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Nicotine signaling and progression of chronic kidney disease in smokers

Gaurav Jain, MD^a and Edgar A Jaimes, MD^{a,b}

^aDivision of Nephrology, University of Alabama at Birmingham, Birmingham, Alabama

^bVeteran Affairs Medical Center, Birmingham, Alabama

Abstract

The deleterious health effects of cigarette smoking are far reaching, and it remains the most important modifiable risk factor for improving overall morbidity and mortality. In addition to being a risk factor for cancer, cardiovascular disease and lung disease, there is strong evidence, both from human and animal studies, demonstrating a role for cigarette smoking in the progression of chronic kidney disease (CKD). Clinical studies have shown a strong correlation between cigarette smoking and worsening CKD in patients with diabetes, hypertension, polycystic kidney disease, and post kidney transplant. Nicotine, in addition to its role in the addictive properties of cigarette smoking, has other biological effects via activation of non-neuronal nicotinic acetylcholine receptors (nAChRs). Several nAChR subunits are expressed in the normal kidney and blockade of the $\alpha 7$ -nAChR subunit ameliorates the effects of nicotine in animal models of CKD. Nicotine increases the severity of renal injury in animal models including acute kidney injury, diabetes, acute nephritis and subtotal nephrectomy. The renal effects of nicotine are also linked to increased generation of reactive oxygen species and activation of pro-fibrotic pathways. In humans, nicotine induces transitory increases in blood pressure accompanied by reductions in glomerular filtration rate and effective renal plasma flow. In summary, clinical and experimental evidence indicate that nicotine is at least in part responsible for the deleterious effects of cigarette smoking in the progression of CKD. The mechanisms involved are the subject of active investigation and may result in novel strategies to ameliorate the effects of cigarette smoking in CKD.

Keywords

Cigarette smoking; chronic kidney disease; nicotine receptors; reactive oxygen species; fibrosis

1. Introduction

There are over a billion cigarette smokers worldwide, with over one third of the population above 15 years of age being smokers[1]. Cigarette smoking is the single most important modifiable risk factor for adverse health outcomes and has been identified as the most

Correspondence to: Edgar A. Jaimes, M.D., ZRB 637, 1720 2nd Avenue South, Birmingham, AL 35294, Tel: 205-934-0544, Fax: 205-975-6288, ejaimes@uab.edu.

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important source of preventable morbidity and mortality in the U.S.[2, 3]. Cigarette smoking associated diseases are responsible for one in every 5 deaths in the developed world, and are the most common cause for premature death in the adult population[1]. Cigarette smoking associated diseases are also responsible for 6–15% of the healthcare costs in the developed world [1]. Cigarette smoking is a major risk factor for different types of cancer, atherosclerotic vascular disease, including coronary artery disease and stroke, obstructive lung disease, cataracts and macular degeneration, osteoporosis and peptic ulcer disease[1, 4]. Moreover, accumulating clinical evidence indicates that cigarette smoking is also a risk factor in the progression of chronic kidney disease (CKD) of different etiologies including diabetes and hypertension[5–10]. Cigarette smoking has also been associated with worsening of renal function after renal transplantation [11–17]. CKD is a major public health problem worldwide, although particularly serious in the United States [18]. Despite improvements in the therapy of major risk factors, such as diabetes and hypertension, the incidence and prevalence of CKD continues to increase with the number of prevalent patients with ESRD surpassing 550,000 in 2009 [18]. Although it is now well established that tobacco smoking accelerates the progression of CKD, the mechanisms involved have not been identified.

Mainstream cigarette smoke contains over 4000 compounds, including reactive oxygen species, stable reactive aldehydes and ketones and carbon monoxide among others [19]. Amid the numerous compounds present in tobacco smoke, nicotine is one of the biologically active and stable compounds present in large concentrations in tobacco that can be acquired through active and passive smoking. In addition to being responsible for the addictive properties of tobacco smoking, nicotine has a variety of biological effects that may play an important role in the pathogenesis of different conditions including renal disease.

This review focuses on the biologic effects of nicotine, with special emphasis on the effects in the kidney, and summarizes the clinical evidence and experimental evidence implicating cigarette smoking and nicotine exposure as major risk factors in the progression of chronic kidney disease.

2. Biologic Effects of Nicotine

Nicotine mediates its effects via the activation of muscle and neuronal nicotinic acetylcholine receptors (nAChRs) [20]. The nAChRs are a family of transmembrane ligand-gated ion-channels consisting of five subunits that result from the combinatorial assembly of different nAChR subunits [20]. The muscle nAChRs consist of five subunits: $\alpha 1$ and 4 non-subunits ($\beta 1, \beta 2, \beta 3, \beta 4$) and have binding capacity in the micromolar range [21]. The neuronal nAChRs are ~50 fold more sensitive to nicotine than the muscle nAChRs with binding capacity in the nanomolar range, and can be homopentamers or heteropentamers. Ten subunits, which can form neuronal nAChRs, have been identified, of which seven are α -like subunits ($\alpha 2-7, 9-10$) and 3 are non- α subunits ($\beta 2-4$). Receptor pentamers can be constructed from various combinations of α and β subunits[20]. The diversity of nAChRs subunits is a major determinant of the specialized properties and functions of the mature receptors. In the central nervous system the high affinity nicotine-binding receptor consists of at least 4 and 2 nAChR subunits[22, 23]. In the brain, the various nAChR subunit combinations differ in their distribution, pharmacological and kinetic properties [24]. The two main types of nAChRs in the brain are the $\alpha 7$ homo-oligomer, characterized by a fast activation, low affinity (binding affinity K_i 400–1,500 nM) and a high Ca^{2+} permeability [25–27]; and the $\alpha 4\beta 2$ hetero-oligomer, which is typified by a high affinity (binding affinity K_i 0.6–1.0 nM) and slow desensitization [24, 28]. The nAChRs are also expressed in non-neuronal cells including bronchial epithelial cells, endothelial cells, vascular smooth muscle cells and human mesangial cells as we have previously demonstrated (Table 1) [29–34]. In

contrast to the central and peripheral nervous systems where the distribution and composition of the nAChRs has been extensively studied, the distribution, composition and binding properties of these receptors in the kidney are not well known.

In addition to its well-recognized role as mediator of the addictive effects of tobacco smoking, nicotine has a variety of other biological effects that may play an important role in the pathogenesis and progression of a variety of pathologic conditions. In the vasculature, nicotine promotes atherosclerosis, angiogenesis and lesion growth in mouse models of lung cancer [35]. In these studies nicotine at a concentration of 10 nM induced the formation of capillary-like structures in cultured endothelial cells and induced endothelial cell proliferation at concentrations between 1 and 10 nM. In vivo the administration of nicotine in the drinking water at a concentration of 100 µg/mL resulted in levels of nicotine similar to those found in the plasma of smokers and increased atherosclerotic plaque in Apo-E deficient mice, tumor size in mice after subcutaneous injection of Lewis lung cancer and neovascularization after limb ischemia. The angiogenic effects of nicotine are inhibited by specific blockade of the $\alpha 7$ -nAChR suggesting an important role of this receptor in these effects [33]. In these studies the angiogenic effects of nicotine were found to be partially dependent on vascular endothelial growth factor (VEGF), and completely dependent on the phosphatidylinositol 3-kinase, mitogen activated protein kinase pathways and NF- κ B activation [33]. Moreover in recent studies we have demonstrated that the administration of nicotine (100 µg/mL in the drinking water) exacerbated the formation of atherosclerotic plaque in Apo E deficient mice but not in mice Apo E deficient and lacking CD36, an important mediator of oxidized LDL (oxLDL) uptake and contributor of macrophage foam formation. In vitro, nicotine (100 nM) had synergistic effects with oxLDL on the expression of pro-inflammatory cytokines including TNF- α , MCP-1, IL-6 and CXCL-9.

In vascular endothelial and smooth muscle cells from bovine aorta, nicotine also induces cell proliferation in a process mediated by increased generation of basic fibroblast growth factor (bFGF) and platelet derived growth factor. In contrast the administration of nicotine resulted in dose dependent reduction in the release of transforming growth factor- β by endothelial cells [36–38]. These effects of nicotine were dose dependent with significant proliferation observed at concentrations of nicotine in the nanomolar range (60 nM); however the peak proliferative response was observed at concentrations of nicotine (600 nM) that are unlikely to occur in vivo. In human vascular smooth muscle cells nicotine, as well as its stable metabolite cotinine (10 and 100 nM), increase the production of bFGF and upregulate the expression of several matrix metalloproteinases involved in cell migration such as collagenase-1, stromelysin-1 and gelatinase A [39]. The effects of cotinine on bFGF production also suggest that some of the observed effects of nicotine might be mediated via its stable metabolites and without the activation of specific nicotine receptors.

In addition to inducing proliferation nicotine has also been shown to increase extracellular matrix deposition in several cell types. Nicotine (10–100 µg/mL) increases fibronectin production in lung fibroblasts in vivo and in vitro in a process that requires protein kinase C activation, MAPKs activation, increased intracellular levels of cAMP and DNA binding of the transcription factor CREB [40]. In dermal fibroblasts nicotine, acting via the $\alpha 3$ nAChR significantly increases the expression of the cell cycle regulators p21, cyclin D1, Ki-67 and PCNA as well as significant increases in type 1 collagen, elastin and matrix metalloproteinase-1 [41]. These studies however were performed utilizing concentrations of nicotine in the micromolar range (10 µM). Studies in non-small cell lung cancer cell lines have shown that nicotine, at concentrations in the micromolar range (1 µM), induces cell proliferation by β -arrestin-mediated activation of Src and Rb-Raf-1 pathways [42]. These effects were prevented by $\alpha 7$ -nAChR blockade suggesting a role for this subunit on these effects. Furthermore, in human coronary artery endothelial cells, nicotine (100 nM to 10

μM) increases the mRNA levels of endothelial nitric oxide synthase (eNOS), angiotensin-I converting enzyme, tissue type plasminogen activator, plasminogen activator inhibitor, von Willebrand factor and vascular cell adhesion molecule-1 [43].

2.1 Effects of Nicotine on Kidney Derived Cells

Studies from our laboratory have demonstrated the presence of the nAChRs subunits $\alpha 4$, $\alpha 5$, $\beta 7$, $\gamma 2$, $\delta 3$ and $\epsilon 4$ in human mesangial cells[44]. In addition we demonstrated that nicotine is a powerful stimulus for human mesangial cell proliferation and fibronectin production. Importantly these effects were evident at concentrations of nicotine (100 nM) which are similar to the peak concentrations found in the plasma of active smokers [45]. These effects of nicotine were prevented by nanomolar concentrations of α -bungarotoxin, suggesting that the $\alpha 7$ -nAChR subunit plays a major role on these effects. Nicotine induced mesangial cell proliferation was also prevented by α -lobelin and dihydro- β -erythroidine indicating that the $\alpha 4$ and $\beta 2$ nAChR subunits also play an important role on these effects[44].

The effects of nicotine on mesangial cell proliferation and fibronectin production were prevented by two different NADPH oxidase inhibitors indicating an important role for NADPH oxidase mediated reactive oxygen species (ROS) generation on the pro-proliferative and pro-fibrotic effects of nicotine[44]. Moreover, nicotine induced the production of reactive oxygen species in human mesangial cells that was prevented by NADPH oxidase inhibition demonstrating that this enzyme is the main source of ROS in response to nicotine.

Protein kinase C (PKC) is required for NADPH oxidase activation in several cell types [46, 47]. Our studies demonstrated that the effects of nicotine on mesangial cell proliferation and fibronectin production are prevented by PKC inhibition suggesting that PKC activation is required for the growth promoting effects of nicotine probably by participating in NADPH oxidase activation. ROS promote the activation of MAPKs[48, 49], a process that is associated with growth related responses in response to a variety of stimuli[46, 50]. ERK1 and ERK2, the best studied MAPKs, participate in cellular growth and differentiation in response to Angiotensin II [46], PDGF[50] and TGF- β [51]. Studies from our laboratory demonstrated that nicotine (100 nM) induces ERK1/2 phosphorylation and that specific ERK1/2 inhibition prevents mesangial cell proliferation and fibronectin production in response to nicotine indicating that ERK1/2 are required for these effects[44, 52]. In other studies we demonstrated that nicotine increases COX-2 expression and activity in human mesangial cells and that COX-2 inhibition significantly reduces nicotine-induced mesangial cell proliferation and fibronectin production, suggesting that COX-2 derived prostaglandins play a major role as mediators of glomerular injury in response to nicotine[53]. Several studies have demonstrated that ROS are mediators of COX-2 induction in response to a variety of stimuli including pro-inflammatory cytokines and Angiotensin II [54, 55]. NADPH oxidase inhibition reduces nicotine stimulated COX-2 expression suggesting a role for nicotine induced ROS as mediators of COX-2 expression[53].

Nicotine (200 μM) also increases the production of ROS from NADPH oxidase and the mitochondria in cultured renal proximal tubule cells[56]. The resultant oxidative stress leads to JNK-mediated activation of the activator protein (AP)-1 transcription factor in vitro and may play a role on the tubular effects of nicotine. In addition, nicotine (200 μM) has been shown to potentiate the effects of TGF- β on α -SMA, vimentin and fibronectin production in proximal tubular cells via increased expression of unphosphorylated form of signal transducer and activator of transcription-3 (U-STAT3), a noncanonical mediator of inflammation and fibrosis, another potential mechanism that may participate on the deleterious effects of nicotine in renal disease[57].

2.2 Effects of Nicotine in Models of Renal Injury in vivo

The effects of nicotine have been investigated in diverse models of renal injury including models of acute kidney injury, models of chronic kidney disease, diabetes and glomerular diseases. In models of warm-ischemia reperfusion in rats the administration of nicotine (200 µg/mL in the drinking water) results in worse morphological changes and renal function and is associated with increased makers of oxidative stress including nitrotyrosine and malondialdehyde[56]. Moreover the administration of nicotine to sham-operated rats also resulted in increased oxidative stress without inducing obvious morphologic alterations. In these studies the authors postulate that smoking may sensitize the kidney to ischemic insults and facilitate the progression of acute kidney injury to chronic kidney injury[56]. Importantly the plasma concentrations of cotinine observed (127–134 ng/mL) were within the range reported in active smokers[58]. In contrast, however, other studies have shown that the administration of nicotine (1 mg/kg IP before injury) attenuates the severity of renal injury in rat models of ischemia reperfusion and in a model of sepsis[59, 60]. In these studies the administration of nicotine was associated with reductions in inflammatory infiltrates, pro-inflammatory cytokines and NF-κB activation[59, 60]. In these studies however the concentrations of the cotinine or stable nicotine metabolites were not assessed and nicotine was administered as a single injection before the induction of injury. These studies however suggest that under specific circumstances nicotine could have important anti-inflammatory effects. Indeed studies in isolated macrophages suggest that nicotine reduces the pro-inflammatory responses[61]. These results also suggest that nicotine may induce a change of macrophages from a pro-inflammatory to a more pro-fibrotic phenotype.

In addition to models of acute kidney injury, the effects of nicotine have also been investigated in models of glomerulonephritis. The anti-Thy1 model of glomerulonephritis is characterized by initial mesangiolysis followed by transitory glomerular cellular proliferation, inflammation and fibronectin production that occurs after a single injection of an anti-Thy1 antibody and resolves spontaneously after four weeks[62, 63]. In this model the administration of nicotine (100 µg/mL in the drinking water) resulted in levels of cotinine of ~ 60 ng/mL and induced glomerular cell proliferation and fibronectin production[53]. In these studies the administration of nicotine was also accompanied by significant increases in the expression of the inducible COX isoform COX-2, a major source of prostaglandins in the kidney[54]. COX-2 expression is increased in conditions associated with glomerular inflammation such as acute glomerulonephritis and ureteral obstruction [64–66]. Moreover COX-2 expression is upregulated in experimental models of diabetes [67, 68] and chronic kidney disease [69, 70]. COX-2 inhibition in a model of renal ablation has been shown to reduce mesangial matrix expansion, glomerular sclerosis and fibronectin and collagen IV expression, suggesting that COX-2 derived prostaglandins play an important role in the pathogenesis of renal injury [71]. These findings would suggest that COX-2 derived prostaglandins might have an important role as mediators of the deleterious effects of nicotine in models of acute nephritis. In support of this hypothesis, COX-2 inhibition in vivo has been shown to reduce the pro-angiogenic and pro-atherosclerotic effects of nicotine[35]. Although the administration of nicotine resulted in worse kidney injury in the anti-Thy1 model of acute nephritis, in the Munich-Wistar-Frömter rat, a model of proteinuria-induced renal inflammation, the long-term administration of nicotine (20 to 100 µg/mL in the drinking water) resulted in improvements in proteinuria and renal function[72]. The plasma levels of cotinine varied from 139 ng/mL for the lower dose of nicotine to more than 500 ng/mL for the higher doses of nicotine. Nicotine reduced the severity of sclerosis, desmin deposition, podocin loss, and density of renal macrophages and myofibroblasts. These changes were accompanied by reductions in the expression of MCP-1 and VCAM[72]. This animal model is characterized by spontaneous albuminuria with age that is under polygenic influence and linked to a trait locus on the rat chromosome 8 [73].

These rats have a reduced number of podocytes that likely participate in the initiation of albuminuria [74]. Chronic albuminuria in this model is associated with progressive glomerulosclerosis and fibrosis. The mechanisms by which albuminuria, in the absence of specific glomerular pathology, lead to glomerular sclerosis in this model are not well understood. We speculate that the effects of nicotine in this model although intriguing may involve pathways specific to this model and probably cannot be generalized to other models. These findings also suggest that the effects of nicotine on renal disease are not universal and dependent at least in part on the animal model, and specific experimental conditions.

The db/db mouse is a model of type II diabetes that after several weeks of hyperglycemia develops significant proteinuria and mesangial expansion that closely resemble diabetic nephropathy in humans[75] including glomerular hypertrophy, mesangial expansion, increased extracellular matrix deposition and proteinuria. These mice have a leptin receptor mutation that leads to defective signaling of the adipocyte hormone leptin[76, 77]. Absence of leptin signaling leads to polyphagia, obesity and insulin resistance followed by diabetes mellitus. In these mice the exposure to environmental tobacco smoke results in significant mesangial expansion associated with increased expression of the extracellular matrix proteins fibronectin and collagen IV, as well as the pro-fibrotic cytokine TGF- β [78]. Moreover the administration of nicotine (100 μ g/mL in the drinking water) to db/db mice does not induce any significant hemodynamic changes but leads to substantial increases in glomerular hypertrophy and mesangial expansion[52]. These changes in mesangial expansion and glomerular hypertrophy are accompanied by concomitant increases in urinary albumin excretion. The administration of nicotine to non-diabetic animals results in a small increase in cortical fibronectin expression, while diabetic mice as expected have a significant increase in cortical fibronectin that was further increased by the administration of nicotine[52]. Several studies have demonstrated that diabetes is associated with increased oxidative stress [79, 80] and that increased expression of NOX4, the most abundant NADPH oxidase in the kidney, is associated to the development of diabetic nephropathy [80]. The administration of nicotine to diabetic mice results in significant increases in cortical NOX4, nitrotyrosine expression and Akt/PKB phosphorylation, a serine-threonine kinase Akt/PKB activated by ROS that plays a major role in cell growth and matrix expansion including diabetic nephropathy [80], suggesting that increased NOX4 mediated generation of ROS exerts an important role as mediator of the deleterious effects of nicotine in diabetic nephropathy[52].

2.3 Role of the $\alpha 7$ -nAChR on the Effects of Nicotine in Renal Disease

Nicotine mediates its effects via the activation of nicotinic acetylcholine receptors (nAChRs) that function as agonist-regulated Ca^{2+} channels and are composed of five subunits and expressed by neuronal as well non-neuronal cells including human mesangial cells [34], endothelial cells and vascular smooth muscle cells[31, 33]. Of the different subunits that can be part of the nAChRs, the $\alpha 7$ subunit has been identified as an one of the most important for several of the cholinergic actions mediated by nAChRs in macrophages, vascular smooth muscle cells and cancer cell lines [81, 82].

Studies from our lab have also examined the role of the $\alpha 7$ -nAChR as mediator of the effects of nicotine in the progression of renal disease. For these studies we utilized a rat model of 5/6 nephrectomy, a well-characterized and validated model of CKD that closely mimics CKD in humans [83]. After a two-step subtotal renal ablation these rats develop progressive glomerulosclerosis and fibrosis similar to the changes observed in humans with CKD[84]. Under basal conditions we observed strong expression of the $\alpha 7$ -nAChR subunit in the renal cortex especially in the proximal tubules and at a lower degree in the distal tubules (Figure 1). In contrast to previous reports of $\alpha 7$ -nAChR expression in the systemic vasculature[33, 35], we did not observe $\alpha 7$ -nAChR expression in the intrarenal vasculature

suggesting regional differences in the vascular expression of the $\alpha 7$ -nAChR. In rats with 5/6Nx we observed that the administration of nicotine (100 μ g/mL in the drinking water) for 12 weeks resulted in small increases in blood pressure in sham animals and resulted in levels of cotinine that were similar to those found in the plasma of active smokers [85]. The administration of nicotine induced significant increases in proteinuria and glomerular injury score in 5/6Nx rats (Figure 2) that were prevented by the concomitant administration of the $\alpha 7$ -nAChR blocker methyllycaconitine (MLA). In addition the administration of nicotine to 5/6Nx rats was accompanied by increased cortical expression of fibronectin and TGF- β a well-recognized mediator of fibrosis and extracellular matrix production [86]. These effects were also prevented by $\alpha 7$ -nAChR blockade demonstrating the role of this receptor on the pro-fibrotic effects of nicotine in kidney disease. Although we observed predominantly tubular expression of the $\alpha 7$ -nAChR, MLA reduced both glomerular injury as well as interstitial fibronectin expression. Given the well-known role for the proximal tubules as a source of pro-fibrotic and pro-inflammatory cytokines [87], we postulate that tubular activation of the $\alpha 7$ -nAChR might result in increased the production of these mediators. In addition, the administration of nicotine increased the cortical expression of nitrotyrosine, a well-validated marker of oxidative stress, and of the NADPH isoform NOX4. These effects were also reduced by $\alpha 7$ -nAChR blockade suggesting that nicotine signaling via the $\alpha 7$ -nAChR plays a major role as mediator of the deleterious effects of nicotine in the progression of chronic kidney disease.

In summary, results from studies in different animal models demonstrate that nicotine exerts important biological effects in various organ systems. In addition, the administration of nicotine worsens renal injury in several animal models of renal disease suggesting that nicotine is at least in part responsible for the deleterious effects of cigarette smoking in the progression of chronic kidney disease. The mechanisms involved are complex, and mediated by activations of specific nAChRs. These animal studies supporting the role of cigarette smoking in renal dysfunction are further supported by multiple studies conducted in humans as described below. It is important to consider the fact that cigarette smoke contains several harmful compounds, including nicotine, and hence some of the adverse effects noted in human subjects may not be completely attributable to nicotine.

3. Role of Cigarette Smoking in human subjects

3.1 Increased albumin excretion and worsening renal function in the general population

A strong association has been noted between cigarette smoking and urinary albumin excretion, both in healthy subjects as well as patients with underlying diseases such as diabetes and hypertension[5–10]. These studies suggest a role of smoking in causing “de novo” renal injury, even in healthy subjects with no pre-existing chronic kidney disease. This early renal injury may not be evident by a noticeable decrease in eGFR or increase in creatinine, though decreased renal plasma flow and increased albumin excretion are well proven surrogate markers for early renal injury. This observation is most likely the result of an increase in fractional filtration rate and consistent with the presence of intraglomerular hypertension, one of the mechanisms implicated in the progression of chronic kidney disease [88]. In an elegant study conducted by Gambaro et al, 24 non-smokers were compared to 30 cigarette smokers (13 current smokers, and 17 former smokers), and renal function was evaluated by clearance of technetium-99m-diethylenetriamine penta-acetic acid (DTPA scan, to evaluate GFR) and clearance of technetium-99m-mercaptoacetyl triglycine (MAG3 scan, to evaluate renal plasma flow)[89]. Interestingly, although GFR did not differ between the two groups, the renal plasma flow as measured by MAG3 was much lower in the smokers (199.20 $\pm$ 58.85 in smokers vs. 256.54 $\pm$ 60.14 ml/min per 1.73 m², $p < 0.001$). A large cross-sectional survey on the general population in Norway was recently performed with the intent of investigating the effects of obesity, smoking and physical inactivity on

chronic kidney disease[90]. Age and sex adjusted regression analysis revealed a relative risk of 1.52 (95% CI 1.13–2.06) for CKD among smokers (>25 pack years). Similar findings were reported in an Australian study on the general population where smoking was noted to have a significant association with CKD in men, with an odds ratio of 3.59[91]. Interestingly, there was no association between CKD and smoking in women. This difference in the risks of smoking and the effects on renal function among men and women has been observed in other studies as well, though the exact reason for this difference remains elusive [92, 93]. However, more recently a study from Iran found current smoking to be an independent risk factor for developing CKD among women[11].

The HOPE (Heart Outcomes and Prevention Evaluation) study also noted smoking to be an independent risk factor for microalbuminuria in both diabetic and non-diabetic patients[94]. The increase in urinary albumin excretion is also associated with the number of cigarettes smoked in a “dose dependent” fashion[6, 10]. Another recent study also showed that smoking increases the risk of CKD in the general population (OR = 1.6, $p = 0.009$, 95% CI = 1.12–2.29), with the risk being highest for patients with preexisting hypertension and diabetes[12]. They also noted a higher risk associated with a higher cumulative quantity of cigarette smoking. In a study investigating risk factors for CKD in over 3500 CKD patients, the Chronic Renal Insufficiency Cohort (CRIC) study also demonstrated a moderate association between cigarette smoking and lower eGFR[13]. Similar deleterious results have been noted in a study evaluating the effects of smoking, as well as second hand cigarette smoking exposure among adolescents[14]. Median (IQR) eGFR in participants unexposed to tobacco was 99.1 (88.1–111.0) mL/minute per 1.73 m² compared with 96.8 (85.0–109.0) mL/minute per 1.73 m² among participants exposed to secondhand smoke and 90.1 (81.3–100.9) mL/minute per 1.73 m² among active smokers. This study has important implications, as it suggests (a) Smoking has deleterious effects on renal function, starting at an early age (b) Passive or second hand smoking can have significant deleterious effects on the renal function as well.

3.2 Role of Cigarette Smoking in Patients with co-morbidities predisposing to CKD

Among patients with coexisting illnesses like diabetes, hypertension and adult polycystic kidney disease (ADPKD), multiple studies have shown smoking to be a risk factor for increased urine albumin excretion, and hence a risk factor for onset of chronic kidney disease. Similarly, there is reasonable evidence to suggest a faster progression of chronic kidney disease in smokers with coexisting diabetes, hypertension and ADPKD.

In patients with hypertension, hypertensive smokers have more microalbuminuria compared to normotensive smokers and hypertensive non-smokers, further emphasizing the added deleterious effects of smoking in patients with underlying vascular disease[95]. The relationship between smoking and increased urine albumin excretion in patients with hypertension has been demonstrated in multiple other studies as well[96, 97]. In a prospective study following hypertensive patients treated to a target MAP of 100 mmHg, and followed for 3 years, smoking was noted to be the strongest predictive factor for predicting a decline in eGFR, in addition to preexisting chronic kidney disease and African-American race[98].

Diabetic nephropathy has also been studied extensively in regards to the deleterious effects of smoking, and it has been well established that smoking is associated with new onset of microalbuminuria, faster progression of microalbuminuria to macroalbuminuria and causes a decrease in GFR in patients with diabetes[9, 94, 95, 99–104]. One of the first reports of the association of smoking and proteinuria described a 2.8 fold higher incidence of increase urine albumin excretion in young patients with Type I DM[102]. Other studies have demonstrated the deleterious effects of smoking on CKD progression in a group of patients

with Type II DM who were followed for 5 years. These patients had reasonable glycemic control, as well as good control of blood pressure, and were being treated with RAAS blocking agents. The progression of diabetic nephropathy with elevation in serum creatinine and increasing urinary albumin excretion occurred much faster in smokers compared to non-smokers, despite appropriate blood pressure control, glycemic control and ACE inhibition[100]. In a recent study that aimed to develop a clinical prediction rule to determine the development of microalbuminuria in patients with Type I Diabetes, “ever smoking” was noted to be a clinically relevant predictor (OR 1.40, CI 1.16, 1.69 in multivariate analysis) for the development of microalbuminuria, in addition to baseline albumin excretion rate, glycosylated hemoglobin, body mass index and waist to hip ratio[105]. In another study conducted on 91 patients with Type II DM and microalbuminuria, of which 39 were non-smokers and 52 were smokers, the effects of smoking and smoking cessation on diabetic nephropathy were investigated[106]. All patients underwent smoking cessation intervention, and 11 of the 52 smokers quit smoking, generating three groups of patients: smokers (S), quitting smokers (Q) and non-smokers (NS). The patients were treated towards recommended BP and glycemic control, and were followed prospectively for 5 years. Annual measurements of eGFR and urinary albumin excretion were performed. Results revealed that 17% of the continuing smokers (S) but none of the other patients progressed to macroalbuminuria ($P < 0.003$). Smokers (S) had a faster eGFR decline rate compared to the non-smokers and the patients who quit smoking [106]. Multiple other studies have demonstrated rapid progression of CKD in smokers compared to non-smokers in both Type I diabetics as well as patients with Type II DM[107, 108].

Cigarette smoking has also been independently associated with proteinuria and rapid progression in patients with autosomal polycystic kidney disease[109, 110]. Among patients with CKD secondary to primary glomerulopathies, two studies have retrospectively reviewed and demonstrated the association between cigarette smoking and CKD progression in older men who smoke, though similar results were not seen in women who smoke[92, 93]. In a retrospective study of 160 patients with lupus nephritis, the time to develop ESRD was much shorter in smokers compared to non-smokers (145 months vs. 273 months)[111]. As expected, atherosclerotic renal artery stenosis is more common in smokers compared to non-smokers[112].

3.4 Cigarette Smoking and its effects on Renal Allografts

Over the past decade, several studies have demonstrated poor outcomes, including death censored allograft loss as well as patient mortality, in renal transplant recipients who smoked in the past, or start smoking after the renal transplant[11–17]. In an administrative dataset of a US population of over 40,000 renal transplant recipients, 9.9% were noted to be prior smokers, and 4.6% were noted to start smoking after the renal transplant[11]. Among the “new onset” smokers, smoking was associated with an increased risk of death-censored graft loss (adjusted hazard ratio 1.46, 95% CI 1.19–1.79, $p < 0.0001$) and death (adjusted hazard ratio 2.26, 95% CI 1.91–2.66, $p < 0.001$). Prior smoking also increased the risk of death censored allograft loss (adjusted hazard ratio of 1.20, 95% CI 1.06–1.35, $P = 0.003$). Other studies have shown a 30% higher risk of graft failure (relative risk 1.30; 95% confidence interval [CI], 1.04 to 1.63; $P = 0.021$) in patients with a 25 pack-year smoking history at the time of the transplant compared to non-smokers and patients who smoked less than 25 pack-years[12]. Sung et al noted reduced kidney graft survival among smokers (at the time of transplant evaluation) of 84%, 65%, and 48% at 1, 5, and 10 years, respectively, compared with graft survival in nonsmokers of 88%, 78%, and 62% ($P = 0.007$)[16]. Another study demonstrated a worse renal allograft function at 3 years among patients who continued to smoke after the renal transplant, compared to non-smokers (1.45 $\pm$ 0.55 mg/dl vs. 1.57 $\pm$ 0.48 mg/dl, $p < 0.05$), and there was a significant correlation between the number of

pack-years and worsening of renal function[13]. In a recent single center study on living donor kidney transplant recipients, smokers were noted to have a worse allograft survival (p 0.01), overall survival (p 0.02), as well as rejection free first year compared to never smokers (p 0.03)[14]. In a prospective population based study on patients older than 60 years who underwent a cadaveric kidney transplant to evaluate modifiable factors predicting patient survival[17], multivariate analysis revealed three modifiable risk factors for patient survival, including smoking (hazard ratio (HR) 2.09, 95% confidence interval (CI) 1.22–3.60), BMI and time on dialysis prior to transplant. Smoking was the only modifiable risk factor associated with renal allograft survival (HR 2.04, 95% CI 1.24–3.30). Interestingly, it has been reported that donor smoking has detrimental effects on both allograft survival (HR 1.05, p <0.05) as well as recipient survival (HR 1.06, P <0.05), while intravenous drug use and alcohol dependency in the donor did not affect these outcomes[113].

4. Smoking, Hypertension and Renal Hemodynamics

Smokers with diabetic nephropathy have higher resting energy expenditure, as well as higher levels of biomarkers of oxidative stress and inflammation, when compared to non-smokers[114]. Multiple recent studies have shown an association between smoking and hypertension, with a higher risk associated with a higher daily number of cigarettes smoked, and a longer duration of smoking[115–117]. Cigarette smoking causes sympathetic stimulation, and a related increase in blood pressure and heart rate that is mediated via direct stimulation of postganglionic sympathetic nerve endings, leading to an increase of plasma concentrations of norepinephrine and epinephrine; these effects are mediated by nicotine, and are not seen in nicotine free cigarettes[118]. Patients with primary hypertension were noted to have elevated adrenocorticotrophic hormone and aldosterone levels during smoking[119]. Administration of nicotine gum leads to transitory elevations in blood pressure accompanied by a decrease in glomerular filtration rate (GFR) and effective renal plasma flow in nonsmokers but not in smokers[120]. It has been proposed that the response of the kidney to increased systemic blood pressure may be impaired in smokers, leading to an increase in GFR and possibly also increased intraglomerular pressure, compared to non-smokers who have the normal reno-protective decrease in GFR and renal plasma flow in this scenario[115]. The role of sympathetic activation in smoking induced increased renal vascular resistance is further supported by the fact that α -1-receptor antagonism can inhibit these effects of smoking on the kidney[121].

5. Conclusion

In summary, there is a vast body of evidence supporting the adverse effects of cigarette smoking on renal function (Table 1), with a significant impact on health care outcomes, as well as health care economics. The cardiovascular and pulmonary adverse effects are well noted, and there has been extensive healthcare debate to encourage smoking cessation to improve cardiovascular, pulmonary and malignancy related outcomes. The last decade has seen several significant studies, both on animal models, as well as human subjects, to strongly suggest the role of cigarette smoking in progression of renal dysfunction. The effects of smoking, both active and passive, start at an early age, and have long lasting effects. The increasing prevalence of chronic kidney disease calls for stronger measures to halt the progression of CKD, and smoking cessation may be a valuable tool in this direction. This is particularly important as patients with chronic kidney disease are also at risk for significant cardiovascular disease, and smoking cessation may offer a combined cardiovascular as well as renal benefit[92]. Further studies are underway to investigate the mechanisms of nicotine induced renal injury, as well as possible ways to prevent this renal dysfunction. Large clinical trials evaluating the effects of smoking in patients with CKD, as

well as effects of smoking cessation on “renal health” are desirable, and may help answer some significant questions including measures to prevent the progression of CKD.

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Figure 1. Cortical localization of the 7-nAChR

Panel A: Control slide incubated with primary antibody but without secondary antibody (20X). **Panel B:** Representative photomicrograph demonstrating expression of 7-nAChR in the proximal tubules as identified by Lotus tetragonolobus lectin (20X). **Panel C:** Representative photomicrograph of a slide showing expression of 7-nAChR in the distal tubules as identified by peanut agglutinin (20X). **Panel D:** Representative photomicrograph demonstrating lack of expression of the 7-nAChR in the intra-renal vasculature (20X).

Figure 2. Glomerular Injury Score

The glomerular injury score was assessed in all groups and the results presented as percent and severity of damaged glomeruli on a 0+ to 4+ scale where 0 is no damage. (* $p < 0.05$ vs. 5/6Nx, 5/6Nx+Nic and 5/6Nx+ Nic+MLA, # $p < 0.05$ vs. 5/6 Nx, † $p < 0.05$ vs. 5/6Nx+ Nicotine)

Table 1

nAChR Subunit	Tissue Distribution in non-Neuronal Tissues
2	Vascular smooth muscle
3	Lymphoid tissue, keratinocytes, bronchial epithelial cells, endothelial cells, vascular smooth muscle, astrocytes
4	Muscle, Lymphoid tissue, alveolar macrophages, alveolar epithelial cells, neuroepithelial bodies, vascular smooth muscle, astrocytes, mesangial cells
5	Muscle, bronchial epithelial cells, endothelial cells, vascular smooth muscle
7	Muscle, lymphoid tissue, macrophages, keratinocytes, bronchial epithelial cells, neuroepithelial bodies, endothelial cells, astrocytes, mesangial cells
9	Keratinocytes
10	Endothelial cells, vascular smooth muscle
2	Muscle, lymphoid tissue, alveolar macrophages, keratinocytes, neuroepithelial bodies, endothelial cells, mesangial cells
3	Astrocytes, mesangial cells
4	Muscle, lymphoid tissue, endothelial cells, astrocytes, mesangial cells

Distribution of specific nAChR subunits in different tissues detected by mRNA or protein expression

Table 2

Clinical Effects of Cigarette Smoking
Increased albumin excretion
Progression of microalbuminuria to macroalbuminuria in diabetic patients
Increased incidence of renal artery stenosis
Decreased eGFR <ul style="list-style-type: none">• Subtle as measured by renal perfusion scans• Frank reduction as measured by serum creatinine
Decreased renal allograft survival
Increased mortality in patients with ESRD
Passive smoking can affect renal function in a similar fashion
All age groups affected, including adolescents