Chapter 3

Diabetic Nephropathy in Childhood: Predictive Tools and Preventive Strategies

Samuel N. Uwaezuoke, Ugo N. Chikani and
Ngozi R. Mbanefo

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.79331

Abstract

Diabetic nephropathy is the commonest microvascular complication in both types 1 and 2 diabetes mellitus. Disease pathogenesis is based on a multifactorial interaction between metabolic and hemodynamic factors. In response to hyperglycemia, which disrupts the body's metabolic milieu, a cascade of complex molecular events occur leading to glomerular hypertrophy, tubular inflammation, mesangial expansion, oxidative stress, and renal fibrosis. Beyond the conventional microalbuminuria, which can predict disease onset, novel biomarkers are now proving more reliable as predictive tools. While several reports show that glomerular and tubular biomarkers are more sensitive than microalbuminuria, tubular markers specifically constitute earlier predictors of the disease. Similarly, biomarkers of inflammation and oxidative stress have been demonstrated as dependable diagnostic tools. As an important cause of mortality from end-stage renal disease (ESRD), diabetic nephropathy constitutes an important challenge in diabetic care. Interestingly, strict glycemic control assessed by glycated hemoglobin (Hb A1 c) estimates, and antihypertensive therapy with angiotensin-converting enzyme inhibitors/angiotensin-receptor blockers (ACEI/ARB) ± calcium-channel blockers form the main strategies for preventing its onset and slowing down its progression. Other strategies include uric acid antagonist, and renin and endothelin inhibitors. This book chapter discusses these predictive tools and possible preventive strategies.

Keywords: diabetic nephropathy, type 1 diabetes mellitus, type 2 diabetes mellitus, biomarkers, predictive tools, preventive strategies

1. Introduction

Type 1 diabetes mellitus is the usual form of diabetes mellitus in children. However, type 2 diabetes mellitus is now observed among them as well, especially in adolescents: with obesity as a putative risk factor. Diabetic nephropathy is the commonest microvascular complication of the kidney in both types of diabetes mellitus [1]. It is a clinical syndrome characterized by persistent macroalbuminuria (or urine albumin excretion rate of ≥200 μg/min) recorded at least twice within a 3- to 6-month interval, progressive decline in glomerular filtration rate (GFR), and hypertension [2]. The presence of microalbuminuria (urine albumin excretion rate of ≤199 μg/min) in type 1 diabetes mellitus is not only strongly predictive of macroalbuminuria (overt diabetic nephropathy) and subsequent end-stage renal disease (ESRD) but also constitutes a risk factor for future cardiovascular disease [3]. Contrary to the previously-held view that diabetic nephropathy is rare in childhood [4], some reports indicate that the disease can actually occur in the pediatric age group [5–7]. While the development of diabetic nephropathy can occur over a period of 10–20 years, beginning with microalbuminuria and ending with ESRD [1], it is currently projected that the rate of progression from microalbuminuria to macroalbuminuria over a 5- to 10-year period is about 15–30%, and may increase to 45% in patients with up to 15 years of diabetes duration [8].

Although microalbuminuria has traditionally remained the gold-standard biomarker for predicting diabetic nephropathy, its draw-backs include the following. Firstly, not all microalbuminuric diabetics will end up with diabetic nephropathy and ESRD [9, 10]. Secondly, microalbuminuria can only occur in the presence of significant renal injury since it is preceded by the appearance of several tubular and glomerular biomarkers in urine [11]. Thirdly, many diabetic patients with diabetic nephropathy can revert to normoalbuminuria, and these patients can also present with a reduction in GFR without progressing from normo- to macroalbuminuria (the concept of 'non-albuminuric' diabetic nephropathy) [12]. Thus, in place of microalbuminuria, these novel biomarkers are now increasingly used as earlier predictors of the disease.

In this book chapter, the use of biomarkers as predictive tools of diabetic nephropathy, and the preventive strategies against its onset and progression to ESRD are discussed.

1.1. Pathogenesis of diabetic nephropathy: a synopsis

In response to hyperglycemia, which disrupts the body's metabolic milieu, a cascade of complex molecular events occur resulting in the key pathogenic components of diabetic nephropathy, namely glomerular hypertrophy, tubular inflammation, mesangial expansion, oxidative stress, and renal fibrosis; several activated pathways individually or collectively influence the onset and progression of this disease [13]. However, it is important to note that genetic predisposition also contributes to the development of diabetic nephropathy because only 30–40% of diabetic patients develop diabetic kidney disease irrespective of glycemic control [14, 15].

In fact, the pathogenesis of the disease is based on a multifactorial interaction between metabolic and hemodynamic factors [16]. Metabolic factors involve glucose-dependent pathways, such as advanced glycation end-products and their receptors while hemodynamic factors

consist of several vasoactive hormones, such as components of the renin–angiotensin system [16]. It is postulated that these metabolic and hemodynamic factors interact through common molecular and signaling pathways, such as protein kinase C leading to generation of reactive oxygen species. Presumably, these contributing factors result in pathological damage not only to the glomerulus, especially the podocytes, but also to the tubulo-interstitium. In other words, hyperglycemia induces vascular injury through complex overlapping pathways, comprising formation of advanced glycation end-products, activation of protein kinase C and generation of reactive oxygen species, which might play a key role in the initiation and progression of diabetic nephropathy [17].

Specifically, resident and nonresident cells of the kidney are stimulated by hyperglycemia to produce humoral mediators, cytokines, and growth factors which are implicated in the structural alterations such as increased deposition of extracellular matrix protein at the glomerulus, and functional alterations such as hyperpermeability of the glomerular basement membrane or shear stress [15]. Increased deposition of extracellular matrix protein results in basement membrane thickening and mesangial expansion while glomerular hyperpermeability leads to progressive albuminuria, which is one of the hallmarks of the disease (**Figure 1**).

1.2. Novel biomarkers as predictive tools for diabetic nephropathy

Novel biomarkers are now useful tools for predicting the onset and progression of diabetic nephropathy. Attempts have been made at classifying these biomarkers into major groups, although these groups overlap with one another. Representative groups include glomerular biomarkers; tubular biomarkers; biomarkers of inflammation; biomarkers of oxidative stress; and miscellaneous biomarkers [18]. Examples of each group of biomarkers include albumin, transferrin, laminin, immunoglobulin G, fibronectin, ceruloplasmin, type IV collagen, lipocalin-type prostaglandin synthase (L-PGDS), and glycosaminoglycans which make up the glomerular biomarkers; neutrophil gelatinase-associated lipocalin (NGAL), cystatin C, kidney molecule injury 1 (KIM-1), liver-type fatty acid binding protein (L-FABP),

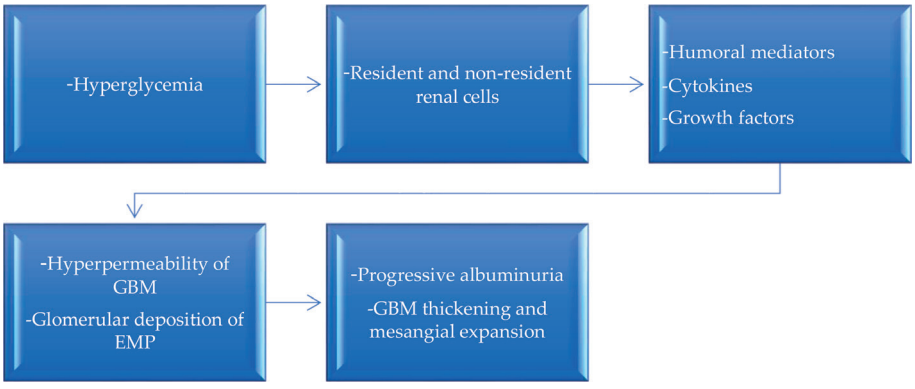

Figure 1. Pathogenetic pathway of albuminuria in diabetic nephropathy. GBM= glomerular basement membrane, EMP= extracellular matrix protein.

N-acetyl-β-D-glucosaminidase (NAG) and α-1-microglobulin constitute the tubular biomarkers; tumor necrosis factor-α (TNF-α), interleukin-1β, interleukin-8, interleukin-18, monocyte chemoattractant protein-1 (MCP-1), eotaxin, orosomucoid, RANTES and granulocyte colony-stimulating factor (G-CSF) represent the biomarkers of inflammation; 8-oxo-7,8-dihydro-2-deoxyguanosine (8oHdG) is a typical example of a biomarker of oxidative stress; nephrin, podocalyxin, advanced glycation end products (AGEs), vascular endothelial growth factor (VEGF), heart fatty-acid binding protein (H-FABP) and retinol-binding protein are listed as miscellaneous biomarkers. However, the overlap of the groups with one another shows that some miscellaneous biomarkers such as podocalyxin, nephrin and VEGF are also considered as glomerular biomarkers, whereas H-FABP and retinol-binding protein can be regarded as tubular biomarkers as well. Similarly, some biomarkers of inflammation such as TNF-α, the interleukins and MCP-1 are also identified as tubular biomarkers.

1.2.1. Glomerular biomarkers versus albuminuria

Several studies have provided evidence which indicate that novel glomerular biomarkers are more sensitive predictors of diabetic nephropathy compared to albuminuria, which has been considered as the conventional biomarker of glomerular injury (**Table 1**).

Firstly, urine transferrin, which is highly sensitive as a biomarker of diabetic nephropathy [19, 20], conversely has poor specificity for the disease because of confounders like primary

Glomerular biomarkers	Predictive ability for diabetic nephropathy
• Microalbuminuria	• Traditional biomarker predicting glomerular injury in T1DM and T2DM
• Urine transferrin	• High sensitivity • Poor specificity • More sensitive than microalbuminuria in predicting diabetic nephropathy in T2DM
• Urine ceruloplasmin	• High sensitivity • More sensitive than microalbuminuria
• Urine type IV collagen	• High sensitivity • More sensitive than microalbuminuria • Early predictor of diabetic nephropathy in T2DM • Can differentiate diabetic nephropathy from non-diabetic nephropathy
• Urine laminin	• Similar predictive ability with urine type IV collagen • Appears before microalbuminuria in T1DM
• Urine fibronectin	• Higher excretion in microalbuminuric than normoalbuminuric T2DM patients • Degradation products correlate with albuminuria

T1DM = type 1 diabetes mellitus, T2DM = type 2 diabetes mellitus.

Table 1. Glomerular biomarkers versus microalbuminuria as predictive tools for diabetic nephropathy.

glomerulonephritis and systemic diseases affecting the glomerulus, which also present with transferrinuria [21]. However, the biomarker is considered more sensitive than albuminuria. For instance, some authors have reported that, in the pre-albuminuric phase, transferrinuria was higher in diabetic subjects than in healthy controls [22, 23]. These findings underscore the superiority of urine transferrin over urine albumin excretion in the early prediction of diabetic nephropathy.

Secondly, urine ceruloplasmin is poorly filtered through the glomerular barrier because of its negative charge. Similar to urine transferrin, this biomarker can predict diabetic nephropathy earlier than albuminuria in patients with type 2 diabetes mellitus [24]. It has also been noted that urine ceruloplasmin together with urine transferrin, immunoglobulin G and orosomucoid are simultaneously elevated prior to the onset of microalbuminuria in type 2 diabetic patients [25].

Another remarkable glomerular biomarker is type IV collagen: a component of the glomerular basement membrane and mesangial matrix. Urine type IV collagen has been reported as a more sensitive marker of renal injury than urine albumin in patients with type 2 diabetes mellitus [26]. It can therefore serve an early predictor of diabetic nephropathy. Furthermore, urine type IV collagen can potentially differentiate diabetic nephropathy from non-diabetic nephropathy because studies show that type 2 diabetics with nephropathy have a significantly higher type IV collagen/albumin ratio in comparison with their non-diabetic cohorts with nephropathy [27, 28].

Laminin, a component of the glomerular basement membrane, is a biomarker with similar predictive properties as type IV collagen [29]. For instance, urine laminin can also discriminate between diabetic and non-diabetic nephropathy as one report showed that type 2 diabetics with nephropathy had significantly higher laminin/albumin ratio in comparison to patients with non-diabetic nephropathy [27]. More importantly, some investigators have documented a higher urine laminin excretion in pediatric patients with type 1 diabetes mellitus when juxtaposed with their healthy controls, even before the appearance of microalbuminuria [30].

Another glomerular biomarker worthy of mention is fibronectin. It is basically an intrinsic component of the glomerular extracellular matrix. Interestingly, urine fibronectin is reportedly higher in diabetic patients compared to their controls, with a significant difference noted only in macroalbuminuric patients [31, 32]. Further evidence supporting the predictive role of urine fibronectin in diabetic nephropathy include its higher excretion in type 2 diabetics with microalbuminuria than in those with normoalbuminuria [33], as well as the correlation of its degradation products with albuminuria [34].

1.2.2. Tubular biomarkers versus albuminuria

When compared to microalbuminuria and other novel glomerular biomarkers, tubular biomarkers are early predictors of diabetic nephropathy because tubulointerstitial lesions are known to occur much earlier in the course of the disease and may actually precede glomerular injury [35]. This characteristic has given this group of biomarkers an edge over glomerular biomarkers as earlier predictors of diabetic nephropathy.

For instance, studies on NGAL as a biomarker have revealed these interesting findings: presence of its elevated urine levels in diabetic patients with normoalbuminuria and its use

in evaluating tubular lesions in the disease [36], its occurrence before microalbuminuria in patients with type 1 diabetes mellitus [37, 38], as well as its role in predicting the progression of diabetic kidney disease in type 2 diabetics [39, 40]. Thus, NGAL may have a better predictive ability for diabetic nephropathy than microalbuminuria.

Secondly, α-1-microglobulin has been identified as an affordable tubular biomarker for the early prediction of diabetic nephropathy [41]. Although α-1-microglobulin undergoes glomerular filtration, its tendency for proximal tubular reabsorption ensures its increased urine excretion in tubular dysfunction because of impaired reabsorption. In type 2 diabetics with normoalbuminuria, some authors were able to demonstrate increased urine levels of this biomarker because, as previously mentioned, tubular injury precedes the onset of microalbuminuria in diabetic nephropathy [42].

On the other hand, there appears to be divergent findings on the usefulness of NAG as a biomarker in the evaluation of diabetic nephropathy. Whereas some authors reported its clinical insignificance as an early biomarker of the disease [43], other reports conversely show that it remains a sensitive tubular biomarker for early detection of renal lesions in patients with both type 1 and 2 diabetes mellitus, as its urinary excretion can occur before microalbuminuria [44–46].

Furthermore, increased urine L-FABP levels have been observed in type 1 diabetics with normoalbuminuria, and is capable of not only predicting the onset of microalbuminuria but also its progression to macroalbuminuria [47]. Type 2 diabetics with normoalbuminuria also present with increased urine levels of this biomarker, which underscores its predictive ability for the onset and progression of diabetic nephropathy [48, 49].

Finally, some authors have reported the predictive ability of cystatin C for the progression of diabetic nephropathy [50], as well as its role in the evaluation of early nephropathy in type 2 diabetes mellitus [51], while increased urine KIM-1 levels have been observed more in diabetic patients with microalbuminuria than in those with normoalbuminuria [52]. This again suggests that tubular injury occurs early in the pathophysiologic trajectory of diabetic nephropathy.

1.2.3. Biomarkers of inflammation

Interestingly, the role of TNF-α (a pro-inflammatory cytokine) in the development of diabetic nephropathy is well documented [53–55]. The predictive ability of this cytokine as a biomarker for the disease is predicated upon the observations of its increased urine levels in diabetic patients who have albuminuria, as well as the significant rise of its urinary excretion during progression of diabetic nephropathy [54]. In fact, elevated urine TNF-α excretion and TNF-α levels in renal interstitial fluid have been noted to precede a significantly raised albuminuria in experimental diabetic rats [56]. This is corroborated by other studies which report a direct association between albuminuria and serum TNF-α in diabetic patients with normal renal function and microalbuminuria on one hand, as well as in those with macroalbuminuria and ESRD on the other hand [57, 58]. Similarly, other biomarkers of inflammation such as IL-8, MCP-1, G-CSF, eotaxin, and RANTES are reportedly higher in microalbuminuric type 2 diabetics than in their normoalbuminuric counterparts [59]. In type 1 diabetes mellitus, urine

orosomucoid was also noted to be raised in normoalbuminuric patients when compared to non-diabetic controls: with increasing levels reported in microalbuminuric and macroalbuminuric diabetic patients [60].

1.2.4. Biomarkers of oxidative stress

8oHdG is the prototype of this group of biomarkers. This molecule is actually a product of oxidative injury to DNA which appears unchanged in the urine. It may represent a dependable clinical biomarker for predicting the onset of diabetic kidney disease because some authors have observed that patients whose nephropathy progressed significantly had higher urinary excretion of this biomarker than those who had lower or moderate urinary excretion [61].

2. Preventive strategies for diabetic nephropathy

Generally, strict glycemic control and painstaking control of hypertension have significant impact on prevention and progression of diabetic nephropathy; a finding noted in adult patients with type 2 diabetes mellitus [62]. In children and adolescents, who are predominantly type 1 diabetics, these measures should be equally applicable as preventive strategies. More importantly, strategies that target the modifiable risk factors for diabetic nephropathy may result in better outcomes. Some of the modifiable and non-modifiable risk factors include poor glycemic control, pubertal growth spurt, hypertension, hyperlipidemia, smoking habits, albuminuria, obesity and genetic predisposition [6, 63–66]. For instance, in type 1 diabetes mellitus, strict glycemic control is the major preventive strategy for diabetic nephropathy, whereas in type 2 diabetes mellitus, modulation of hypertension, dyslipidemia, and obesity (modifiable risk factors) constitute important strategies [63]. Notably, the current recommendations/strategies for preventing the onset and retarding the progression of diabetic nephropathy are essentially evidence-based, and are summarized in **Table 2**.

Firstly, the measurement of hemoglobin A1 c (Hb A1 c) has been adopted as a reliable tool for assessing glycemic control. It is a marker for average glycemic levels over the previous 3 months. The goal of glycemic control is basically to maintain Hb A1 c level of less than 7% while avoiding hypoglycemia. In fact, strict glycemic control reduces the risk of microvascular complications in both type 1 and type 2 diabetes mellitus [67, 68]. Reports show that diabetic nephropathy rarely occurs when the Hb A1 c level is consistently less than 7.5–8.0% [2, 65]. Notably, results of two major clinical trials have buttressed the effectiveness of tight glycemic control as a preventive strategy [68, 69]. In one of the studies conducted on type 2 diabetics, there was a 34% reduction in the risk of microalbuminuria [68], while in type 1 diabetics, the incidence of microalbuminuria was decreased by 39% in the primary prevention group with a 54% reduction in its progression to macroalbuminuria in the secondary prevention group [69].

Secondly, the control of hypertension is renoprotective in type 1 and type 2 diabetes mellitus, as it reduces albuminuria and delays the onset of nephropathy [70]. The renin-angiotensin system constitutes the target of the most effective strategy for blood-pressure control and for reducing the pathophysiologic abnormalities which lead to albuminuria. Specifically, it

Preventive strategies	Mechanism of action
• Strict or tight glycemic control	• Reduces risk of microalbuminuria • Reduces progression of microalbuminuria to macroalbuminuria
• Antihypertensive therapy with ACEI/ARB ± calcium-channel blockers	• Reduces albuminuria and delays the onset of diabetic nephropathy • Prevents progression of diabetic nephropathy in microalbuminuric patients
• Vitamin D	• Ameliorates nephropathy by reducing albuminuria
• Allopurinol (uric acid antagonist)	• Reduces urinary TGF-β in diabetic nephropathy
• Aliskiren (renin inhibitor)	• Reduces albuminuria and serves as an antihypertensive in T2DM
• Atrasentan (endothelial inhibitor)	• Reduces residual albuminuria in type 2 diabetic nephropathy
• Dietary protein/phosphate restriction	• Retards progression of diabetic nephropathy

ACEI = angiotensin-converting enzyme inhibitor, ARB = angiotensin-receptor blocker, TGF-β = transforming growth factor-beta, T2DM = type 2 diabetes mellitus.

Table 2. Preventive strategies for the onset and progression of diabetic nephropathy: an evidence-based summary.

does appear that blood pressure elevation directly correlates with the degree of albuminuria in type 2 diabetes mellitus [71]. Thus, antihypertensive therapy, especially with angiotensin converting enzyme inhibitors (ACEI) or in combination with other antihypertensive agents, is an effective strategy for preventing the progression of diabetic nephropathy [71]. While either ACEI or angiotensin-II type 1 receptor blockers (ARB) is recommended in patients with microalbuminuria and type 2 diabetes mellitus [72], none of them is recommended in normotensive, normoalbuminuric diabetics for the primary prevention of diabetic nephropathy. Apart from ACEI which are regarded as first-choice treatment for hypertension in diabetics, other effective alternative antihypertensive drugs include calcium-channel blockers: either alone [73], or in combination with ACEI [74].

Thirdly, the use of novel treatments has been tried as strategies for preventing the onset and progression of diabetic nephropathy. These therapeutic agents include the following: vitamin D, allopurinol (uric acid antagonist), aliskiren (renin inhibitor), and atrasentan (endothelin antagonist or l inhibitor) [75–78]. In experimental murine models, vitamin D is thought to activate an antioxidant pathway and ameliorate diabetic nephropathy by reducing albuminuria [75]. Furthermore, vitamin D deficiency is known to be a common disorder in diabetics and probably constitutes a risk factor for ischemic heart disease, worsening of chronic kidney disease and diabetic nephropathy [79]. Vitamin D metabolites may inhibit the renin-angiotensin system and exert renoprotective effect by preventing glomerulosclerosis and reducing albuminuria in diabetic nephropathy; in addition, administering the vitamin is reported to have resulted in a reduction in insulin resistance and blood pressure [80, 81]. In fact, findings from several studies support the beneficial effect of dietary or supplemental vitamin D in retarding the progression of diabetic nephropathy through reduction of albuminuria [82–85]. Another

novel therapeutic agent- allopurinol- was reported to reduce albuminuria in patients with type 2 diabetes [86]. Evidence point to a greater serum uric acid level in patients with diabetic nephropathy than in normal subjects [87]. Uric acid specifically plays a mediatory role in the pathogenesis of diabetic nephropathy, as it leads to endothelial dysfunction, increased activity of the renin-angiotensin-aldosterone system (RAAS), and induction of inflammatory cascades, as well as profibrotic cytokine activation which synergistically contribute to progression of microvascular disease and extracellular matrix deposition; these events result in kidney injury in diabetic nephropathy [87]. Obviously, hyperuricemia may thus impair glomerular function, and promote albuminuria. Interestingly, allopurinol has also been observed to reduce urinary transforming growth factor-beta (TGF-β) in diabetic nephropathy, apart from improving endothelial dysfunction [76]. Furthermore, the direct renin inhibitor-aliskiren-reportedly reduces albuminuria and also serves as an antihypertensive in type 2 diabetes mellitus either as a monotherapy [77], or in combination with an angiotensin receptor blocker (ARB) [88]. Endothelin-1 is regarded as the most potent vasoconstrictor which plays a role in renal regulation of fluid and salt balance. More importantly, excessive renal production of endothelin-1 is associated with proteinuria and tubulointerstitial injury [89]. Although trials are still going on to validate the endothelin antagonist or inhibitor (atrasentan), this novel agent has been shown to lower residual albuminuria in type 2 diabetic nephropathy besides its antihypertensive action [78].

Finally, dietary modification has proved to be an effective tool in retarding the progression of diabetic nephropathy. In diabetics, high dietary protein has renal hemodynamic effects which comprise elevated GFR, hyperfiltration, and raised intraglomerular pressure which are probably accentuated by poor glycemic control [79]. Thus, it has been observed that dietary protein restriction slows down the deterioration of kidney function in type 1 and type 2 diabetes mellitus [90, 91]. For instance, a prospective study which compared type 1 diabetics placed on dietary protein/phosphate restriction with those on unrestricted diet revealed a differential reduction in GFR, as the GFR in the former group was progressively reduced by only 0.26 ml/min/month compared with 1.01 ml/min/month seen in the latter group [92].

3. Conclusion

Both glomerular and tubular biomarkers are sensitive predictors of diabetic nephropathy when compared to microalbuminuria, with tubular biomarkers serving as earlier predictors of the disease. Other groups of novel biomarkers have also been shown to be effective predictive tools. As the major microvascular complication of both type 1 and type 2 diabetes mellitus and an important cause of ESRD-related mortality, the onset and progression of diabetic nephropathy therefore constitute an important challenge in diabetic care. Fortunately, strict glycemic control and antihypertensive therapy with ACEI/ARB ± calcium-channel blockers form the main strategies for preventing the onset and slowing the progression of diabetic nephropathy. Other novel treatment options include dietary protein restriction, use of vitamin D, uric acid antagonist, as well as renin and endothelin inhibitors. As newer strategies emerge, prospects for better outcomes in diabetic nephropathy are getting brighter.

Acknowledgements

The authors acknowledge the useful information obtained from the cited paper- SN Uwaezuoke (2015) Prevention of Diabetic Nephropathy in Children and Adolescents: How Effective are the Current Strategies? Int J Diabetol Vasc Dis Res, S5:001, 1-5.

Conflict of interest

The authors declare no conflict of interests in this work.

Author details

Samuel N. Uwaezuoke[1,2]*, Ugo N. Chikani[1,2] and Ngozi R. Mbanefo[2]

*Address all correspondence to: samuel.uwaezuoke@unn.edu.ng

1 College of Medicine, University of Nigeria/University of Nigeria Teaching Hospital, Enugu, Ituku-Ozalla, Nigeria

2 University of Nigeria Teaching Hospital, Enugu, Ituku-Ozalla, Nigeria

References

[1] Koulouridis E. Diabetic nephropathy in children and adolescents and its consequences in adults. Journal of Pediatric Endocrinology and Metabolism. 2001;**14**:S1367-S1377. PMID: 11964036

[2] Deferrari G, Repetto M, Calvi C, Ciabattoni M, Rossi C, Robaudo C. Diabetic nephropathy: From micro- to microalbuminuria. Nephrology, Dialysis and Transplantation. 1998;**13**:S11-S15. PMID: 9870419

[3] McKenna K, Thompson C. Microalbuminuria: A marker to increased renal and cardiovascular risk in diabetes mellitus. Scottish Medical Journal. 1997;**42**:99-104

[4] Danne T, Kordonouri O, Hövener G, Weber B. Diabetic angiopathy in children. Dia betic Medicine. 1997;**14**:1012-1025. DOI: 10.1002/(SICI)1096-9136(199712)14:12<1012:AID-DIA479>3.0.CO;2-H

[5] DeClue TJ, Campos A. Diabetic nephropathy in a prepubertal diabetic female. Journal of Pediatric Endocrinology. 1994;**7**:43-46. PMID: 8186823

[6] Maghribi H, Abu-Odeh A. Early diabetic nephropathy in a pediatric patient. Journal of the Royal Medical Services. 2006;**13**:51-53

[7] Francis J, Rose SJ, Raafat F, Milford DV. Early onset of diabetic nephropathy. Archives of diseases in childhood. 1997;**77**:524-525. PMCID: PMC1717409

[8] Caramori ML, Fioretto P, Mauer M. Enhancing the predictive value of urinary albumin for diabetic nephropathy. Journal of the American Society of Nephrology. 2006;**17**:339-352. DOI: 10.1681/ASN.2005101075

[9] Remuzzi G, Schieppati A, Ruggenenti P. Nephropathy in patients with type 2 diabetes. New England Journal of Medicine. 2002;**346**:1145-1115. DOI: 10.1056/NEJMcp011773

[10] Adler AI, Stevens RJ, Manley SE, Bilous RW, Cull CA, Holman RR; UKPDS GROUP. Development and progression of nephropathy in type 2 diabetes: The United Kingdom prospective diabetes study (UKPDS 64). Kidney International. 2003;**63**:225-232. DOI: 10.1046/j.1523-1755.2003.00712.x

[11] Matheson A, Willcox MDP, Flanagan J, Walsh BJ. Urinary biomarkers involved in type 2 diabetes: A review. Diabetes Metabolism Research and Reviews. 2010;**26**:150-171. DOI: 10.1002/dmrr.1068

[12] Currie G, McKay G, Delles C. Biomarkers in diabetic nephropathy: Present and future. World Journal of Diabetes. 2014;**5**:763-776. DOI: 10.4239/wjd.v5.i6.763

[13] Arora MK, Singh UK. Molecular mechanisms in the pathogenesis of diabetic nephropathy: An update. Vascular Pharmacology. 2013;**58**:259-271. DOI: 10.1016/j.vph.2013.01.001

[14] The Diabetes Control and Complications (DCCT) Research Group: Effect of intensive therapy on the development and progression of diabetic nephropathy in the diabetes control and complications trial. Kidney International. 1995;**47**:1703-1720. PMID: 7643540

[15] Schena FP, Gesualdo L. Pathogenetic mechanisms of diabetic nephropathy. Journal of American Society Nephrology. 2005;**16**:S30-S33. PMID: 15938030

[16] Cao Z, Cooper ME. Pathogenesis of diabetic nephropathy. Journal of Diabetes Investi gation. 2011;**2**:243-247. DOI: 10.1111/j.2040-1124.2011.00131.x

[17] Forbes JM, Coughlan MT, Cooper ME. Oxidative stress as a major culprit in kidney disease in diabetes. Diabetes. 2008;**57**:1446-1454. DOI: 10.2337/db08-0057

[18] Uwaezuoke SN. The role of novel biomarkers in predicting diabetic nephropathy: A review. International Journal of Nephrology and Renovascular Disease. 2017;**10**:221-231. DOI: 10.2147/IJNRD.S143186

[19] Bernard AM, Amor AA, Goemaere-Vanneste J, et al. Microtransferrinuria is a more sensitive indicator of early glomerular damage in diabetes than microalbuminuria. Clinical Chemistry. 1988;**34**:1920-1921. PMID: 3416456

[20] O'Donnell MJ, Martin P, Cavan D, et al. Increased urinary transferrin excretion in exercising normoalbuminuric insulin-dependent diabetic patients. Annals of Clinical Biochemistry. 1991;**28**:456-460. DOI: 10.1177/000456329102800506

[21] Yaqoob M, McClelland P, Patrick AW, Stevenson A, Mason H, Bell GM. Tubular damage in microalbuminuric patients with primary glomerulonephritis and diabetic nephropathy. Renal Failure. 1995;**17**:43-49. PMID: 7770643

[22] Martin P, Walton C, Chapman C, Bodansky HJ, Stickland MH. Increased urinary excretion of transferrin in children with type 1 diabetes mellitus. Diabetic Medicine. 1990;**7**: 35-40. PMID: 1688749

[23] Cheung CK, Cockram CS, Yeung VT, Swaminathan R. Urinary excretion of transferrin by non-insulin-dependent diabetics: A marker for early complications? Clinical Chemistry. 1989;**35**:1672-1674. PMID: 2758634

[24] Wang C, Li C, Gong W, Lou T. New urinary biomarkers for diabetic kidney disease. Biomarker Research. 2013;**1**:9. DOI: 10.1186/2050-7771-1-9

[25] Narita T, Sasaki H, Hosoba M, et al. Parallel increase in urinary excretion rates of immunoglobulin G, ceruloplasmin, transferrin, and orosomucoid in normoalbuminuric type 2 diabetic patients. Diabetes Care. 2004;**27**:1176-1181. PMID: 15111541

[26] Kotajima N, Kimura T, Kanda T, et al. Type IV collagen as an early marker for diabetic nephropathy in non- insulin-dependent diabetes mellitus. The Journal of Diabetic Complications. 2000;**14**:13-17. PMID: 10925061

[27] Banu N, Hara H, Okamura M, Egusa G, Yamakido M. Urinary excretion of type IV collagen and laminin in the evaluation of nephropathy in NIDDM: Comparison with urinary albumin and markers of tubular dysfunction and/or damage. Diabetes Research and Clinical Practice. 1995;**29**:57-67. DOI: 10.1016/0168-8227(95)01119-X

[28] Kado S, Aoki A, Wada S, et al. Urinary type IV collagen as a marker for early diabetic nephropathy. Diabetes Research and Clinical Practice. 1996;**31**:103-108. DOI: 10.1016/0168-8227(96)01210-7

[29] Haiyashi Y, Makino H, Ota Z. Serum and urinary concentrations of type IV collagen and laminin as a marker of microangiopathy in diabetes. Diabetic Medicine. 1992;**9**:366-370. PMID: 1600709

[30] Miyake H, Nagashima K, Yagi H, Onigata K. Urinary laminin P1 as an index of glycemic control in children with insulin-dependent diabetes mellitus. Diabetes Research. 1992;**23**:131-138. PMID: 7536137

[31] Fagerudd JA, Groop PH, Honkanen E, Teppo AM, Grönhagen-Riska C. Urinary excretion of TGF-β1, PDGF-BB and fibronectin in insulin-dependent diabetes mellitus patients. Kidney International. 1997;**51**:S195-S197

[32] Kanauchi M, Nishioka H, Hashimoto T, Dohi K. Diagnostic significance of urinary transferrin in diabetic nephropathy. Nihon Jinzo Gakkai Shi. 1995;**37**:649-654. PMID: 8583702

[33] Takahashi M. Increased urinary fibronectin excretion in type II diabetic patients with microalbuminuria. Nihon Jinzo Gakkai Shi. 1995;**37**:336-342. PMID: 8583702

[34] Kuboki K, Tada H, Shin K, Oshima Y, Isogai S. Relationship between urinary excretion of fibronectin degradation products and proteinuria in diabetic patients, and their

suppression after continuous subcutaneous heparin infusion. Diabetes Research and Clinical Practice. 1993;**21**:61-66. PMID: 8253024

[35] Mise K, Hoshino J, Ueno T, et al. Prognostic value of tubulointerstitial lesions, urinary N-acetylβ-d-glucosaminidase, and urinary β2-microglobulin in patients with type 2 diabetes and biopsy-proven diabetic nephropathy. Clinical Journal of the American Society of Nephrology. 2016;**11**:593-601. DOI: 10.2215/CJN.04980515

[36] Lacquaniti A, Donato V, Pintaudi B, et al. "Normoalbuminuric" diabetic nephropathy: Tubular damage and NGAL. Acta Diabetologica. 2013;**50**:935-942. DOI: 10.1007/s00592-013-0485-7

[37] Bolignano D, Lacquaniti A, Coppolino G, et al. Neutrophil gelatinase-associated lipocalin as an early biomarker of nephropathy in diabetic patients. Kidney and Blood Pressure Research. 2009;**32**:91-98. DOI: 10.1159/000209379

[38] Yürük Yıldırım Z, Nayır A, Yılmaz A, Gedikbası A, Bundak R. Neutrophil gelatinase-associated lipocalin as an early sign of diabetic kidney injury in children. Journal of Clinical Research and Pediatric Endocrinology. 2015;**7**:274-279. DOI: 10.4274/jcrpe.2002

[39] Yang YH, He XJ, Chen SR, Wang L, Li EM, Xu LY. Changes of serum and urine neutrophil gelatinase-associated lipocalin in type-2 diabetic patients with nephropathy: One year observational follow-up study. Endocrine. 2009;**36**:45-51. DOI: 10.1007/s12020-009-9187-x

[40] de Carvalho JA, Tatsch E, Hausen BS, et al. Urinary kidney injury molecule-1 and neutrophil gelatinase-associated lipocalin as indicators of tubular damage in normoalbuminuric patients with type 2 diabetes. Clinical Biochemistry. 2016;**49**:232-236. DOI: 10.1016/j.clinbiochem.2015.10.016

[41] Shore N, Khurshid R, Saleem M. Alpha-1-microglobulin: A marker for early detection of tubular disorders in diabetic nephropathy. Journal of Ayub Medical College Abbottabad. 2010;**22**:53-55

[42] Hong CY, Hughes K, Chia KS, Ng V, Ling SL. Urinary alpha-1-microglobulin as a marker of nephropathy in type 2 diabetic Asian subjects in Singapore. Diabetes Care. 2003;**26**:338-342. PMID: 12547859

[43] Ambade V, Singh P, Somani BL, Basannar D. Urinary N-acetyl β-glucosaminidase and γ-glutamyl transferase as early markers of diabetic nephropathy. Indian Journal of Clinical Biochemistry. 2006;**2**:142-148. DOI: 10.1007/BF02912930

[44] Patel DN, Kalia K. Efficacy of urinary N-acetyl-β-D glucosaminidase to evaluate early renal tubular damage as a consequence of type 2 diabetes mellitus: A cross-sectional study. International Journal of Diabetes in Developing Countries. 2015;**35**:449-457. DOI: 10.1007/s13410-015-0404-2

[45] Assal HS, Tawfeek S, Rasheld EA, El-Lebedy D, Thabet EH. Serum cystatin C and tubular urinary enzymes as biomarkers: A renal dysfunction in type 2 diabetes mellitus. Clinical Medicine Insights: Endocrinology and Diabetes. 2013;**6**:7-13. DOI: 10.4137/CMED.S12633

[46] Jones AP, Lock S, Griffiths KD. Urinary N-acetyl-β-glucosaminidase activity in type I diabetes mellitus. Annals of Clinical Biochemistry. 1995;**32**:58-62. DOI: 10.1177/000456329503200104

[47] Nielsen SE, Sugaya T, Hovind P, et al. Urinary liver-type fatty acid-binding protein predicts progression to nephropathy in type 1 diabetic patients. Diabetes Care. 2010;**33**:1320-1324. DOI: 10.2337/dc09-2242

[48] Kamijo-Ikemori A, Sugaya T, Yasuda T, et al. Clinical significance of urinary liver-type fatty acid-binding protein in diabetic nephropathy of type 2 diabetic patients. Diabetes Care. 2011;**34**:691-696. DOI: 10.2337/dc10-1392

[49] Panduru NM, Forsblom C, Saraheimo M, et al., Finn Diane Study Group. Urinary liver-type fatty acid binding protein and progression of diabetic nephropathy in type 1 diabetes. Diabetes Care. 2013;**36**:2077-2083. DOI: 10.2337/dc12-1868

[50] Kim SS, Song SH, Kim IJ, et al. Urinary cystatin C and tubular proteinuria predict progression of diabetic nephropathy. Diabetes Care. 2013;**36**:656-661. DOI: 10.2337/dc12-0849

[51] Jeon YK, Kim MR, Huh JE, et al. Cystatin C as an early biomarker of nephropathy in patients with type 2 diabetes. Journal of Korean Medical Sciences. 2011;**26**:258-263. DOI: 10.3346/jkms.2011.26.2.258

[52] Petrica L, Vlad A, Gluhovschi G, et al. Proximal tubule dysfunction is associated with podocyte damage biomarkers nephrin and vascular endothelial growth factor in type 2 diabetes mellitus patients: A cross-sectional study. PLoS One. 2014;**9**:e112538. DOI: 10.1371/journal.pone.0112538

[53] Elmarakby AA, Sullivan JC. Relationship between oxidative stress and inflammatory cytokines in diabetic nephropathy. Cardiovascular Therapeutics. 2012;**30**:49-59. DOI: 10.1111/j.1755-5922.2010.00218.x

[54] Navarro JF, Mora C. Diabetes, inflammation, proinflammatory cytokines, and diabetic nephropathy. The Scientific World Journal. 2006;**6**:908-917. DOI: 10.1100/tsw.2006.179

[55] Hasegawa G, Nakano K, Sawada M, et al. Possible role of tumor necrosis factor and interleukin-1 in the development of diabetic nephropathy. Kidney International. 1991;**40**:1007-1012. PMID: 1762301

[56] Kalantarinia K, Awas AS, Siragy HM. Urinary and renal interstitial concentrations of TNF-α increase prior to the rise in albuminuria in diabetic rats. Kidney International. 2003;**64**:1208-1213. DOI: 10.1046/j.1523-1755.2003.00237.x

[57] Navarro JF, Mora C, Maca M, Garca J. Inflammatory parameters are independently associated with urinary albumin excretion in type 2 diabetes mellitus. American Journal of Kidney Diseases. 2003;**42**:53-61. PMID: 12830456

[58] Navarro JF, Mora C, Rivero A, et al. Urinary protein excretion and serum tumor necrosis factor in diabetic patients with advanced renal failure: Effects of pentoxifylline administration. American Journal of Kidney Diseases. 1999;**33**:458-463

[59] Liu J, Zhao Z, Willcox MD, Xu B, Shi B. Multiplex bead analysis of urinary cytokines of type 2 diabetic patients with normo- and microalbuminuria. Journal of Immunoassay and Immunochemistry. 2010;**31**:279-289. DOI: 10.1080/15321819.2010.524860

[60] Jiang H, Guan G, Zhang R, et al. Increased urinary excretion of orosomucoid is a risk predictor of diabetic nephropathy. Nephrology. 2009;**14**:332-337. DOI: 10.1111/j.1440-1797.2008.01053.x

[61] Hinokio Y, Suzuki S, Hirai M, Suzuki C, Suzuki M, Toyota T. Urinary excretion of 8-oxo-7,8-dihydro-2-deoxyguanosine as a predictor of the development of diabetic nephropathy. Diabetologia. 2002;**45**:877-882. DOI: 10.1007/s00125-002-0831-8

[62] O'Connor PJ, Spann SJ, Woolf SH. Care of adults with type 2 diabetes mellitus: A review of the evidence. Journal of Family Practice. 1988;**47**:S13-S22. PMID: 9834750

[63] Molnár M, Wittmann I, Nagy J. Prevalence, course and risk factors of diabetic nephropathy in type-2 diabetes mellitus. Medical Science Monitor. 2000;**6**:929-936. PMID: 11208433

[64] Bogdanović R. Diabetic nephropathy in children. Nephrology, Dialysis and Transplanta tion.2001;**16**:S120-S122. PMID: 11568268

[65] Di Landro D, Catalano C, Lambertini D, et al. The effect of metabolic control on develop ment and progression of diabetic nephropathy. Nephrology, Dialysis and Transplanta tion. 1988;**13**:S35-S43. PMID: 9870424

[66] Sochett E, Daneman D. Early diabetes-related complication in children and adolescents with type 1 diabetes. Implications for screening and intervention. Endocrinology and Metabolism Clinics of North America. 1999;**28**:865-882. PMID: 10609124

[67] Molitch ME. The relationship between glucose control and the development of diabetic nephropathy in type I diabetes. Seminars in Nephrology. 1997;**17**:101-113. PMID: 9148376

[68] UK Prospective Diabetes Study Group. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). Lancet. 1998;**352**:837-853. PMID: 9742976

[69] The Diabetes Control and Complications Trial Research Group. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. New England Journal of Medicine. 1993;**329**:977-986. DOI: 10.1056/NEJM199309303291401

[70] Parving HH. Is antihypertensive treatment the same for NIDDM and IDDM patients? Diabetes Research and Clinical Practice. 1998;**39**:S43-S47. PMID: 9649959

[71] Mogensen CE. Drug treatment for hypertensive patients in special situations: Diabetes and hypertension. Clinical and Experimental Hypertension. 1999;**21**:895-906. PMID: 10423111

[72] Jerums G, MacIsaac RJ. Treatment of microalbuminuria in patients with type 2 diabetes mellitus. Treatment in Endocrinology. 2002;**1**:163-173. PMID: 15799209

[73] Rossing P. Promotion, prediction and prevention of progression of nephropathy in type 1 diabetes mellitus. Diabetic Medicine. 1998;**15**:900-919. DOI: 10.1002/(SICI)1096-9136(1998110)15:11<900::AID-DIA709>3.0.CO;2-0

[74] Bakris GL, Weir MR, DeQuattro V, McMahon FG. Effects of an ACE inhibitor/calcium antagonist combination on proteinuria in diabetic nephropathy. Kidney International. 1998;**54**:1283-1289. DOI: 10.1046/j.1523-1755.1998.00083.x

[75] Nakai K, Fujii H, Kono K, et al. Vitamin D activates the Nrf2-Keap1 antioxidant pathway and ameliorates nephropathy in diabetic rats. American Journal of Hypertension. 2014;**27**:586-595. DOI: 10.1093/ajh/hpt160

[76] Talaat KM. el-sheikh AR. The effect of mild hyperuricemia on urinary transforming growth factor beta and the progression of chronic kidney disease. American Journal of Nephrology. 2007;**27**:435-440. DOI: 10.1159/000105142

[77] Persson F, Rossing P, Schjoedt KJ, et al. Time course of the antiproteinuric and antihypertensive effects of direct renin inhibition in type 2 diabetes. Kidney International. 2008;**73**:1419-1425. DOI: 10.1038/ki.2008.68

[78] de Zeeuw D, Coll B, Andress D, et al. The endothelial antagonist atrasentan lowers residual albuminuria in patients with type 2 diabetic nephropathy. Journal of American Society of Nephrology. 2014;**25**:1083-1093. DOI: 10.1681/ASN.2013080830

[79] Diaz VA, Mainous AG 3rd, Carek PJ, Wessell AM, Everett CJ. The association of vitamin D deficiency and insufficiency with diabetic nephropathy: Implications for health disparities. Journal of American Board of Family Medicine. 2009;**22**:521-527

[80] Zhang Z, Sun L, Wang Y, et al. Renoprotective role of the vitamin D receptor in diabetic nephropathy. Kidney International. 2008;**73**:163-171. DOI: 10.1038/sj.ki.5002572

[81] Schwarz U, Amann K, Orth SR, Simonaviciene A, Wessels S, Ritz E. Effect of 1,25 (OH)2 vitamin D3 on glomerulosclerosis in subtotally nephrectomized rats. Kidney International. 1998;**53**:1696-1705. DOI: 10.1046/j.15231755.1998.00951.x

[82] Kim MJ, Frankel AH, Donaldson M, et al. Oral cholecalciferol decreases albuminuria and urinary TGF-beta1 in patients with type 2 diabetic nephropathy on established renin angiotensin-aldosterone system inhibition. Kidney International. 2011;**80**:851-860

[83] Bonakdaran S, Hami M, Hatefi A. The effects of calcitriol on albuminuria in patients with type-2 diabetes mellitus. Saudi Journal of Kidney Diseases and Transplantation. 2012;**23**:121520. DOI: 10.4103/1319-2442.103562

[84] de Zeeuw D, Agarwal R, Amdahl M, et al. Selective vitamin D receptor activation with paricalcitol for reduction of albuminuria in patients with type 2 diabetes (VITAL study): A randomized controlled trial. Lancet. 2010;**376**(9752):1543-1551. DOI: 10.1016/S01406736(10)61032-X

[85] Huang Y, Yu H, Lu J, et al. Oral supplementation with cholecalciferol 800 IU ameliorates albuminuria in Chinese type 2 diabetic patients with nephropathy. PLoS One. 2012;**7**(11):e50510. DOI: 10.1371/journal.pone.0050510

[86] Momeni A, Shahidi S, Seirafian S, Taheri S, Kheiri S. Effect of allopurinol in decreasing proteinuria in type 2 diabetic patients. Iranian Journal of Kidney Diseases. 2010;**4**:128-132

[87] Jalal DI, Maahs DM, Hovind P, Nakagawa T. Uric acid as a mediator of diabetic nephropathy. Seminars in Nephrology. 2011;**31**:459-465. DOI: 10.1016/j. semnephrol.2011.08.011

[88] Parving HH, Persson F, Lewis JB, Lewis EJ, Hollenberg NK. Aliskiren combined with losartan in type 2 diabetes and nephropathy. New England Journal of Medicine. 2008;**358**:243346. DOI: 10.1056/NEJMoa0708379

[89] Egido J, Rojas-Rivera J, Mas S, et al. Atrasentan for the treatment of diabetic nephrop athy. Expert Opinion on Investigational Drugs. 2017;**26**:741-750. DOI: 10.1080/13543784. 2017.1325872

[90] Evans TC, Capell P. Diabetic nephropathy. Clinical Diabetes. 2000;**18**:1-16

[91] Barsotti G, Cupisti A, Barsotti M, et al. Dietary treatment of diabetic nephropathy with chronic renal failure. Nephrology, Dialysis and Transplantation. 1998;**13**:49-52. PMID: 9870426

[92] Toeller M, Buyken AE. Protein intake–new evidence for its role in diabetic nephropathy. Nephrology, Dialysis and Transplantation. 1998;**13**:1926-1927. PMID: 9719140