Review Article

Cardiovascular Complications in CKD Patients: Role of Oxidative Stress

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Starting with the early stages, patients with chronic kidney disease (CKD) experience higher burden of cardiovascular disease (CVD). Moreover, CVD complications are the major cause of mortality in CKD patients as compared with complications from chronic kidney failure. While traditional CVD risk factors, including diabetes, hypertension, hyperlipidemia, obesity, physical inactivity, may be more prevalent among CKD patients, these factors seem to underestimate the accelerated cardiovascular disease in the CKD population. Search for additional biomarkers that could explain the enhanced CVD risk in CKD patients has gained increasing importance. Although it is unlikely that any single nontraditional risk factor would fully account for the increased CVD risk in individuals with CKD, oxidative stress appears to play a central role in the development and progression of CVD and its complications. We will review the data that support the contribution of oxidative stress in the pathogenesis of CVD in patients with chronic kidney failure.

1. Introduction

Cardiovascular disease (CVD) is a major cause of morbidity and mortality in patients with chronic kidney disease (CKD) [1, 2]. Even early stages of CKD that are characterized by relatively preserved or minimally decreased overall renal function are associated with increasing incidence of *de novo* and recurrent CVD events [3, 4]. As glomerular filtration rate (GFR) diminishes, the prevalence and severity of CVD abnormalities have been reported to be increasing [5, 6]. Among patients with stages III-IV CKD, the prevalence of CVD is 4- to 5-fold higher than that observed for the general population. CKD patients are known to be affected by diabetes, hypertension, and obesity—which are known traditional CVD risk factors in general population [7, 8]. However, cross-sectional studies demonstrated that the Framingham Risk Score which is based on the traditional CVD risk factors failed to determine the extent of CVD risk in subjects with CKD and those with end-stage renal disease (ESRD) [9–11], and other factors must be involved in the increased CVD prevalence in this high-risk population [12, 13]. Before a new biochemical marker could be considered as CVD risk factor, it must meet the following conditions: (a) evidence of the biological plausibility as to why the factor may promote CVD risk; (b) demonstration that the risk factor level increases with severity of kidney disease; (c) evidence of an association between the risk factor and cardiovascular disease in CKD patients; (d) demonstration in double-blind, randomized placebo-controlled clinical trials that treatment of the risk factor decreases CVD outcomes. Available data on the importance of oxidative stress as one of the contributing factors in the prevalence and severity of CVD abnormalities in patients with chronic kidney disease will be summarized in the following sections.

2. Role of Oxidative Stress in CVD

Oxidative stress (OxStress) is recognized as a critical factor in the development of atherosclerotic cardiovascular disease

(ACVD) [14, 15]. According to the oxidation hypothesis of atherosclerosis, low-density lipoprotein (LDL) in its native state is not atherogenic [16, 17]. LDL must undergo oxidative modification before it can contribute to the initiation and progression of atherosclerosis [16, 17]. Data from animal models of atherosclerosis, both diet-induced and genetically altered models, have demonstrated the presence of oxidized LDL (oxLDL) in plasma as well as in atherosclerotic lesions [18, 19]. Presence of oxLDL, autoantibodies against malondialdehyde-modified LDL, and of LDL-IgG immune complexes has also been reported in human plasma and human atherosclerotic lesions [18, 19]. The pathways involved in the formation of these oxidative markers and the relationship between these markers and disease progression remain to be elucidated.

In case-control studies, some reports have suggested a positive relationship between autoantibodies against MDA-LDL (one form of oxLDL) and disease severity [20, 21], while others have noted no relationship [22] or inverse relationship [23] between autoantibody levels to oxLDL and the extent of atherosclerosis. In the Watanabe rabbit, animals with high autoantibody levels following immunization with oxLDL were found to have less severe lesions than animals with low antibody levels [24]. We have reported that independent of fasting levels of autoantibodies, patients with diseased endothelium demonstrated a characteristic acute and transient reduction in autoantibody levels following meal consumption [25]. Such a reduction in autoantibody levels was not observed in young healthy controls with one or less than one risk factor. We also reported that this meal-induced reduction in autoantibody levels is unique to meal challenges that are enriched in polyunsaturated fatty acids. Isocaloric meal challenges containing primarily saturated or monounsaturated fatty acids failed to induce the transient reduction in autoantibody levels in these same individuals [26, 27].

A large body of evidence suggests that oxidized low-density lipoprotein (oxLDL) is the leading candidate in the pathogenesis of atherosclerosis [28] and may serve as a unique mediator of oxidative status in the vascular environment [28]. While the emphasis is on the oxidative modification of LDL, all plasma lipoproteins are subjected to oxidative modification [29]. Case-control studies as well as a limited number of prospective studies have linked the level of oxLDL to disease severity. Due to the heterogeneity of oxLDL and the available epitopes that are recognized by the various antibodies, a number of different immunoassays are available for oxLDL, and the correlation among these measurements is quite poor [30]. Several studies have reported strong and independent relationship between measures of oxLDL and CVD [31–33], including metabolic syndrome [34]. Data from progression/regression studies with nonhuman primates actually complicated the relationship between indices of LDL oxidation and disease status. Using antibodies that recognized the oxidative epitopes of phospholipids moiety of LDL (oxPL), diet-induced atherosclerosis is associated with increased levels of oxLDL and increased levels of total oxPL as well as oxPL/apoB [35]. With regression, as LDL-cholesterol is reduced, there was a modest reduction in total oxPL but a statistically significant increase in oxPL/apoB [35]. In humans, data from clinical trials (REVERSAL, MIRACL) with aggressive LDL-cholesterol reduction also reported and unexpected increase in oxPL/apoB [36, 37].

Oxidative modification of LDL resulted in the formation of a lipid-rich particle with specific characteristics that contribute to the development of early atherosclerosis [38]. By inducing adhesion molecules (VCAM-1) and specific receptors, oxLDL stimulates the adhesion of circulating monocytes to endothelial cells [39]. oxLDL can also stimulate the production and release of monocyte chemotactic protein-1 (MCP-1) by endothelial cells and smooth muscle cells resulting in the enhanced migration of monocytes into the arterial intima [40]. oxLDL has also been reported to inhibit in vitro proliferation and survival of vascular cells [41] as well as alterations in the normal function of endothelial cells [42]. Additionally, oxLDL has prothrombotic activity and increases platelet activation and expression of tissue factor and PAI-1 (plasminogen activator inhibitor 1) by endothelial cells [43].

3. Oxidative Stress in CKD

Oxidative stress is a state of imbalance between free radicals production and their degradation by antioxidant systems with increased accumulation of the radicals. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are examples of free radicals. Over 90% of ROS formation occurs "accidentally" in mitochondria during metabolism of oxygen when some of electrons passing "down" the electron transport chain leak away from the main path and go directly to reduce oxygen molecules to the superoxide anion [44]. In addition, ROS could be "deliberately" synthesized in phagocytic cells, as well as in vascular wall and various other tissues by enzymes such as NAD(P)H oxidase, myeloperoxidase, xanthine oxidase, cyclooxygenase, and lipoxygenase [45]. At low concentrations, ROS (superoxide anion, hydrogen peroxide, hydroxyl radical, hypochlorite ion, and hydroperoxyl radical) involved the vast array of physiologic functions. For example, ROS are known regulators of nitric oxide synthesis, intracellular signaling cascades, including cytokines, growth factors, MAPK, and NF-κB, and also involved in the modulation of immune responses, apoptosis, and mutagenesis [46–48]. Additionally, ROS produced during phagocytic burst is a key defense mechanism against environmental pathogens. However, when ROS are made in excess, they can react with various molecules such as lipids, carbohydrates, proteins, and DNA altering their structure and function, resulting in cellular damage that leads to pathologic processes including, but not limited to, atherosclerosis development. These potentially deleterious reactions are controlled by a system of enzymatic and nonenzymatic antioxidants which eliminate pro-oxidants and scavenge-free radicals. Superoxide dismutase (SOD), glutathione peroxidase, glutathione reductase, and catalase are among the main enzymatic antioxidants. Glutathione, thiols, ascorbic acid, alpha-tocopherol (vitamin E), mixed carotinoids, and bioflavonoids are among the nonenzymatic antioxidant defense processes.

Direct measurement of free radicals in vivo is difficult due to the highly reactive nature of these compounds and minute concentrations in biological fluids. Instead, we rely on measurement of stable end products of oxidation of different molecules. Numerous biomarkers of oxidative stress have been shown to be elevated in patients with CKD. These include products of lipid oxidation (lipid peroxides, malondialdehyde, and thiobarbituric acid reactive substances) and oxidized LDL [49, 50], advanced oxidation protein products (AOPPs) [51], F2 isoprostanes and isolevuglandins (a family of reactive γ-ketoaldehydes generated by free radical oxidation of arachidonate-containing lipids through the isoprostane pathway) [52, 53], and 8-hydroxyl 2′-deoxyguanosine—marker of oxidative DNA damage [54]. Furthermore, indices of OxStress are increased with severity of kidney disease. For example, glomerular filtration rate has been reported to be inversely associated with levels of AOPP [51], malondialdehyde (MDA) [55], and F2 isoprostanes [56, 57], suggesting that decline in renal function may have direct effect on worsening of oxidative stress.

The nature of oxidative stress in chronic kidney disease remains to be elucidated. Impaired oxidative balance in CKD is likely to come from a combination of increased ROS production and reduced clearance as well as an ineffective antioxidant defense mechanism. Several important antioxidant pathways have been reported to be altered in patients with CKD, including reduced erythrocyte SOD activity [55], reduced plasma thiol groups [58], diminished plasma glutathione, and glutathione peroxidase function [59]. However, total antioxidant capacity (TAC) of plasma remains normal or even becomes elevated as CKD progresses [57]. Elevated concentrations of uric acid in CKD patients have been suggested to account for the high capacity for peroxyl radicals that constitute the substrate for the in vitro TAC assay [60].

CKD patients are typically affected by multiple concomitant diseases such as diabetes and hypertension (HTN) which are also associated with oxidative stress [61, 62]. Increased activity of baseline and stimulated NAD(P)H oxidase are responsible for overproduction of superoxide anion, in circulating peripheral mononuclear (PMN) cells isolated from patients with stage I-II CKD [63]. The presence of CKD appears to further enhance the oxidative stress independently from underlying conditions. Agarwal [64] reported that urinary MDA excretion and protein carbonylation were increased in hypertensive patients with concomitant CKD as compared with patients with HTN and no CKD. The renin-angiotensin-aldosterone system (RAAS) plays an important role in activation of NAD(P)H oxidase in vascular smooth muscle cells and the kidney [65, 66]. Additionally, NAD(P)H oxidase could be activated by cytokines (*TNFα*), hyperglycemia, and mechanical stress [65]. Therefore, rigorous studies examining the relation between oxidative stress, CKD, and underline diabetes and HTN are needed in humans to clearly establish if oxidative stress is a marker of severity of underline condition leading to CKD or that CKD independently further promotes OxStress.

Hemodialysis—a procedure utilized as part of usual care in the management of terminal chronic kidney disease, has also been demonstrated to contribute to OxStress. Contact of blood with dialysis membrane during extracorporeal blood purification can lead to PMN cell activation and generation of ROS [67, 68]. Presence of inadequately removed endotoxin in water used for dialysate preparation also influences PMN activation and ROS production in hemodialysis patients [69]. Myeloperoxidase (MPO) activity has been found to be increased during hemodialysis, especially with the use of bioincompatible dialysis membranes [70]. Moreover, heparin that is routinely used for anticoagulation during hemodialysis is known to activate MPO leading to increased ROS production [71]. Intravenous administered heparin can also displace extracellular-superoxide dismutase (EC-SOD) from vascular endothelium [72] by interfering with the binding of the antioxidant enzyme to type C heparin sulfate proteoglycans. It has been suggested that EC-SOD is the major determinant of nitric oxide bioavailability in blood vessels, and loss of EC-SOD from vascular wall may contribute to endothelial dysfunction [73]. Additionally, plasma ascorbic acid and lipid-soluble alpha-tocopherol, both potent components of an antioxidant defense system, were significantly reduced after single hemodialysis session [74–76]. However, the hemodialysis procedure was also reported to have beneficial effects on total antioxidant capacity by increasing plasma thiol content [76, 77].

4. Association between Oxidative Stress Biomarkers and CVD in CKD Patients

Strong correlation between oxidative markers and the presence and extent of cardiovascular diseases was found in the general population as outlined in the recent paper [78]. Moreover, oxidative markers were shown to be important predictors of cardiovascular outcomes in prospective analyses [79–83]. Unfortunately, the limited number of studies examined the relationship between oxidative stress markers and cardiovascular disease in patients with kidney failure. Drüeke et al. [84] found that levels of AOPP strongly correlated with carotid artery intima-medial thickness (IMT) in 79 patients with ESRD. Similar finding was reported by Yang et al. in 109 patients with CKD [85]. Additionally, significant positive correlation between carotid artery IMT and TBARS [86] and MPO [87] and negative correlation between carotid artery IMT and reduced SOD and plasma sulfhydryl were reported in patients with ESRD [86]. Shoji et al. [88] observed a statistical trend in correlation between carotid artery IMT and autoantibodies against oxidized LDL. A stronger correlation was observed between femoral artery IMT and autoantibodies against oxidatively modified LDL in ESRD [88].

Prospective studies that examine the association between oxidative stress markers and clinical outcomes in hemodialysis patients are scarce. MPO levels have been shown to predict all-cause mortality in 356 chronic hemodialysis patients [89] with a hazard ratio of 1.14 (95% confidence interval 1.03–1.26) for each 1,000 pmol/L increase in MPO level. Interestingly, MPO gene polymorphism was also demonstrated to be associated with presence of CVD and higher CVD-related

mortality in ESRD patients [90]. Levels of autoantibodies against oxLDL were found to strongly predict cardiovascular mortality during 4 years of follow up in 94 hemodialysis patients [91].

5. Antioxidant Interventions and Cardiovascular Outcomes in Patients with CKD

Several small studies have examined the impact of antioxidant interventions on oxidative stress markers in patients with CKD [92–94]. Unfortunately, randomized controlled clinical trials addressing the impact of antioxidant interventions on CVD outcomes in patients with CKD are scarce. The SPACE (Secondary Prevention with Antioxidants of Cardiovascular Disease in End-Stage Renal Disease) was a clinical trial involving 196 patients with ESRD who were randomized to either 800 IU of alpha-tocopherol per day or placebo [95]. During a median followup period of 519 days, statistically significant reduction in the primary composite outcome, consisting of myocardial infarction (fatal and nonfatal), ischemic stroke, peripheral vascular disease, and unstable angina, was observed in patients receiving vitamin E supplementation [95]. The relative risk (RR) was 0.46 (95% confidence interval (CI) 0.27–0.78) in the vitamin E group as compared to the placebo.

In the second randomized controlled trial, N-acetyl-cysteine at oral dose 600 mg twice daily over a period of approximately 15 months also significantly reduced primary composite variable consisting of cardiac events including fatal and nonfatal myocardial infarction, cardiovascular disease death, need for coronary angioplasty or coronary bypass surgery, ischemic stroke, peripheral vascular disease with amputation, or need for angioplasty [96]. The relative risk was 0.6 (95% CI 0.38–0.95). However, no beneficial effects of vitamin E or N-acetylcysteine administration were observed on all-cause mortality, suggesting that exploration of additional strategies is needed to improve overall survival in dialysis patients. Subgroup analysis of some lipid-lowering studies which have included CKD patients has suggested that statin therapy may also reduce inflammatory and oxidative markers [97]. Additionally, subgroup of patients with CKD taking atorvastatin for a median period of 3.3 years had a statistically significant decrease in cumulative incidence for fatal and nonfatal stroke, total coronary events, total cardiovascular events, and all-cause mortality as compared to placebo in the Anglo-Scandinavian Cardiac Outcomes Trial—Lipid Lowering Arm (ASCOT-LLA) [98]. However, it should also be noted that two large randomized clinical trials using atorvastatin [99] and rosuvastatin [100] failed to demonstrate any reduction of CVD events or all-cause mortality in patients with ESRD. Results from the Study of Heart and Renal Protection (SHARP) might shed more light on cardiovascular benefits of statin use in CKD patients [101]. Additional studies that address the efficacy of novel antioxidants including endogenous antioxidants such as hemeoxygenase-1 and bilirubin [102, 103] in reducing oxidative stress CKD patients are needed.

In summary, cardiovascular disease is an important cause of morbidity and mortality in patients with impaired kidney function. Increasing evidence strongly supports oxidative stress as plausible independent cardiovascular risk factor in patients with CKD. Nevertheless, several important questions remain unanswered. The exact processes underlying increased oxidative stress in CKD remain to be elucidated. Furthermore, identification of biochemical and/or functional biomarkers that could be used to monitor oxidative imbalance in CKD may allow the development of optimal intervention strategy to reduce oxidative stress in CKD.

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