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Invited critical review

Diabetic nephropathy: Traditional to proteomic markers

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ABSTRACT

Diabetic nephropathy (DN) is one of the major microvascular complications of diabetes and it is defined as a rise in the urinary albumin excretion (UAE) rate and abnormal renal function. Currently, changes in albuminuria are considered a hallmark of onset or progression of DN. However, some patients with diabetes have advanced renal pathological changes and progressive kidney function decline even if urinary albumin levels are in the normal range, indicating that albuminuria is not the perfect marker for the early detection of DN. The present article provides an overview of the literature reporting some relevant biomarkers that have been found to be associated with DN and that potentially may be used to predict the onset and/or monitor the progression of nephropathy. In particular, biomarkers of renal damage, inflammation, and oxidative stress may be useful tools for detection at an early stage or prediction of DN. Proteomic-based biomarker discovery represents a novel strategy to improve diagnosis, prognosis and treatment of DN; however, proteomics-based approaches are not yet available in most of the clinical chemistry laboratories. The use of a panel with a combination of biomarkers instead of urinary albumin alone seems to be an interesting approach for early detection of DN, including markers of glomerular damage (e.g., albumin), tubular damage (e.g., NAG and KIM-1), inflammation (e.g., TNF-α) and oxidative stress (e.g., 8-OHdG) because these mechanisms contribute to the development and outcomes of this disease.

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Abbreviations: 2-DE, Two-dimensional gel electrophoresis; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; A1 MG, α_1-Microglobulin; AGEs, Advanced glycation end products; AKI, Acute kidney injury; ALP, Alkaline phosphatase; CD40 L, CD40 ligand; CE-MS, Capillary electrophoresis coupled with mass spectrometry; CKD, Chronic kidney disease; CRP, C-reactive protein; CVD, Cardiovascular disease; DM, Diabetes mellitus; DN, Diabetic nephropathy; ESRD, End-stage renal disease; GFR, Glomerular filtration rate; GGT, γ-Glutamyltransferase; IL-1, Interleukine-1; IL-6, Interleukine-6; IL-8, Interleukine-8; INF-γ, Interferon-γ; KIM-1, Kidney injure molecule-1; L-FABP, Liver-type fatty acid binding protein; MCP-1, Monocyte chemoattractant protein-1; MS, Mass spectrometry; NADPH, Nicotinamide adenine dinucleotide phosphate; NAG, N-acetyl-β-D-glucosaminidase; NF-κB, Nuclear factor-kappa B; NGAL, Neutrophil gelatinase-associated lipocalin; NO, Nitric oxide; NOS, Nitric oxide synthase; RBP, Retinol-binding protein; ROS, Reactive oxygen species; sCD40 L, Soluble CD40 ligand; SELDI, Surface-enhanced laser desorption/ionization; TNF-α, Tumor necrosis factor-α; TOF, Time of flight; UAE, Urinary albumin excretion; β2 MG, β_2-Microglobulin.

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1. Introduction

Diabetic nephropathy (DN) is defined as a rise in urinary albumin excretion (UAE) rate, and abnormal renal function as recognized by as abnormal plasma creatinine level, glomerular filtration rate (GFR) or calculated creatinine clearance [1]. DN is considered one of the major microvascular complications of diabetes [2] and has become the most common single cause of end-stage renal disease (ESRD) in the United States and Europe. Approximately 20–30% of patients with type 1 or type 2 diabetes develop evidence of nephropathy, but in type 2 diabetes, a considerably smaller fraction of these patients progress to ESRD [3]. Currently, DN has considerable impact on society in the areas of public health and social economy. In this context, many scientists are involved in research to elucidate the pathogenesis of DN and the prevention, as well as, the cure of this disease [4].

The exact pathogenesis of DN is complex and not completely understood [5]. Several mechanisms contribute to the development and outcomes of DN, such as the interaction between hyperglycemia-induced metabolic and hemodynamic changes and genetic predisposition, which sets the stage for kidney injury [6]. Glucose and its metabolites subsequently activate protein kinase C, the polyol pathway and non-enzymatic glycation, and these pathways contribute to renal functional and structural changes [7]. Therefore, hyperglycemia plays a central role in a cascade of damaging effects mediated by cytokines and growth factors that produces oxidative stress, abnormal glycosylation, lipid peroxidation, and the production of further inflammatory elements [8]. Superimposed on these mechanisms are transient and/or long-term changes in blood pressure and hemodynamics [7]. The hemodynamic changes of glomerular hyper-perfusion and hyperfiltration become evident before the earliest measurable clinical signs of nephropathy [8].

The earliest renal manifestation of diabetes is glomerular hyperfiltration [5], which results from functional changes in the nephron at the level of the glomerulus. Subsequently, thickening of the glomerular basement membrane, glomerular hypertrophy and mesangial expansion take place [8] and lead to a decline in the GFR accompanied by albuminuria usually 5 or more years after the onset of diabetes mellitus (DM) [5]. Finally, overt albuminuria develops and the GFR continues to fall. Microalbuminuria, however, has a variable course. Its progression to macroalbuminuria (UAE > 300 mg per day) is unpredictable and it does not always lead to the development of nephropathy [9]. In addition, some patients with diabetes have advanced renal pathological changes and progressive kidney function decline even if urinary albumin levels are in the normal range, indicating that albuminuria is not the perfect marker for the early detection of DN [10]. Therefore, there is a need for a more sensitive and specific biomarker than urinary albumin. In this context, identifying the sensitive biomarkers that can predict the microalbuminuria or DN in the early stage of diabetes might provide not only meaningful information regarding early pathophysiology but also an earlier clinical approach to the diagnosis. Therefore, this article provides an overview of the literature available in the PubMed database that reports on biomarkers that can be measured in the urine, plasma or serum and are associated with DN. These biomarkers were divided into groups according to the major pathways in the development or progression of DN.

2. Diabetic nephropathy and biomarkers of glomerular damage

Renal damage in DN is characterized by changes in glomerular permeability and structure. The glomerular wall contains three layers: endothelial cells, basement membrane, and epithelial cells. Much of the selectivity of filtration occurs in the basement membrane where the barrier excludes proteins on the basis of both their size and their charge [11,12]. However, in DN the permeability barrier is damaged and leads to proteinuria [13] of plasma proteins, such as albumin and transferrin, that are normally not freely filtered through the glomerulus [10]. Structural changes in diabetic kidney disease include the accumulation of mesangial matrix and thickening of the basement membrane in the glomeruli [14], as well as renal tubular hypertrophy and associated basement membrane alterations in the tubulointerstitium with tubulointerstitial fibrosis [15]. These abnormalities are associated with the renal overproduction of extracellular matrix proteins, such as type IV collagen [16] and other proteins. Table 1 presents some clinical studies that have evaluated urinary biomarkers associated with glomerular damage in patients with diabetes.

2.1. Albumin

Albumin (molecular weight of 65 kDa) filtered in the glomeruli is considered to be a major source of urinary albumin. Filtration of albumin is followed by tubular reabsorption; the resulting albuminuria reflects the combined contribution of these two processes. Dysfunction of both of these processes may result in increased excretion of albumin. Both glomerular injury and tubular impairment have been implicated in the initial events that lead to proteinuria (urinary protein excretion above 0.5 g/24 h) [17]. Therefore, a change in the UAE is one the first asymptomatic clinical manifestations of microvascular damage in diabetes [20] and has been used to categorize DN into two stages: microalbuminuria (UAE > 20 μg/min and <200 μg/min or UAE > 30 mg/day and < 300 mg/day) and macroalbuminuria (UAE > 200 μg/min or UAE > 300 mg/day) [1].

Microalbuminuria, traditionally equated with incipient nephropathy, is a common feature of diabetes [18]. An increase in albuminuria should not be considered a risk factor for DN, but rather as evidence of early organ damage that is related to specific risk factors for this complication of diabetes [19]. A gradual increase in the UAE progressing to macroalbuminuria leads to overt proteinuria and a rapid worsening of renal function [20]. Therefore, microalbuminuria has been recognized as a predictor of progression to ESRD in type 2 [21–23] and in type 1 diabetic patients [24,25]. Furthermore, urinary albumin levels are an important prognostic indicator of cardiovascular and renal outcomes in diabetic patients [26–28]. Although the measurement of microalbuminuria has been the mainstay for the detection of early DN [29] for many years, some evidence indicates that some patients may develop DN in the absence of microalbuminuria [30,31]. In addition, structural