Review

The role of miRNAs in the regulation of oxidative stress and microvascular reactivity in chronic kidney disease

Justina Mihaljević¹, Dubravka Mihaljević², Ines Drenjančević³*, Zvonimir Sitaš⁴

¹Clinical Department of Diagnostic and Interventional Radiology, University Hospital Centre Osijek, J. Huttlera 4, 31000 Osijek, Croatia
²Department of Nephrology, University Hospital Centre Osijek, J. Huttlera 4, 31000 Osijek, Croatia; and Department of Internal medicine and history of medicine, Faculty of Medicine Osijek, Josip Juraj Strossmayer University of Osijek, J. Huttlera 4, 31000 Osijek, Croatia
³Department of Physiology and Immunology, Faculty of Medicine Osijek, Josip Juraj Strossmayer University of Osijek, J. Huttlera 4, 31000 Osijek, Croatia; and Scientific Center of Excellence for Personalized Health Care, Josip Juraj Strossmayer University of Osijek, Trg Svetog Trojstva 3, 31000 Osijek, Croatia
⁴Department of Nephrology, University Hospital Centre Osijek, J. Huttlera 4, 31000 Osijek, Croatia

Chronic kidney disease (CKD) is an initially asymptomatic, but chronic condition characterized by a progressive loss of kidney function over the time. Etiology of CKD includes diabetes, hypertension, autoimmune diseases, polycystic kidney disease and other genetic diseases, nephrotic syndrome, etc. The development of complications such as hypertension, anemia, bone diseases, and cardiovascular complications (like heart failure, coronary artery disease, arrhythmias, valvular heart disease, cardiac arrest etc.) with an increased risk of death and hospitalization is common. Due to the significant rate of morbidity and mortality from CKD, early detection and primary prevention are extremely important.

Oxidative stress affects microvascular reactivity and is considered to be one of the most important causes of endothelial dysfunction, underlying CKD. Recently, the role of miRNA, a non-coding approximately 22 nucleotides long RNA molecules which mediate post-transcriptional gene silencing, in oxidative stress has also been investigated. Individual miRNA molecules, such as miRNA-335-5p, miR-92a, miR-92a-3p relate to endothelial dysfunction. This opens new diagnostic and therapeutic possibilities and requires further research in the field of CKD. The aim of this review article is to systemize recent knowledge on the role of miRNA in the regulation of oxidative stress and microvascular reactivity in CKD.

Keywords: Chronic kidney disease, cardiovascular complications, microvascular reactivity, miRNA, oxidative stress.
**Chronic kidney disease and microvascular reactivity**

Chronic kidney disease (CKD) is a chronic condition that is manifested by progressive and irreversible changes in the structure and function of the kidneys. The disease has 5 stages based on the eGFR value, where the 1st stage is the mildest form and is characterized by the preservation of the glomerular filtration value (eGFR >90ml/min/1.73m²), while the 5th stage is the most severe with exceptional damage to glomerular function, manifesting itself in reduced glomerular filtration (eGFR<15 ml/min/1.73m²) and resulting in renal failure [1]. In addition to damage to kidney function, patients are at the increased risk of complications and mortality, primarily cardiovascular. Cardiovascular complications, such as coronary artery disease, congestive heart failure, heart rhythm disorders and cardiac arrest are responsible for the majority of mortality and morbidity in patients with CKD [2]. Among those with CKD the incidence of traditional risk factors (e.g. advanced age, high blood pressure, valvular disease, smoking, lipid disorders, diabetes, male gender, etc.) and non-traditional risk factors (e.g. albuminuria, anemia, atherosclerosis, reduced glomerular filtration, inflammation, extracellular volume load, oxidative stress, endothelial dysfunction) for cardiovascular diseases is increased [3], and the risk of death from cardiovascular disease is bigger than the risk of eventual need for renal replacement therapy [4]. Approximately half of dialysis patients have more than two comorbidities; the number of hospitalizations and the number of days spent in hospital is 1.9 and 12.8 per patient-year, and the subjective assessment of the quality of life is lower compared to the healthy population [5]. The progress of the disease is faster as the patient is in a higher stage [6]. CKD is a huge burden on society, since the global prevalence is 13.4% (11.7-15.1%) [7]. It was estimated that between 4,902 and 7,083 million patients in the end-stage renal disease (ESCD) worldwide need renal replacement therapy [7]. One of the main causes of the association between CKD and cardiovascular events is endothelial dysfunction. Risk factors for endothelial dysfunction in CKD is acute and chronic inflammation and nitric oxide deficit [8]. Like most chronic diseases, CKD is characterized by a persistent inflammatory state. The causes of inflammation in CKD can be found in reduced kidney function, volume load on the kidneys, the dialysis process, and numerous comorbidities. Inflammation is manifested by increased markers of inflammation, among which are cytokines, acute phase proteins and adhesion molecules. This is presented as schematic in Figure 1. The production of pro-inflammatory cytokines is increased, acidosis is common, and infections are frequent, all of which lead to chronic inflammation in CKD [9]. Research links inflammation with many complications of CKD such as atherosclerosis, heart failure, left ventricular hypertrophy, calcification of blood vessels, atrial fibrillation, and increased mortality. Inflammation also accelerates the progression of CKD, resulting in insulin and erythropoietin resistance, bone pathologies, oxidative stress, anemia and dysfunction of endothelium [10]. The most important markers are C-reactive protein (CRP), interleukin-1 (IL-1), interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), adhesion molecules, adipokines, and CD40 ligand [10]. They are positively associated with the severity of CKD. Adhesion molecules (ICAM-1 and VCAM-1), glycoproteins responsible for the adhesion between cells or cells and the extracellular matrix, are induced by inflammation and up-regulated in CKD patients as a result of their reduced clearance and increased synthesis, resulting in endothelial dysfunction in CKD patients [11].

**Figure 1. Etiopathogenesis of inflammation in CKD;**

CKD: Chronic kidney disease, CRP: C-reactive protein, ICAM-1: Intercellular Molecule 1, IL-6: Interleukin 6, IL-1: Interleukin 1, TNF-α: Tumor necrosis factor alpha, VCAM-1: Vascular cell adhesion molecule 1 (based on references: [8 – 11])
The most significant mechanisms that contribute to the occurrence and development of CKD are tissue hypoxia and reduced tissue perfusion due to dysfunctional endothelium. Unregulated angiogenesis, endocrine, metabolic and immune disorders along with hypertension and apoptosis of endothelial cells contribute to the pathogenesis of CKD. As a result, the development of fibrosis and eventual organ failure occurs. Due to progressive disruption of homeostasis, progressive organ damage ultimately occurs. Despite the notion that adverse alterations of the vascular function are most pronounced distal to the level of small arterioles [12], most of studies focus on macrovascular complications, while microvascular complications remain neglected. Macrovascular complications result in cardiovascular diseases such as heart attack, cardiac arrest, heart failure and heart rhythm disorders as previously mentioned. Also, the risk for cerebrovascular disease is increased [13]. This is due to traditional risk factors such as high blood pressure and diabetes and non-traditional ones associated with uremia, such as oxidative stress and abnormal calcium and phosphorus metabolism, as well as cerebral hypoperfusion and changes in the structure of the heart [13]. Microvascular complications coexist and precede macrovascular ones, which is why it is important to detect them early and act with the aim of preventing the development of macrovascular complications. Both types of complications are mediated by the same mechanisms, such as accumulation of uremic toxins, chronic inflammatory state, and oxidative stress, indicating that preventing the development of one type would directly contribute to preventing the other [14].

Alterations in blood vessels

Earlier research shows that in CKD patients, compared to healthy controls, the microvascular density in skeletal muscles and the heart was reduced by 32%, which was proven by biopsy, autopsy, and imaging [12]. The decrease in microvascular density occurs due to reduced blood supply of micro vessels. However, the most significant vascular changes are atherosclerosis and vascular calcifications (VC). Vascular changes in CKD are presented as schematic in Figure 2. CKD affects the medial layer of blood vessels by increasing the formation of calcifications. VC are the result of a multifactorial process that leads to a change in the phenotype of vascular smooth muscle cells into cells like osteoblasts [14]. Known mechanisms are mineral dysregulation and hyperphosphatemia, which occurs as CKD progresses. A study conducted on mice proved that SIRT6, a protein deacetylase and mono-ADP ribosyltransferase enzyme which is secreted in states of stress, has a defensive function in patients with CKD [15]. SIRT6-transgenic (SIRT6-Tg) mice had reduced VC, whereas vascular smooth muscle cell-specific (VSMC-specific) SIRT6 knockdown mice had severe VC in CKD. The results showed that SIRT6 prevents VC by repressing the osteogenic transdifferentiation of VSMC, which can be a potential site of therapeutic action for VC in CKD.

Figure 2. Blood vessels alterations in CKD. CKD: chronic kidney disease; (based on references: [12, 14, 16])

As already noted, endothelial dysfunction underlies development of vascular component and complications in CKD. The method of choice for measuring endothelium-dependent microvascular reactivity is non-invasive laser Doppler flow (LDF) measurement. Patients with developed CKD demonstrated significant changes in thermal hyperemic reaction [16]. Significant deviations were observed in LDF parameters such as the amplitude of thermal hyperemia (TH) and the area under the TH curve and deviations in the level of C reactive protein (CRP) [16]. Microvascular reactivity depends on the equilibrium of many factors, among which endothelin, prostanoids and nitric oxide stand out [17]. To be able to understand the pathophysiological mechanisms in CKD, the evaluation of microvascular function is extremely significant.
Association between oxidative stress and microvascular reactivity in CKD

Oxidative stress is an important factor in the pathophysiology of CKD due to the reduction of antioxidants as well as the increased production of ROS. This imbalance leads to toxic effects through the generation of peroxides and free radicals that harm all component of cell, including DNA molecules, proteins, and lipids. Bases are damaged and DNA chains are broken. Base damage is indirect and is caused by reactive oxygen species, for example superoxide, hydrogen peroxide and hydroxyl radical [18]. Since kidney is an organ characterized by a high metabolism, it is abundant in mitochondrial oxidation reactions. Because of that, it is prone to damage by oxidative stress. In CKD, an increase in the concentration of oxidative stress markers such as mitochondrial superoxide and oxidized LDL, homocysteine was recorded and deficiency of SOD and GSH was also reported [19]. Complications of CKD, such as hypertension, inflammation, anemia, and atherosclerosis, are strongly associated with oxidative stress [19]. It is considered that oxidative stress is one of the most significant causes of endothelial dysfunction, which is the underlying mechanism of the origin and development of many chronic diseases, including CKD. Patients treated with hemodialysis (HD) are at particular risk of developing microcirculatory disorders, which is directly connected with increased oxidative stress. A study of 33 patients treated with HD who do not show signs of peripheral arterial disease (PAD), along with a control group of 20 healthy individuals, demonstrated that microcirculation disorder in patients undergoing HD procedures was associated with endothelial damage due to oxidative stress [20]. In both groups, the transcutaneous oxygen tension (TcPO2) of the microcirculation of the dorsum of the foot was measured, and vitamin C (200 mg per day) and vitamin E (600 mg per day) supplementation was used in 8 patients for a period of 6 months to examine the effect of antioxidants on TcPO2. The results indicated a significantly lower value of TcPO2 in patients with HD compared to healthy controls. Vitamin supplementation has led to a significant raise in the value of TcPO2 and significantly reduced serum levels of markers of endothelial damage and lipid peroxidation such as thrombomodulin and thiobarbituric acid reactants [20]. There are some indications that in CKD, calcification of blood vessels occurs under the influence of oxidative stress. For example, celasterol, a natural plant ingredient, has been shown to reduce oxidative stress resulting in a reduction in calcification of arterial rings in rats and humans [21]. Celasterol increases mRNA and levels of heme oxygenase-1 (HMOX-1) and reduces reactive species of oxygen, which indicates that celasterol exerts an inhibitory effect on vascular calcification through HMOX-1, which may be a possible therapeutic site for treatment of CKD. Oxidative stress promotes remodeling of blood vessels in kidneys and increases preglomerular resistance, which favors the development of acute and chronic kidney injury, diabetic nephropathy, and increased blood pressure. An important role in this mechanism is played by the NADPH oxidase family through the production of superoxide [22]. Superoxide is the main reactive oxygen species (ROS). An excess of superoxide and related ROS reduces the biological effects of NO, whose role is to regulate systemic blood pressure and kidney function itself through vasodilation [23]. ROS are an important link in the pathophysiology of hypertension. Blood vessels are abundant with NADPH oxidase, which produces ROS that damage the kidney vasculature. Oxidative stress leads to dysfunction of endothelium, inflammatory state, hypertrophy, apoptosis, migration of cell, development of fibrosis and angiogenesis [24]. An interesting therapeutic goal is the regulatory role of NO in the control of renal microcirculation. Similarly, oxidative stress and cardiovascular events are associated with paraoxonase-1 (PON1) enzyme polymorphisms [25]. Paraoxonase-1 (PON1) is a lipoprotein-associated esterase whose role is to hydrolyze oxidized LDL-cholesterol, and its low activity increases risk of cardiovascular events [26]. In addition, immunohistochemistry demonstrated increased expression of PON1 on capillary endothelium in patients with diabetes or hypertension [26]. An important factor in oxidative stress is hypoxia, which leads to apoptosis of kidney tubular cells, resulting in kidney fibrosis with the loss of peritubular capillaries, which loop back to hypoxia and forms a vicious circle that ultimately leads to the final stage of CKD [27]. Hypoxia can also occur in the earlier stages of CKD due to an imbalance of vasoactive substances, where the renin-angiotensin system (RAS) plays an important role. Local activation of the RAS leads to spasm of efferent arterioles, which consequently leads to hypoperfusion and hypoxia of postglomerular peritubular capillaries. Another mechanism by which hypoxia affects endothelial dysfunction is directly, via NADPH oxidase activated by angiotensin II [27]. Thus, angiotensin II has a dual role in oxidative stress, through hemodynamic and non-hemodynamic mechanisms, both mediated by inducing hypoxia. For this reason, in the treatment of hypertension and CKD, great attention is paid to blocking the RAS effects. Vascular reactivity is also affected by CKD, which was proven in a study where skin vasodilation after local heating and glomerular filtration rate (eGFR) were measured. Compared to healthy controls, patients with CKD had an increased ratio of isofuran to F2-isoprostane, an oxidative stress biomarker, associated with renal, hepatic, and coagulation failure, and weakened vasodilation [28]. In addition, it was determined that the relative lack of L-arginine and oxidative stress lead to reduced skin vasodilation due to local heating in patients with CKD. In that study skin vascular conductance was weakened in CKD compared to healthy controls [29]. Another link between higher rates of
oxidative stress and endovascular dysfunction was discovered in the study where endothelial cells were cultured with the serum of diabetics with CKD, where a significant increase in expression of aldose reductase mRNA was noted [30]. The use of vitamin E and probucol, anti-hyperlipidemic drug, prevented the increase of aldose reductase, cytosolic NADPH-dependent oxidoreductase, in endothelial cells, which indicates the role of glycation, polyol pathway and oxidative stress in the progression of microvascular dysfunction [30]. All previously mentioned links between oxidative stress and microvascular reactivity are shown schematically in Figure 3.

![Figure 3: The connection between oxidative stress and chronic kidney disease. ROS: Reactive oxygen species (based on references: [18-30])](image)

Considering all the mentioned studies, the importance of oxidative stress in microvascular damage leading to the advancement of CKD is indisputable. However, there is a lack of studies that address the missing link of potentially altered antioxidative mechanisms and CKD. Furthermore, the misregulation of antioxidative mechanisms could occur at the level of gene transcription and expression.

**MicroRNAs (miRNAs)**

MicroRNAs (miRNAs) are RNA molecules approximately 22 nucleotides long. They are non-coding, and their role is post-transcriptional gene silencing [31]. They arise from RNA precursors by sequential cleavage of transcripts by the enzyme’s ribonuclease III (Dicer and Drosha). The gene silencing complex is formed with the help of the Argonaute effector protein, which creates the miRNA-induced silencing complex (miRISC) [31]. This complex leads to suppression of translation and/or destabilization of mRNA by the mechanism of sequence complementarity [31]. A vast of more than 1,800 miRNA molecules in the human genome target approximately 60% of human mRNAs [31]. Because of their role in the process of translation and destabilization of mRNA, they are associated with various diseases. Several miRNA molecules have recently been promoted as an interesting therapeutic target and an area that requires further research, related to endothelial function and CKD. For example, through a series of in vivo and in vitro experiments, the importance of miR-124-3p in the regulation of renal mitochondrial function and in the progression of CKD was suggested, in study on db/db mice to db/m mice [32]. In db/db mice, a reduced expression of miR-124-3p and an increase in FOXQ1 and a downregulation of Sirt4 (mitochondrial protein with ribosyltransferase, deacetylase, lipoamidase, and deacylase enzymatic activities) were found, which ultimately results in mitochondrial dysfunction and leads to the progression of CKD [32]. Importantly, this study directly linked a specific miRNA molecule to the progression of CKD. Furthermore, there is an association of miR-21-5p expression with the regulation of lipids, peroxidation process and mitochondrial respiration in H9C2 cells [33]. Particularly interesting is that the increased expression of miR-21-5p reduces the content of cellular lipids and their peroxidation, which indicates a reduction in the amount of oxidative stress [33]. The reason for this is a decrease in the intake and utilization of lipids in the cells and an increased utilization of the glycolytic pathway [33]. The same authors previously found that nephrectomy in CKD influences miR-21-5p in the left ventricle via receptor-α and by influencing oxidation of fatty acids and glycolysis trough the transcripts expression [34]. Another miRNA molecule, specifically miRNA-335-5p, is also associated with endothelial aging, which is considered the initial event in the development of atherosclerotic cardiovascular disease (ASCVD). MiRNA-335-5p expression is increased in endothelial cells under the influence of
oxidative stress and high levels of glucose, hydrogen peroxide and tumor necrosis factor lead to endothelial aging by reducing the expression of SIRT7, NAD+ dependent deacetylase [35]. Taken together these findings enabled a better understanding of the pathophysiology of the aging process of endothelial cells and open a new venue of therapeutic possibilities. Oxidative stress and dysfunction of endothelium, which favor the development of cardiovascular incidents, are linked to the accumulation of uremic toxins. In conditions of oxidative stress in kidney endothelial cells, the expression of miR-92a increases and leads to the formation and progression of atherosclerosis and the process of angiogenesis, and the expression of the miR-92a molecule itself is enhanced by uremic toxins that accumulate in CKD [36]. These conclusions were drawn based on the measurement of miR-92a levels in human CKD serum compared to healthy controls.

For many miRNAs, a change in expression due to oxidative stress has been demonstrated. Ten such miRNA molecules were identified on cultured HK-2 kidney tubule cells [37]. For example, the level of miR-205 was significantly reduced in states of oxidative stress, and it was concluded that miR-205 has a protective role in oxidative and ER stress through the suppression of Egl nine homolog 2 (EGLN2) and reduced intracellular ROS, which can open new therapeutic possibilities in acute kidney injury (AKI) and CKD. The direct connection between CKD and miRNA molecules is manifested by differences in urinary miRNAs of CKD patients compared to healthy controls. A significant increase in miR-21, miR-126 and miR-141 and a decrease in miR192 and miR204 were detected in CKD patients [38]. MiR-21 has a role in control of the proliferation and apoptosis of smooth muscle cells by influencing autophagy via PARP-1/AMPK/mTOR signalling pathway [39], and is a marker of at least 29 diseases [40]. MiR-126 through the AKT2/HK2 axis affects the mechanisms of proliferation, migration, invasion and apoptosis of non-small cell lung cancer cells [41], and its lower levels are observed in CKD patients compared to healthy controls [42]. MiR-141 expression is increased under oxidative stress and ischemia-reperfusion injury (IRI). Since the development of acute kidney injury is most often caused by IRI and since there is no adequate treatment, it is necessary to continue investigating molecular mechanisms and find diagnostic and therapeutic options for prevention of the onset of AKI and its progression in CKD. With this idea, research was conducted with the aim of determining the role of miR-92a-3p in the pyroptosis of tubular epithelial cells (TEC). Kidney cell damage, which ultimately leads to CKD, is associated with the pyroptosis process through the classical pathway mediated by caspase-1 and the non-classical pathway mediated by caspase-4/5/11 [43]. Interestingly, it appears that there is a possibility to decrease oxidative stress by altering specific miRNAs. E.g., the study, conducted in vitro and in vivo, linked the inhibition of miR-92a-3p with reduced oxidative stress of tubular epithelial cells, and pyroptosis through the action of Nrf1 in kidney IRI [44]. Increased expression of miR-19-5p and miR-20-5p for mesenchymal stem cells originated from human inducible pluripotent stem cells (iPS-MSC) also plays an important role in the preservation of renal function. Compared to iPS-MSCs alone, therapy with double overexpression of the mentioned miRNAs proved to be more successful in preserving renal function in the presence of CKD and ischemia-reperfusion injury [45]. The study was conducted on rats, as was the study in which chronic intermittent hypoxia (CIH) was investigated, which contributes to the worsening of renal function and is the main pathophysiological mechanism of obstructive sleep apnea (OSA) [46]. This study also linked a miRNA molecule, specifically miR155, and kidney damage. Increased expression of miR155 was demonstrated in kidney cells exposed to CIH [46]. In the same study, the influence of miR-155 in the modulation of the NLRP3 inflammasome, which participates in kidney damage by activating caspase-1 and pro-interleukin-1β cytokines, was studied. Overexpression of miR-155 has been shown to promote NLRP3 pathway activation and CIH-induced inflammasome activation. These results open the possibility for pharmacological blockade of the NLRP3 pathway in the therapy of CKD and OSA. The schematic diagram (Figure 4) presents the association of the mentioned miRNAs with oxidative stress and CKD.
MicroRNAs (miRNAs) and antioxidative defense systems

MiRNAs, in addition to ROS production, also affect antioxidant systems. They act on the ROS level using the enzymes NADPH oxidase 2 (NOX2), NADPH oxidase 4 (NOX4) and proline oxidase (POX), where individual miRNA molecules such as miR-3, miR-21, miR-34a, miR-23b they have an activating effect, while miR-124-5p, miR-21-a-23p, miR-253, miR-182 have an inhibitory effect [47]. MiRNAs act on important antioxidant systems such as catalase (CAT), which is inhibited by miR-30b, miR-146a and miR511b, superoxide dismutase (Cu/Zn SOD and MnSOD), inhibited by miR-21 and miR-206, and glutathione peroxidase (GPX) and glutathione reductase (GR) affected by miR-144 [47]. This is shown schematically in figure 4 Interestingly, models for these studies were mouse models of cardiac injury, renal tumors, glioma cells, mesenchymal stem cells (MSCs) from aged mice, macrophages derived from bone marrow (BMDM) from wild type and p47phox−/− mice etc. And for the effect of miRNAs in altering oxidative stress in CKD, there are no such studies yet. Taken all together, the role of miRNA in the regulation of oxidative stress is indisputable and manifests itself on several levels.

In conclusions: The main cause of death in CKD patients are pathologies related to the cardiovascular system. The causes of exceptional cardiovascular morbidity and mortality can be found in endothelial dysfunction, pronounced vascular calcifications, and increased arterial stiffness. Although vascular reactivity is altered compared to the non-CKD population, it is not given enough attention in clinical practice. Therefore, improving the endothelial function of patients with CKD is an important challenge for clinicians and an aspect in which great progress in treatment is possible. The combined approach of analysis of different markers and research could be the starting point for the development of optimal therapeutic tools. The present findings may provide better understanding of the underlying mechanisms of endothelial aging in CKD. According to our knowledge, the role of miRNAs in the regulation of antioxidant systems that affect microvascular function in CKD has not been investigated at all. By researching these pathways, an exceptional contribution could be made to the prevention, early detection, and treatment of CKD. The goal is a personalized approach to each patient, which achieves maximum treatment efficiency.
References


