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Review

Interleukin 8 and diabetic nephropathy

[1]Corina D. Ene, [2]Amalia E. Anghel, [3]Monica Neagu, [4]Ilinca Nicolae

[1] Department of Nephrology, "Dr. Carol Davila" Nephrology Clinical Hospital, Bucharest, Romania; [2] Department of Dermatology, SKINMED CLINIC, Bucharest, Romania; [3] Department of Immunology "Victor Babes" National Institute of Pathology, Bucharest, Romania; [4] Research Department in Dermatology, „Dr. Victor Babeş" Clinical Hospital, Bucharest, Romania.

Abstract. Diabetic nephropathy is a chronic, progressive, multifactorial, kidney disorder. Between known etiopathogenic factors - genetic, metabolic, hemodynamic and environment- there are complex interactions that induce immune disorders to patients with glomerulopathies. An important role in developing renal response to various internal and external insults, lies with interleukin 8. In diabetic nephropathy, interleukin 8 has a central role in the accumulation of neutrophils, basophils and T lymphocytes, in the affected areas and in the progression to end stage renal disease. Interleukin-8 expression is increased in the inflammatory sites and is regulated by depression of promoter gene, transcriptional activation of the gene by NFkB, JNK MAPK. This paper will present literature data regarding the involvement of interleukin 8 in the induction and progression of diabetic glomerulopathy. Current researches provide information on the mechanisms by which interleukin 8 may interfere in controlling proliferation, inflammation, permeability, angiogenesis, apoptosis, fibrogenesis in diabetic nephropathy. Identifying indicators that would help early detection of microvascular complications associated with diabetes, could be useful in early administration of appropriate therapy and in the prevention of progression to diabetic renal disease.

Key Words: diabetic nephropathy, interleukin 8, inflammation, angiogenesis.

Corresponding Author: A. E. Anghel, e-mail: amalia.anghel@yahoo.com.

Introduction

Diabetes is the most common endocrine disorder, affecting in the year 2013, about 382 million people worldwide and it is estimated that in 2035, over 592 million cases of diabetes will be registered (Diabetes FD 2013). Diabetic nephropathy, is a chronic, progressive kidney disease and represents a complication of type 1 or 2 diabetes, being the first cause of terminal chronic kidney failure and affects 30-40% of patients with diabetes (Diabetes FD 2013). In the scientific literature, many recent researches present the diversity of risk factors, the multitude of cellular and molecular alterations in diabetic nephropathy, estimated factors that could prevent or ameliorate complications associated with diabetes (Gnudi et al 2012, Ahluwaha et al 2009, Anand et al 2014, Lim et al 2012, Ene et al 2015). It is considered that chemokines play an important role in various inflammatory circumstances. In the last years, evidence was gained regarding the intervention of interleukin 8 (IL-8) in glomerular architectural conservation, within the modulation mechanisms of the renal response to various internal and external insults. (Anand et al 2014, Lim et al 2012, Gnudi et al 2012, Giunti et al 2006, Liu et al 2015, Tashirok et al 2002)

In this paper, the authors' interest is focused on identifying possible interventions of IL-8, in the promotion and development of diabetic nephropathy.

Multifactorial pathogenesis of diabetic nephropathy

The etiopathogeny of diabetic nephropathy is multifactorial, being involved genetic, metabolic, hemodynamic and environment factors. Between this etiologic agents, are complex interactions, that cause glomerular and molecular alterations, inducing a state of immune alteration in patients with diabetic nephropathy (Gnudi et al 2012, Sharma et al 2013, Nazir et al 2014, Liu et al 2015, Ene et al 2015).

Metabolic abnormalities in diabetic nephropathy, occur sequentially and are represented by: absolute or relative insulin deficiency and/or insuline ressistance, hyperglicemia, glucose toxicity, hyperlipidemia (Gnudi et al 2012, Sharma et al 2013). Hyperglycemia affects three major signaling pathways: mTOR (mammalian target of rapamycin), AMPK (activated proteinkinase), Sirt (NAD-dependent histone deacetykase) (Liu et al 2015). Hemodinamic disturbances of diabetic nephropathy are represented by blood arterial hypertension, increased glomerular capillary pressure and hyperfiltration. Metabolic insults and hemodinamic perturbations are functionally linked in diabetic nephropathy (Giunti et al 2006).

Genetic predisposition is suggested by populational studies (ethnic), family related and experimental models. The risk of diabetic nephropathy is increased in patients with: variants of genes involved in inflammatory cytokines and angiogenesis, in GPCR (G protein-coupled receptor) signaling, in receptor binding pathways, variants of genes coding extracellular matrix components, renal function components, endothelial function and oxidative stress, glucose and lipid metabolism, growth factors, transcription factors, cytoscheletal proteins, components of immune system (Nazir et al 2014, Rizvi et al 2014, Ene et al 2015). On the other hand, regulation of IL-8 expression is performed by multiple mechanisms: repression of gene promoter, transcriptional activation of the gene by NFKb and

JNK/SARK, stabilization of the messenger RNA by p38MAPK (Hoffmann et al 2002).

Chemokines associated with diabetic glomerulopathy

Recent researches are focused on individualizing markers or their patterns with implications in controlling the proliferation, apoptosis, permeability, fibrogenesis, angiogenesis and inflammation, associated with diabetic nephropathy (Ahluwalia et al 2009, Liu et al 2012, Nazir et al 2014).

An essential role in solving this challenge is represented by the searching the genetic and molecular mechanisms involved in progression of diabetic nephropathy (figure 1). In recent decades, clear evidence of the involvement of inflammation and angiogenesis, in diabetic nephropathy onset and accelerating its cellular and molecular alterations (Ahluwalia et al 2009, Lim et al 2012, Liu et al 2015, Lee et al 2014, Nazir et al 2014) was evidenced. Glomerular and molecular manifestations, reported in patients with diabetic nephropathy, have an increased incidence during exacerbation periods and do not totally disappear even if the disease is therapeutically controlled.

The most reported renal injuries (Giunti et al 2006, Gnudi et al 2012, Lim et al 2012) are: alterations of glomerular cells: endothelial, mesangial cells, podocytes, glomerular basement membrane, renal structural changes (fibronecting colagen 4), loss of the endothelial glycocalix, increased basal membrane permeability with urinary albumin loss and glomerular sclerosis with declining glomerular filtration rate, excessive extracellular matrix deposition, formation of areas of bare glomerular basement membrane and thickening of the peripheral glomerular basement membrane, glomerular hypertrophy, tubulointerstitial fibrosis, increased infiltration of leucocytes, monocytes / macrophages in the pathological lesions.

Molecular injury has a major importance in diabetic nephropathy, influencing long term prognosis of patients. The most common molecular alterations are presented as follows:

- activation of intracellular metabolic pathways: stimulation of hexosinase pathway, polyol pathway, non-enzymatic glycation, glucose-autooxidation, PKC (Protein kinase C) pathway and increased DAG(diacylglycerol), AGEs (advanced glycation end products), ROS/RNS (reactive oxygen species/ reactive nitrogen species) production (Giunti et al 2006, Gnudi et al 2012, Liu et al 2015 , Ene et al 2015);
- decreased antioxidant response (Liu et al 2015);
- activation signaling pathways: p38MAPK (mitogen-activated protein kinase), JNK/SARK (c-jun –N-terminal kinase/stress-activated protein kinases), NFKb (nuclear factor kappa light chain enhancer of activated B cells), FOXO (O-forkhead box transcription factor) (Lim et al 2012, Hoffman et al 2002);
- activation of immune-mediated inflammatory processes (Lim et al 2012, Rizvi et al 2014, Sharma et al 2013, Anand et al 2014);
- up-regulation growth factors, cytokines, chemokines: TGF beta (Transforming growth factor beta), TNFalpha (Tumor necrosis factor alpha), Angiotensin, VEGFs (Vascular endothelial growth factors), PDGF (Platelet-derived growth factor), CTGF (Connective tissue growth factor), IGFs (Insulin-like growth factors), MCP-1 (Monocyte chemoattractant protein-1), Angiopoietins, Endothelin, MMPs (Matrix metalloproteinases), tromboxane, IL-1beta, IL-6, IL-8, IL-18, ICAM-1 (Intercellular adhesion molecule-1), VCAM-1 (Vascular cell adhesion molecule-1), adipokines (Lim et al 2012, Nazir et al 2014, Ahluwahia et al 2009, Papaoikonomou et al 2013, Sharma et al 2013, Hoffman et al 2008)
- genetic variants of VEGFA , CCRs (C-C chemokine recep[tors), CCLs (C-C chemokine ligands), IL-1, MMP-9, EPO (Erythropoietin), IL-8, ADIPOQ (Adiponectin), IL-10, IL-6 associated significantly positive with diabetic nephropathy. IL-10, VEGFA, EPO, IL-1, IL-8 are a part of GPCR (G protein –coupled receptor) signaling. VEGFA, EPO, IL-1, IL-8, IL-10, ADIPOQ and CCL2 were considered as receptor binding, while the others inflammatory cytokines (Nazir et al 2014, Ahluwahia et al 2009, Lee et al 2014) ;
- genetic variants of VEGFA and EPO showed association with angiogenesis pathway.

T allele of IL-10 rs 1800871, C allele of VEGFArs833061, G allele of CCR5rs1799987 showed protective effect for diabetic nephropathy. VEGFA rs833061T, ADIPOQ rs17300539A, CCL2 rs3917887,was involved in the development of diabetic nephropathy. MMP9rs17576 leads to over-accumulation of extracellular matrix. The polymorphism CCR5rs333 promote renal fibrosis (Nazir et al 2014, Ahluwalia et al 2009) ;

- in CC homozygotes (174G>C polymorphism of IL-6 gene) were observed lower levels of albuminuria and, though, protection from renal diabetic disease in comparison with GC genotypes. The IL6 -174G>C may contribute to increased albumin-to-creatinine ratio as to poor glycemic control and hyperlipidemia. The IL6-634G allele has been suggested as an aggravating factor in the progression of renal disease (Nazir et al 2014, Ryu et al 2012, Papaoikonomon et al 2013);
- IL-8 rs 4073 presence was associated with increased protein excretion levels in diabetic nephropathy patients. The IL-8T-251A variant increased risk of diabetic nephropathy. IL-8 is a marker for evaluating the degree of renal injury in early stage of diabetic nephropathy. IL-8 polimorphysm (rs1126647 and rs 4073) affect overall survival in advanced renal cell carcinoma (Nazir et al 2012, Niemir et al 2004);
- IL10-1082A/G polymorphism might contribute to the susceptibility for diabetic nephropathy. Changes in IL-10 levels correlatted with high urine albumin/creatinine ratio and extended renal damage in diabetic nephropathy (Nazir et al 2014, Ururahy et al 2014, Ress et al 2002 , Peng et al 2015).

Clinical phenotype of diabetic nephropathy

Disruptions of angiogenesis, permeability, proliferation, apoptosis in patients with diabetic nephropathy may be due to inflammatory, immune and fibrotic phenomena. The expression of this disease is characterized by albuminuria, reduced glomerular filtration rate and glomerulosclerosis (IFD Diabete 2013, Sharma et al 2013, Tang et al 2003, Lim et al 2012, Giunti et al 2006, Liu et al 2015, Ene et al 2015).

Identification of indicators that permit the early detection of diabetic nephropathy lesions, followed by adequate therapy, could prevent the progression of renal pathology (Figure 1).

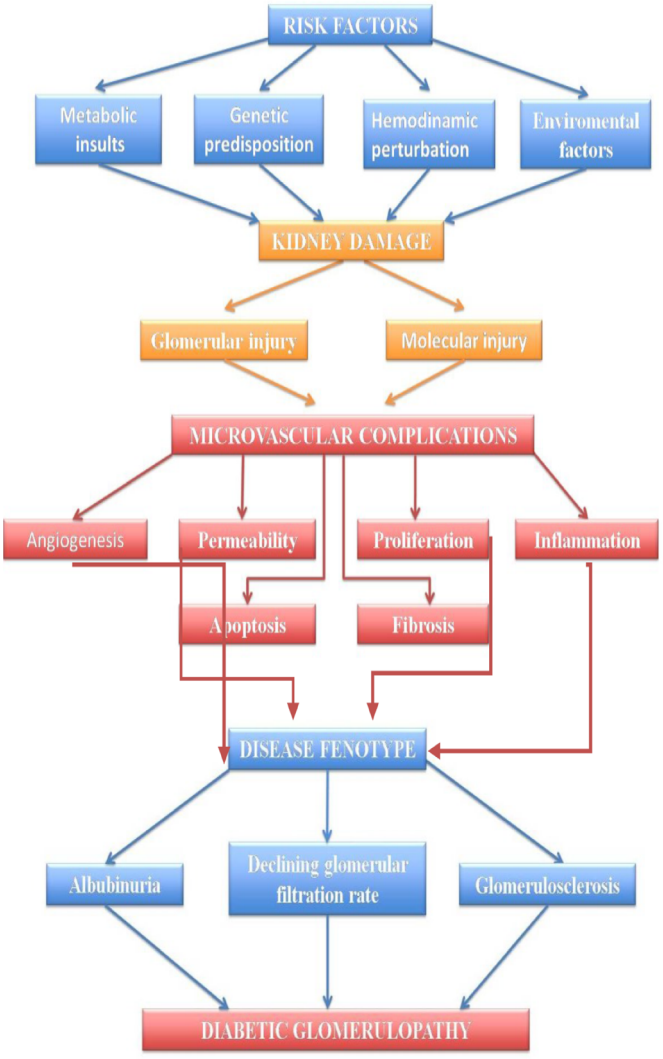

Figure 1. Genetics and molecular factors involved in evolution of diabetic nephropathy to end stage renal disease

The role of IL-8 in glomerular architecture

IL-8 chemokine is secreted as a monomer by endothelial cells, epithelial cells, fibroblasts, neutrophils, monocytes, T lymphocytes and is released in response to many stimuli. Two types of active functional IL-8, made up of 77 and, respectively 72 amino acid residues are known. By in situ hybridization, the specific RNAm IL8 protein, consisting of 77 amino acids, was detected in the normal glomeruli and tubulointerstitial compartiment. The major sources of IL8 are represented by the podocytes and the endothelial cells of the interstitial vessels. Tubular epithelial cells, express small amounts of IL8 version of 72 amino acids. In inflammatory renal diseases specific IL-8 of 72 amino acids RNAm, secreted mainly by podocytes and endocapilare, increases 5 times compared to the normal structures. IL-8 increases at the endothelial cells level, near the inflammatory site and guides activated leukocytes to inflammatory sites. IL-8 facilitates the activated leukocytes to cross the endothelium, by altering the expression of adhesion molecules. 72 molecular variant amino acid of IL-8 has a capacity much stronger than the 77 amino acid IL-8 in inhibiting adhesion of leukocytes to activated endothelial cells. Consequently, IL-8 consisting of 77 amino acids could play an important role in preserving glomerular architecture (Niemir ZI et al 2004, Borst C et al 2015). Angiogenic effects of IL-8 are mediated by the interaction with specific receptors, CXCR1 and CXCR2, which are expressed on neutrophils and T lymphocytes. CXCR1 are activated by IL-8 and GCP2 (granulocyte chemotactic protein-2). CXCR2 interacts with IL8, NAP(neutrophil attractant/activation protein-1), GRO alpha, beta, gamma(a growth factor and chemokine) (Hoffman et al 2002).

Altered profile of IL-8 in kidney damage

IL-8 showed qualitative and quantitative changes in glomerulopathies, CKD (chronic kidney disease), AKI (acute kidney injury), ADPKD (autosomal dominant polycystic kidney disease), oncocitom, RCC (renal carcinoma cells), kidney transplant. IL-8 interferes in proliferative, inflammatory, angiogenic, apoptotic, necrotic, fibrogenetic circumstances. In literature, we found contradictory stories regarding the involvement of IL-8 in renal dysfunction (Borst et al 2015, Hoffman et al 2002, Lee et al 2014, Liu et al 2015, Niemir et al 2004, Xu et al 2015). These discrepancies could be due to the diversity of biological models used, methodological approaches and variability of biological samples analyzed. Thus, they obtained information on the biological role of IL-8, rating preclinical models (cellular models, animal models) and clinical ones. Genomic studies were performed (single nucleotide polymorphism, gene mapping, gene splicing, transcription factor binding, messenger RNA), pharmacokinetic and pharmacodynamic evaluations, immunohistochemical investigations, molecular dosing in various biological samples (serum, plasma, urine, tissue homogenates, mononuclear cells). In diabetic nephropathy, IL-8 variant of 72 amino acids was detected (Niemir et al 2004). Hyperglycemia stimulates the synthesis of IL8. It was found a statistically significant relationship between urinary excretion of IL-8 and the levels of glycosylated hemoglobin, in patients with diabetic nephropathy. The IL8 increase occurs in the early stages of the disease (Tashiro et al 2002, Sirota et al 2013).

Albumin stimulates IL-8 expression, in proximal tubular cells, in vitro and in vivo (Tang 2003). IL8 plasma and urinary profiles have altered profiles in glomerular diseases and congenital anomalies of the kidney (Vianna et al 2013). IL-8 was negatively associated with estimated glomerular filtration rate and positively with body mass index (Vianna et al 2013). The local production of IL-8 stimulates neutrophil recruitment and goes to renal injury. Instead, in the circulatory compartment, IL-8 sequesters the neutrophils and blocks transmigration of target cell to the tissue lesions. Hypoxia, ischemia, reperfusion induce the release of a large number of chemokines that activate infiltration of mononuclear cells in affected renal tissue. Mononuclear cells recruited in these areas, release cytotoxic cytokines, tissue proteolytic enzymes and stimulate transcription of IL-8 in patients with post-transplant kidney failure. Local transcription of IL-8 is associated with delayed graft functionality after transplantation. IL-8 was significantly increased in patients whose renal transplant rejection was recorded. Compared with the pre-implant IL-8 level, this chemokine transcript was significantly lower one hour after reperfusion in transplanted patients. IL-8 correlates with the variation of serum creatinine

concentration and makes the difference between patients who received kidneys from living and deceased donors. As a result, IL-8 is a early predictor of the functionality of allografts, after renal transplant (Borst et al 2015).

The increase of IL-8 in serum has been associated with acute kidney injury in children undergoing cardiac surgery (Tashiro et al 2002). High urinary levels of IL-8 were identified in patients who developed acute renal injury after orthotopic liver transplantation (Borst et al 2015). Urinary levels of IL-8 / CXCL8 were elevated in AKI (Tashiro et al 2002, Sirota et al 2013, Borst et al 2015). IL-8 shows different profiles in kidney damage caused by hypoxia, ischemia, reperfusion. Tubular damage leads to activation of the innate immune system, IL-8 overexpression, apoptosis (peritubular capillary loss, loss glomerular, tubular atrophy), necrosis, interstitial fibrosis (nephron loss). Modulation of IL-8 expression can be achieved by various pharmacological strategies: CCL8/CXCR1/2 neutralizing antibodies, CXCR1/2 antagonists, inhibitors of signal transduction pathway (NFKb, PI3K, MAPK) and RNA (Tashiro et al 2002, Borst et al 2015, Sirota et al 2013).

Conclusions

Interleukin-8 plays an important role in the development of renal response to various internal and external insults, by recruiting neutrophils, basophils and T lymphocytes in the affected areas of the kidney. Dynamics of interleukine synthesis might be an early predictor of renal failure. The modulation of interleukin 8 could reduce or prevent the development of end stage renal disease.

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Authors

Corina D. Ene, Department of Nephrology, "Dr. Carol Davila" Nephrology Clinical Hospital, 4 Calea Grivitei, 010701, Bucharest, Romania, EU, email: koranik85@yahoo.com

Amalia E. Anghel, Department of Dermatology, SKINMED CLINIC, 8 Splaiul Unirii, 40032, Bucharest, Romania, EU, email: amalia.anghel@yahoo.com

Monica Neagu, Department of Immunology "Victor Babes" National Institute of Pathology, 99-101 Splaiul Independentei, 050096, Bucharest, Romania, EU, email:neagu.monica@gmail.com

Ilinca Nicolae, Research Department in Dermatology, „Dr. Victor Babeş" Clinical Hospital, 281 Mihai Bravu Str., 030303, Bucharest, Romania, EU, email: drnicolaei@yahoo.ro

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