is involved in increasing plaque vulnerability by the inhibition of collagen production, thereby contributing to an increased risk of rupture. The aforementioned XO involved in the LOX-1 expression has been indicated as a receptor, involved in VSMC migration [42, 43]. These cells play a role in the different stages of atherosclerosis by secreting chemokines, monocyte attraction, and conversion to foam cells, which leads to their apoptosis and cholesterol release, thereby enlarging its generally accessible source. Moreover, they take part in fibrous cap formation and VSMC viability, which is essential in maintaining plaque stability. Those cells are also a source of calcifying microvesicles, and via these VSMCs participate in vessel calcification—another hallmark of atherosclerosis [44]. The above observations lead to the conclusion that reactive oxygen species are the starters that begin a cascade of events leading to atherosclerosis development.

2.1. Crosstalk between CKD and Atherosclerosis. It is well known that cardiovascular disease is one of the main causes of premature death in patients suffering from chronic kidney disease [45]. Observation of atheromatous plaques in CKD and non-CKD patients showed differences between them in terms of calcification level and increased the hydroxyapatite content in the first group. A study of end stage renal disease patients from 2018 showed that atherosclerosis developed in a majority of end stage renal disease (ESRD) patients [46]. Accelerated atherosclerosis development was observed also in research from the same year that compared atherosclerotic calcification in ESRD hemodialysis patients and healthy controls [47]. Kopel et al. reported greater impairment in vascular function in CKD patients than in the control group. Moreover, using the measure of nitroglycerine-mediated dilatation, they revealed that vascular dysfunction appeared in a mechanism dependent on smooth muscle cell malfunction [48]. In a study conducted by Pawlak et al., elevated OS levels and endothelial injury markers were revealed in hemodialysis patient with coexisting CVD, compared with control [49]. Blood samples derived from children with CKD were characterized by increased levels of endothelial microparticles, markers of endothelial dysfunction, when compared with healthy controls [50]. Similar results were obtained in research focusing on hemodialysis in children with end stage renal disease. Increased levels of TG, cholesterol, and LDL and decreased levels of HDL, which is a marker of premature atherosclerosis development, were detected in their blood samples [51]. Vorm et al. showed increased von Willebrand factor levels, another indicator of endothelium impairment in CKD and ESRD patients [52]. The gathered observation remains in line with the inference made by Gennip et al., who reported increased serum levels of endothelial dysfunction biomarkers in ESRD as compared with controls [53]. Altogether, these reports lead one to the conclusion that changes occurring in CKD are responsible for worsening the vascular condition and can lead to atherosclerosis development. Since traditional risk factors, such as hypertension or diabetes, are not sufficient to explain those pathological changes, new agents involved in those processes are widely sought after. Oxidative stress and reactive oxygen species are gaining more and more interest as nontraditional risk factors of CVD associated with CKD. Establishing their role as atherosclerosis mediators in CKD could be useful in developing a therapy to improve patient outcomes and extend their lifespan. Decreased efficacy of glomerular filtration with subsequent metabolite accumulation leads to the hypothesis that within those substances some of them have an impact on antioxidant compounds or ROS generation.

2.2. Indoxyl Sulfate, a Product of Tryptophan Degradation and Its Biological Activity. Tryptophan is an essential amino acid, which needs to be supplied with nourishment. It is

metabolized via three major catabolic pathways: the indole pathway (Figure 1), the kynurenine pathway (Figure 2), and the serotonin pathway [54].

TRP to indole conversion depends on the host microorganism and takes place in the intestine. After this step, indole undergoes metabolic changes in hepatocytes with indoxyl sulfate (IS) production [55]. This agent is one of the best described uremic toxins.

IS is a small molecule that in at least 90% binds to plasma proteins. The remaining free fraction contributes to numerous pathological conditions, including the induction of oxidative stress and inflammation [56]. By NF-κB p65 phosphorylation, IS leads to an increase of p21 and p53 expression. Concomitantly, release of TGF-β1, a monocyte chemoattractant protein-1, ET-1, and osteopontin, which increase the biological activity of TGF-β manifested by the stimulation of metallopeptidase inhibitor-1 and collagen biosynthesis, is observed [57]. These changes are often accompanied by systemic disturbances, such as cardiovascular disorders, cardiac fibrosis, arterial calcification, osteodystrophy, and kidney tissue damage [58–60]. Several of the biological effects exerted by the free fraction of IS occur via the activation of the aryl hydrocarbon receptor (AhR). This interaction impairs vascular structures through inhibiting endothelial cell proliferation and decreasing DNA synthesis in those cells [61]. Moreover, it enhances the expression of monocyte chemoattractant protein-1, which is involved in atherosclerosis development [62]. Additionally, AhR activation by IS leads to the AhR-NF-κB/MAPK cascade activation followed by the induction of inflammation [63]. The IS-AhR interaction also impairs the skeletal system. Liu et al. indicated the IS/AhR/MAPK signaling pathway as a mediator of impaired osteoblastogenesis [64]. Another experiment revealed that the effect of IS on osteoclastogenesis depends on the concentration and exposure time and that osteoclast differentiation decreases under the chronic impact of IS. It was confirmed that the observed effect occurs through AhR activation [65]. In the central nervous system, IS activates AhR in astrocytes, which leads to increased ROS production [66]. Moreover, experimental outcomes indicate that this interaction leads to inflammation in primary astrocytes and mixed glial cells [67].

Due to the systemic toxicity of IS, methods of its elimination in patients with impaired renal function are in constant development. The high degree of binding with albumins makes hemodialysis an inefficient means of removing IS from the blood. For this reason, different ways of increasing the effectiveness of blood purification are being contemplated. One possibility is the use of binding competitors that displace IS from albumin binding, thus augmenting its free form, which can be dialyzed. The effectiveness of salvianolic acids as a factor that enhances IS removal from blood was examined in an experimental animal model [68]. Another binding competitor that can be used to increase IS elimination is ibuprofen. Its arterial infusion during dialysis treatment significantly decreased the remaining IS amount in serum [69]. Ibuprofen increased the free fraction of IS approximately three-fold in uremic plasma, higher than the free fraction generated by furosemide. Moreover, the addition of furosemide to ibuprofen intensified its displacement effect [70]. In turn, Shi et al. proved both an increase in the free form of IS in rat plasma and higher reduction ratio in a group treated with intravenous lipid emulsion (ILE) than in the control [71]. Free fatty acids, which are the components of ILE that have a high affinity for albumins, so they also act as binding competitors. Another approach in increasing the efficacy of hemodialysis is based on using an adsorbent that binds IS. The addition of poly-cyclodextrin, which binds IS via hydrophobic interactions and hydrogen bonds, to dialysate of the exterior dialyzer resulted in a significant increase in uremic toxin removal from plasma [72]. Yamaguchi et al. demonstrated that the administration of oral adsorbent AST-120 also decreased IS plasma content [73]. An alternative way of improving the efficacy of hemodialysis is modifying the dialysis fluid through the replacement of buffer solution. Hyšpler et al. indicated that use of acetate buffer predominates over the citrate one in terms of enhancing IS elimination [74].

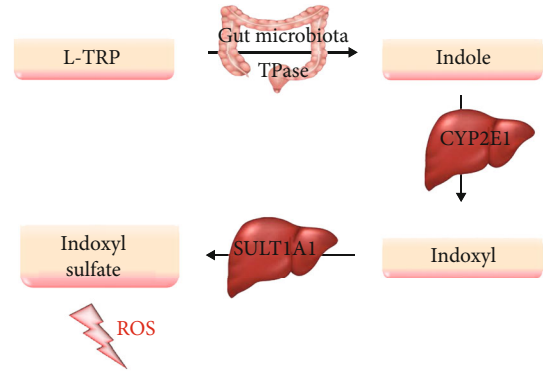

Figure 1: Tryptophan metabolism pathways, including a derivative involved in the production of reactive oxygen species. CYP2C1: cytochrome P450 2C1; L-TRP: L-tryptophan; ROS: reactive oxygen species; SULT1A1: sulfotransferase 1A1; TPase: tryptophanase.

The aspect that cannot be omitted in the attempts of decreasing IS levels is the fact that it is a metabolite of an exogenous amino acid. Thus, diet modifications may be crucial in IS management of CKD patients. It was proved that a very low protein diet (VLPD) helps reduce total and free IS in patients with renal impairment [75]. Comparison of a low protein diet with a very low protein diet showed differences in the efficiency, with a predominance of the latter, indicating that protein content is a key factor in decreasing plasma IS levels [76]. Altogether, these data indicate that there is no unique perfect method that could be used as an effective way to sufficiently eliminate accumulated IS in CKD patients, and for this reason, new approaches need to be found.

2.3. Indoxyl Sulfate in Chronic Kidney Disease. Cumulation of IS in kidney impairment was confirmed by Yeh et al., who indicated its increased serum levels in CKD patients when compared to healthy volunteers [77]. Moreover, it was reported that along with CKD advancement serum IS levels increase, which emphasizes reduced IS excretion together with progressive renal failure [78]. A meta-analysis from

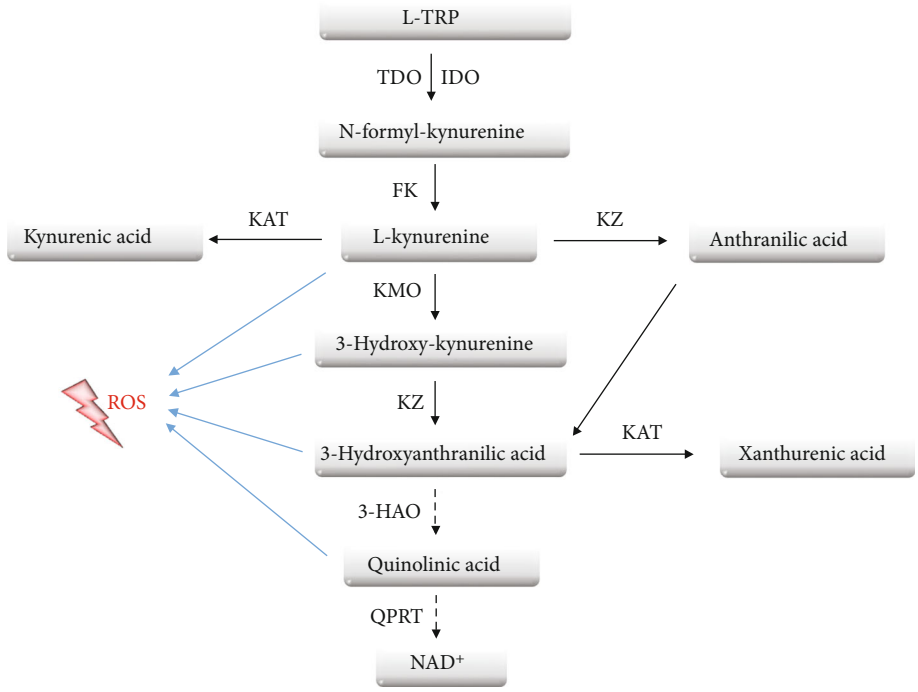

Figure 2: Tryptophan metabolism pathways, including derivatives involved in the production of reactive oxygen species. 3-HAO: 3-hydroxyanthranilate-3,4-dioxygenase; FK: formidase; IDO: indoleamine 2,3-dioxygenase; KAT: kynurenine aminotransferase; KMO: kynurenine 3-monooxygenase; KZ: kynureninase; L-TRP: L-tryptophan; QPRT: quinolinic acid phosphoribosyltransferase; ROS: reactive oxygen species; TDO: tryptophan dioxygenase.

2015 showed an association between elevated levels of free form IS and increased mortality in CKD patients, and comparison of clinical outcomes of hemodialysis patients revealed an elevated risk of all-cause mortality in those who had higher serum IS levels [79, 80]. These facts lead one to consider that IS not only accumulates under kidney impairment but also enhances its progression by damaging renal structures, thus confirming its toxic character (Figure 3(a)). Ellis et al. showed increased expression of proapoptotic protein in proximal tubular epithelial cells (PTECs) and human renal tubular epithelial cells (HK-2) under the impact of IS. Additionally, in the same conditions, they indicated increased hypertrophy and expression of profibrotic molecules, well known hallmarks of CKD, in tested cells [81]. An *in vitro* study of HK-2 cells reveled increased expression of α-smooth muscle actin (α-SMA), N-cadherin, and fibronectin—markers of epithelial-mesenchymal transition, which is another pathological process leading to CKD progression [82]. Enhanced EMT was observed as well in rat renal tubular epithelial cells (NRK-52E) after IS stimulation, thereby confirming its involvement in disease development [83]. This leads to the question as to which IS mechanism causes its toxic effects. The available data show that the administration of an antioxidant and NADPH inhibitor attenuates the proinflammatory effects exerted by IS in proximal tubular cells [84, 85]. Wang et al. discovered that indoxyl sulfate induces oxidative stress, upregulates of NF-κB with following increase in the CYP24 expression in renal tubule epithelial cells (Figure 3(a)) [85, 86]. That remains in accordance with a previous observation that IS enhances ROS production and activates NF-κB in HK-2 cells and rat models, confirming the role of oxidative stress in the toxic mechanism of the described molecule [87]. Further intensified energy consumption, changes in mitochondrial membrane potential and IV complex activity, with consequent reduction of mitochondrial mass on the HK-2 cell line and impairment of mitochondrial functions in nephrectomized mice was demonstrated by Sun and colleagues. According to the authors' conclusions, IS enhances mitochondrial oxidative stress in HK-2 cells, which contributes to cellular damage present in CKD progression [88]. The key role of oxidative stress in the damaging mechanism of IS was also confirmed by Edamatsu. The obtained results found a decrease in total glutathione levels in porcine renal tubular cells and their higher vulnerability to oxidative stress (OS) with enhanced apoptosis after the administration of mixed uremic toxins containing IS [89]. The above data indicate that IS is an important mediator of ROS production in renal structures.

2.4. Indoxyl Sulfate in Atherosclerosis. Observations of IS dealing with ROS generation lead one to assume that this uremic toxin contributes to vascular injuries via a similar mechanism, especially considering the endothelium is highly vulnerable to oxidative stress. The existing data serve as evidence that IS has an effect on endothelial cell viability, permeability, activation, and calcification. Namely, it is involved in multiple steps leading to atherosclerosis (Figure 4(a)). Limited viability of human umbilical vein endothelial cells (HUVECs) under the influence of IS was

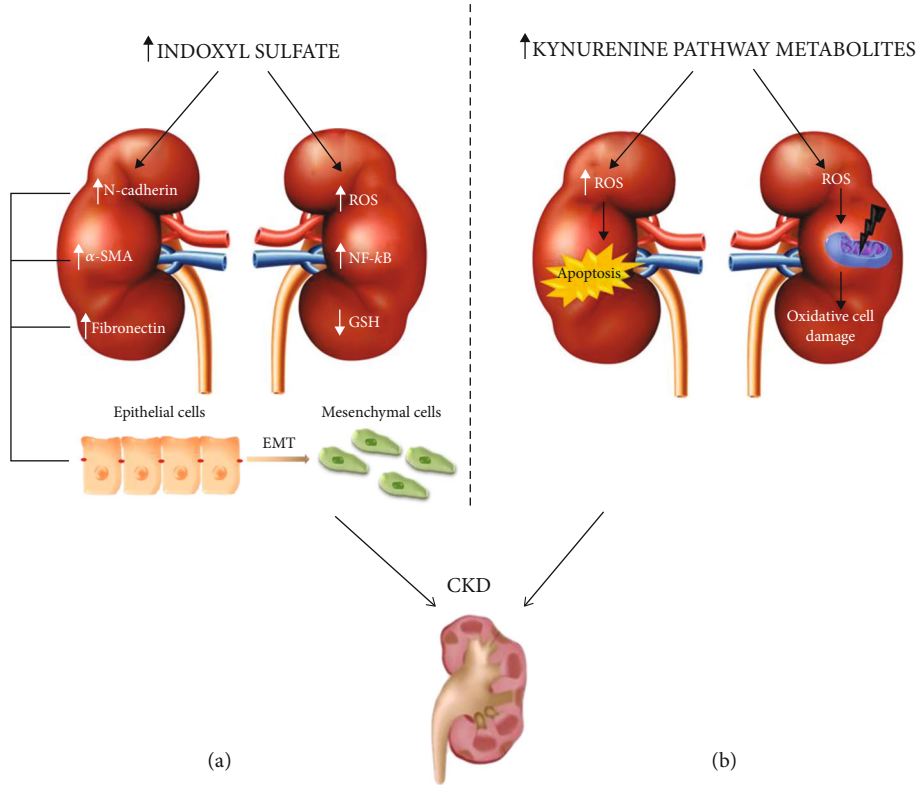

Figure 3: Contribution of tryptophan metabolites, indoxyl sulfate (a) and kynurenines (b), in the development of chronic kidney disease, including reactive oxygen species as a damaging factor. CKD: chronic kidney disease; EMT: epithelial mesenchymal transition; NF-κB: nuclear factor kappa-light-chain-enhancer of activated B cells; ROS: reactive oxygen species.

observed by Li and colleagues [90]. That remains in line with outcomes obtained by Lee et al., who reported not only decreased HUVEC viability after IS treatment but also detected parallels between higher dosage of IS and escalating ROS generation. Moreover, research identified impaired mitochondrial function, reduction in mtDNA copy number, and their decreased mass in IS-treated HUVECs, when compared to untreated controls. These effects have been reversed after the administration of antioxidants such as vitamin C or NAC, which indicates oxidative stress, derived from mitochondria, involvement in the toxic action of IS [91]. Searching of other sources of ROS in endothelial cells after IS exposure led to outcomes indicating enhanced NADPH oxidase activity with simultaneous decreasing eNOS activity and inhibition of NO production in human aortic endothelial cells [92]. Dou et al., who additionally proved a negative impact on glutathione levels in HUVECs exposed to IS, also indicated an NADPH oxidase-depended mechanism [93]. Shen et al., who reported enhanced activity of NADPH oxidase in IS-treated HUVECs, with subsequent intensification of E-selectin expression, obtained similar results. They also found that IS activates NF-κB, which leads to the same effect on the abovementioned protein. Thus, the authors discuss the existing cascade, activation of NADPH oxidase–ROS generation–activation of NF-κB–upregulation of E-selectin expression, as a mechanism through which IS triggers its effect [94]. The activation of NF-κB by IS was also reported by Tumur et al., who indicated that NF-κB enhances the expression of ICAM-1 in HUVECs and that the administration of an antioxidant (NAC) attenuates this effect [95]. That strengthens the hypothesis of oxidative stress involvement in mediating IS toxicity toward endothelial cells. Enhanced ICAM-1 and VCAM-1 expression, thus endothelial activation involved in atherosclerosis, was reported after acute and chronic distribution of IS by Six and colleagues [96]. Those results remain in line with outcomes obtained by Lu et al., who documented morphological changes and HUVEC degeneration after IS treatment [97]. The same authors point to increased intracellular ROS production and decreased eNOS and VE-cadherin expression after IS stimulation. Altogether, these data highlight the role of oxidative stress as a mediator of the IS toxic effect exerted toward vascular structures.

2.5. Kynurenine and Its Biological Activity. TRP undergoes extensive metabolism along several pathways, of which kynurenine is one of the most significant and occurs in the highest level (Figure 2) [98]. To date, three enzymes have been identified to take part in the first step of TRP conversion—two isozymes of indoleamine 2,3-dioxygenase (IDO1, IDO2) and tryptophan dioxygenase (TDO) [99]. All of them take part in the oxidative degradation of the aromatic ring with kynurenine production, and differences between them were observed in catalytic activity [100]. The newly created kynurenine is further converted into active metabolites, 3-hydroxykynurenine (3-HK), kynurenic acid (KYNA), and

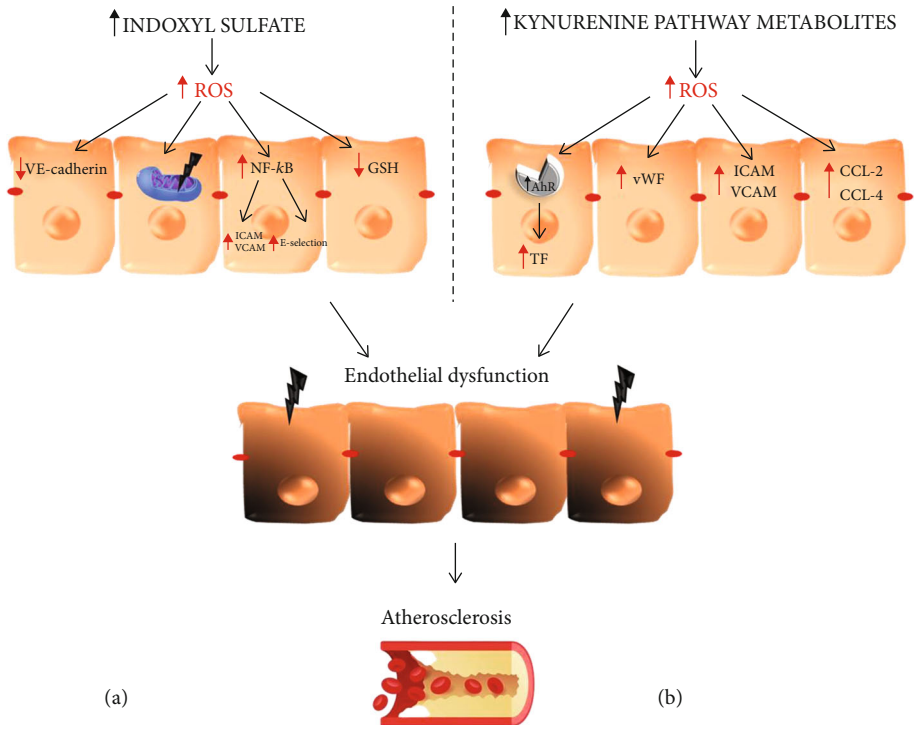

Figure 4: Contribution of tryptophan metabolites, indoxyl sulfate (a) and kynurenines (b), in the development of atherosclerosis, including reactive oxygen species as a damaging factor. AhR: aryl hydrocarbon receptor; CCL-2, -4: macrophage inflammatory protein 2, 4; GSH: glutathione; ICAM-1: intercellular adhesion molecule; NF-κB: nuclear factor kappa-light-chain-enhancer of activated B cells; ROS: reactive oxygen species; VCAM-1: vascular cell adhesion molecule-1; VE-cadherin: vascular endothelial cadherin; vWF: von Willebrand factor.

hydroxyanthranilic acid (3-HAA), together called kynurenines [101]. Kynurenines gained researchers' interest due to alternations in their metabolic pathway and level in disparate medical conditions, such as schizophrenia, Alzheimer's disease, Parkinson's disease, different types of cancer, diabetes mellitus, cardiovascular disorders, or chronic kidney disease [102–110]. Metabolites of the kynurenine pathway exert diverse, sometimes opposite, roles on many biological processes, including inflammation, redox homeostasis, gluconeogenesis, and apoptosis [111]. Their accumulation may affect numerous cellular signaling pathways through AhR activation, leading to the disruption of the homeostasis of various organs. AhR is a ligand-activated transcription factor and has recently been highlighted as playing a critical role in the maintenance of cellular homeostasis. Therefore, its overactivation by the higher concentration of KYN and its metabolites may enhance cell aging processes and their death rate. Moreover, numerous KYN derivatives demonstrate toxic properties related to their ability to induce oxidative stress or the formation of excitotoxic complexes with insulin [111–114]. These alternations may manifest clinically in the form of systemic disorders, such as osteodystrophy, insulin resistance, neurological disorders, changes in blood pressure, anemia, hypercoagulability, atherosclerosis, and kidney tissue damage.

The effectiveness of hemodialysis for kynurenines elimination is limited. It has been reported that they are present in the dialysate, and their plasma level in hemodialyzed patients was higher compared with healthy volunteers [115]. Moreover, Pawlak et al. reported that metabolites of the kynurenine pathway accumulate in hemodialyzed patients [115]. Comparison of three renal replacement therapies (hemodialysis peritoneal dialysis, kidney transplantation) showed a significant increase of IDO activity in all three groups, when compared with the control group under peritoneal dialysis characterized by a meaningful increase of kynurenine levels when compared to others [116]. This indicates the inability of renal replacement therapy to eliminate KYN metabolites. The aspect that needs to be taken under the consideration is the fact that KYN content does not grow proportionally to the decrease in the GFR, which may indicate another mechanism involved in its elimination [111].

2.6. Kynurenines in Chronic Kidney Disease. The metabolites of the kynurenine pathway play a significant role in the modulation of physiological, as well as pathological processes, including redox homeostasis. KYN, the first product of TRP degradation, exerts prooxidant effects, and the aerobic irradiation of KYN produces superoxide radicals and leads to cytochrome C reduction [112]. Moreover, increased levels of KYN result in cell death through the ROS pathway in natural killer (NK) cells [117]. Accumulated KYN metabolites, via the ability to induce/potentiate oxidative stress, show a negative or toxic effect on many cellular processes, which may lead to cell damage, an increased rate of apoptosis, or triggering the inflammatory processes that reflect a disturbance of homeostasis of various organs and systems [98, 111, 112, 118, 119]. The impact of kynurenine metabolites on body

homeostasis cannot be forgotten in the course of diseases where significant changes in their activity are observed [112, 120]. Alterations in the kynurenine system have been linked to CKD and atherosclerosis. The inflammatory process observed in the course of CKD causes an increase in IDO activity. Since IDO is involved in tryptophan metabolism, its induction leads to a decrease in its tissue and plasma concentration with a simultaneous increase in kynurenine pathway metabolite synthesis [111, 114, 120, 121]. In addition, a permanent reduction in the glomerular filtration rate observed in CKD contributes to the increasing level of KYN metabolites in the plasma and tissues. Pawlak et al. reported their accumulation both in experimental models of CKD and in uremic patients. In patients with uremia, the concentrations of KYN, KYNA, and QA were increased by 37-105%, 84-428%, and 394-1018% of the control values, respectively. These changes were accompanied by a significant increase in KYNA/KYN and QA/KYN ratios, reflecting the increased activity of kynurenine pathway enzymes [115, 122, 123]. A positive correlation between the level of KYN metabolites, examined in patients' blood or animal tissues and the degree of renal insufficiency, was previously confirmed [115, 122, 124, 125]. Moreover, KYN metabolites, particularly 3-hydroxykynurenine, are associated with oxidative stress in ESRD patients. The same authors also demonstrated strong positive associations between KYNA, QA, and SOX markers in uremic syndrome. The values of KYN, QA, the QA/KYN ratio, total peroxide, Cu/Zn superoxide dismutase, and malondialdehyde were significantly higher than in healthy people [126]. The biological activity of QA is mainly related to the agonistic action at N-methyl-D-aspartate (NMDA) receptors, which are located both in the central nervous system and in many cells on the periphery [111]. NMDA receptors and free radical processes are involved in the excitotoxic mechanism of QA activity, mainly related to the accumulation of peroxynitrite (ONOO-) in the cell, an increase in nitric oxide synthase activity, a decrease in superoxide dismutase activity, and intensification of the lipid peroxidation process. Increased NO synthesis causes a cellular energy deficit dependent on the reaction of the peroxynitrite (ONOO-) molecule with the enzymes of the tricarboxylic acid cycle, mitochondrial respiratory chain, mitochondrial calcium metabolism, or by DNA damage [127].

As mentioned above, the accumulation of KYN metabolites in the course of CKD may induce oxidative cell damage, which leads to inflammatory processes. Okuda et al. reported that the excess of 3-HKYN enhances ROS generation, leading to mitochondria function impairment [128]. It also dysregulates the respiratory chain parameters, reduces the respiratory control index, decreases the adenosine diphosphate/oxygen and glutamate/malate ratio in mitochondria, and uncouples the respiratory chain and oxidative phosphorylation [128, 129]. These alternations are probably due to the increase in ROS concentration associated with the KYN intensified auto-oxidation process as a result of its accumulation in CKD. Moreover, their ability to produce ROS through increased oxidative stress level severity in renal cells leads to exacerbated cell damage and accelerated rate of apoptosis in renal tissues during CKD progression (Figure 3(b)) [111, 129, 130]. The above data indicate that the accumulated KYN metabolites may participate in the development of kidney cell dysfunction leading to their damage resulting in organ failure. Therefore, the inhibition of TRP metabolic pathway activity, thanks to a slow or even stop in the destructive processes in the kidney, could be a potential therapeutic goal in uremic patients.

2.7. Kynurenines in Atherosclerosis. The available data demonstrated that the accumulation of toxic TRP metabolites in the body seems to be an important factor affecting vascular endothelial dysfunction leading to atherosclerosis (Figure 4(b)) [110, 112]. It has been reported that the KYN/TRP ratio is associated with carotid intima-media thickness, a presymptomatic predictor of atherosclerosis [131, 132]. Elevated levels of 3-HKYN have also been documented in patients with cardiovascular diseases. In addition, the accumulation of QA was independently related with the progression of atherosclerosis in the plasma of uremic patients [126]. The long-term accumulation and biological properties of KYN metabolites negatively affected the parameters of the erythrocytic system in patients [133]. KYN and its metabolites were also associated with hyperfibrinolysis. Kaminski et al. reported that AA is negatively correlated with the tissue plasminogen activator during severe-to-end-stage CKD [134]. Disturbances of the fibrinolytic system cause an increase in the prothrombotic potential, responsible for the pathogenesis of atherosclerosis and cardiovascular disorders. Wang et al. provided evidence that the overactivation of the kynurenine pathway was associated with increased oxidative stress and inflammation [112]. It was proven that the activation of the aryl hydrocarbon receptor AhR and also the promotion of oxidative stress by KYN, 3-KYN, 3-HAA, and QA may be important players in the initiation and progression of atherosclerosis [119, 124, 126, 135]. Pawlak et al. documented that KYN and 3-HKYN were positively associated with inflammation and SOX markers in uremic syndrome. They concluded that a link between kynurenine pathway activation and increased oxidative stress, inflammation, and the progression of atherosclerosis exists [133]. Watanabe et al. showed that overexpression of indoleamine 2,3-dioxygenase in coronary atherosclerotic plaques via increased oxidative stress level, and the AhR pathway stimulation enhanced tissue factor expression (TF) in activated macrophages [136]. The induction of oxidative stress and AhR activation by KYN and its metabolites also increased inflammation [131–133]. Inflammation is crucial to atherosclerosis, since it contributes to coronary plaque instability, increased vulnerability to rupture or erosion, leading to thrombosis and myocardial infarction (MI) [137]. A positive correlation between KYN, 3-HKYN, AA, and QA with crucial factors associated with the development of atherosclerosis such as TF, von Willebrand factor (vWF), thrombomodulin, and prothrombin fragments F(1+2) concentration as well as sICAM-1 (soluble intercellular adhesion molecule-1) and sVCAM-1 (soluble circulating vascular cell adhesion molecule-1) level was noticed [114, 133, 138, 139]. KYN was also independently and significantly associated with elevated sICAM-1, whereas 3-hydroxyanthranilic acid

was positively correlated with the concentration of CCL-2 and CCL-4 chemokines. Additionally, IDO plays a proinflammatory role in human diseases. It has been shown that it was recognized as a novel marker of immune activation in the early stages of atherosclerosis [110]. Its downstream metabolites induce overexpression of proinflammatory factors [140]. Kynurenine 3-monooxygenase has also been documented as another crucial regulator of inflammation [141]. The abovementioned relationship may represent one of the mechanisms involved in the development of atherosclerosis [141]. These data confirm that the aggressive nature of kynurenines may contribute to the induction and progression of atherosclerosis; it has a proinflammatory and prooxidative effect and causes endothelial dysfunction [142, 143]. It is worth mentioning that not all metabolites of the kynurenine pathway have a proatherogenic effect. It has been shown that KYNA concentration and the KYNA/KYN ratio were significantly lower in patients without cardiovascular disease, and they were positively associated with homocysteine levels [144, 145]. The KYNA mechanism of action seems to be related with the inhibition of homocysteine-induced cytotoxicity [144]. Moreover, increased KYNA in plasma concentration caused a decrease in triglyceride and cholesterol levels and an inhibition of the uptake of oxidized low-density lipoproteins by macrophages, which resulted in an inhibition of atherogenesis in a murine model [146, 147].

2.8. Changes of TRP Metabolism in the Course of CKD. As mentioned above, under normal conditions, TRP is metabolized mostly in the liver. Nevertheless, during pathological processes, like chronic kidney disease, its conversion changes significantly. Depletion of TRP in CKD patient serum has been reported multiple times [148, 149]. Moreover, the kynurenine to tryptophan ratio is elevated in those patients, which indicates the overactivity of enzymes that take part in TRP catabolism [150]. The increased activity of IDO1 is due to elevated IFN-γ levels, the main inductor of this protein. Also, increased cytokine levels can affect the hypothalamo-pituitary-adrenal axis, which in turn enhances TDO activity [150]. Altogether, those modifications of TRP metabolism lead to increased levels of its derivatives. This is consistent with scientific reports that indicate a correlation between increased KYN, 3-HKYN, XA, KYNA, AA, and QA and decreased GFR. The kidneys contribute to those changes in two ways. This organ is characterized by high expression of IDO, enhancing tryptophan depletion. Moreover, during kidney dysfunction development, excretion of TRP metabolites is insufficient, which leads to their accumulation. In the context of cardiovascular disorder occurrence in the progress of CKD, it is worth noting that alternation in TRP metabolism varies among groups with varied risk of CVD development. Konje et al. indicated a correlation between decreased basal levels of TRP and increased risk of CVD incident, which could suggest that changes in TRP metabolism are factors contributing to cardiovascular complications occurring in renal impairment [151].

2.9. TRP Pathway Metabolites—a Link between CKD and Atherosclerosis. The abovementioned experimental outcomes indicate IS and kynurenines as mediators of ROS generation, taking part in enhancing both CKD and atherosclerosis. Since it is known that atherosclerosis develops often in patients suffering from CKD, it is logical to suspect TRP metabolites as agents that by accumulating in kidney failure lead to vascular impairment. In nephrectomized rats, augmentation of aortic media thickness and decrease of the aortic lumen diameter was observed. The authors reported higher endothelial dysfunction, reduction of the eNOS expression, and diminution of NO production in nephrectomized rats on additional IS treatment when compared to controls. In this study, the authors confirmed ROS involvement in the IS mechanism of action, by showing a significant elevation of superoxide levels in the aortic walls of rats with kidney impairment [152]. Analysis of samples derived from CKD children also confirms the association between CKD severity, increased IS, and carotid artery intima-media thickness. Further, a correlation between this uremic toxin and marker of endothelial dysfunction persisted for 12 months, separately from other risk factors [153]. Similar results were obtained in uremic patients in whom KYN, QA, and the QA/KYN ratio were positively associated with IMT values (intima-media thickness). The authors observed that kynurenine pathway metabolites, via propagation of oxidative stress, are responsible for both endothelial dysfunction and IMT values in patients with chronic kidney disease [126]. Claro et al. evaluated the connection between kidney impairment and vascular response in CKD patients. Their outcomes indicate a relation between elevated IS levels, markers of vascular inflammation, and endothelial dysfunction, such as sFAS, sVCAM-1, and MCP-1, in those patients [154]. An experiment on mice with induced CKD showed that IS causes loss of endothelial cells and enhances the expression of adhesive molecules (ICAM-1, VCAM-1) [96]. Kynurenines were also positively associated with vWF, TM, sICAM-1, and sVCAM-1, which have been implicated as markers of endothelial cell dysfunction and intima-media thickness in the early stage of systemic atherosclerosis in the course of CKD [110]. The study conducted by Kamiński et al. shows a positive correlation between high levels of IS in CKD patients and TM, a marker of endothelium functionality. Moreover, the parallel between increased IS content and markers of oxidative stress, Cu/Zn SOD and H_2O_2, has been pointed out, which proves the predicted mechanism of action [155]. Another support of the thesis that IS links these two diseases comes from a study on predialysis CKD patients. Administration of oral IS adsorbent (AST-120) significantly improved flow-mediated dilation after diminution of serum IS levels. In this experiment, the authors indicated an NADPH-depended mechanism in ROS generation, which contributes to endothelial impairment mediated by IS [156].

Hypertension, a constant symptom of CKD, appears to have a complex association with endothelial dysfunction. In turn, hyperkynureninemia increases the risk of hypertension occurrence in patients with chronic kidney disease (CKD) [157]. In addition, Martinsons et al. pointed out that hyperkynureninemia may lead to the progression of cardiovascular disease, including hypertension, a known factor in the development of atherosclerosis [158, 159]. The above information

serves as an argument weighted in favor of TRP metabolites as agents enhancing oxidative stress, which increases the risk of atherosclerosis in the progression of chronic kidney disease. These observations could help develop therapies targeting components of the tryptophan-kynurenine pathway in CKD to prevent cardiovascular consequences and improve patient outcomes.

3. Conclusion

The available data provide evidence that the accumulation of TRP toxic metabolites in the body seems to be one of the key factors underlying the development of uremic symptoms, including impaired lipid metabolism and vascular endothelial dysfunction, leading to atherosclerosis in the course of CKD. The main reason is their ability to induce oxidative stress, leading to the exacerbation of simmering inflammation. Complete understanding of the impact of TRP active metabolites on overall body homeostasis, as well as on the condition of many organs, may provide the basis for the development of innovative therapeutic options that improve the diagnosis and treatment of CKD and systemic disorders related with its progression, such as atherosclerosis.

Abbreviations

3-HAA: Hydroxyanthranilic acid
3-HK: 3-Hydroxykynurenine
AhR: Aryl hydrocarbon receptor
Akt: Protein kinase B
ATP: Adenosine triphosphate
CCL-2, -4: Macrophage inflammatory protein 2, 4
CKD: Chronic kidney disease
CVD: Cardiovascular disease
DNA: Deoxyribonucleic acid
ECM: Extracellular matrix
EMT: Epithelial mesenchymal transition
eNOS: Endothelial nitric oxide synthase
ESRD: End stage renal disease
EUTox: European Uremic Toxins Work Group
FAAs: Free fatty acids
FADH2: Flavin adenine dinucleotide
GFR: Glomerular filtration rate
GSH: Glutathione
HDL: High-density lipoproteins
HK-2: Human renal tubular epithelial cells
HUVECs: Human umbilical vein endothelial cells
ICAM-1: Intercellular adhesion molecule
IDO1, IDO2: Indoleamine 2,3-dioxygenase type 1,2
IFN-γ: Interferon gamma
IL-12, 18: Interleukin 12, 18
IMT: Intima-media thickness
IS: Indoxyl sulfate
KYNA: Kynurenic acid
LDL: Low-density lipoproteins
LOX-1: Lectin-like oxidized low-density lipoprotein receptor-1
MCP-1: Monocyte chemoattractant protein-1
NAC: Acetylcysteine
NADH: Nicotinamide adenine dinucleotide
NADPH: Nicotinamide adenine dinucleotide phosphate
NF-κB: Nuclear factor kappa-light-chain-enhancer of activated B cells
NK: Natural killer
NO: Nitric oxide
NOS: Nitric oxide synthase
NOX: NADPH oxidases
NRK-52E: Rat renal tubular epithelial cells
OS: Oxidative stress
oxLDL: Oxidized low-density lipoproteins
p38MAPK: p38 mitogen-activated protein kinases
PI3K: Phosphoinositide 3-kinases
Plg: Plasminogen
PTECs: Proximal tubular epithelial cells
QA: Quinolinic acid
ROS: Reactive oxygen species
sICAM-1: Soluble intercellular adhesion molecule-1
SMC: Smooth muscle cells
SOX: Increased oxidative stress
VCAM-1: Vascular cell adhesion molecule-1
TDO: Tryptophan dioxygenase
TF: Tissue factor
TGF-β1: Transforming growth factor β
TM: Thrombomodulin
TNF-α: Tumor necrosis factor alpha
TRP: Tryptophan
VCAM-1: Vascular cell adhesion molecule-1
VE-cadherin: Vascular endothelial cadherin
VSMCs: Vascular smooth muscle cells
vWF: von Willebrand factor
XO: Xanthine oxidase
α-SMA: Alpha smooth muscle actin.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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