Trakia Journal of Sciences, No 2, pp 163-168, 2025
Available online at:
https://trakia-uni.bg

ISSN 1313-3551 (online) doi:10.15547/tjs.2025.02.012

Mini Review

BIOMARKERS FOR EARLY DETECTION OF DIABETIC NEPHROPATHY

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ABSTRACT

Diabetic nephropathy (DN) is the most common complication of type 2 diabetes mellitus and is the leading cause of end-stage renal disease worldwide. Early detection of diabetic nephropathy (DN) is critical because timely intervention can help slow the loss of kidney function and mitigate negative outcomes. It is crucial to diagnose patients at an early stage of DN, which is reversible and with the possibility of better control of the progression of the complication. As an early marker of DN, microalbuminuria is used in routine practice for screening and onset of the complication. On the other hand, kidney damage can occur even without microalbuminuria. There are several significant biomarkers of kidney damage in diabetes that allow detection of diabetic nephropathy at an early stage. This is crucial for timely treatment that can delay the development of DN and its progression. Therefore, this review focuses on laboratory biomarkers that are early, specific and that could potentially enable early diagnosis, treatment and delay of progression of diabetic nephropathy.

Key words: diabetes mellitus, diabetic nephropathy, microalbuminuria

INTRODUCTON

A significant challenge to the investigation of diabetes is the nature of the etiopathogenetic pathways driving the development of diabetic complications. Diabetes micro- and macroangiopathy are complex, polyetiological disorders that are influenced by both external and endogenous causes (1). Diabetic nephropathy (DN) is the most prevalent consequence of type 2 diabetes mellitus and the primary cause of end-stage renal disease globally. Since DN is reversible and can be better controlled in terms of how the problem progresses, patients must receive a diagnosis as soon as possible (2). Microalbuminuria is frequently applied as a precursor of DN to screen for the earliest stages of the disease. However, renal damage is still detectable in the absence of microalbuminuria (3). The focus of the present research is on early, specific laboratory biomarkers that may help delay the evolution of diabetic nephropathy and allow for early identification and treatment. Prompt intervention can help delay kidney function loss and minimize negative outcomes, early detection of diabetic nephropathy (DN) is essential. The earliest indicator of the onset of diabetic kidney disease (DN) is recognized to be the presence of minute amounts of the protein albumin in the urine, or microalbuminuria (4). It's interesting to note that renal damage can happen even in the absence of microalbuminuria. A progressive decrease in glomerular filtration rate (eGFR), which frequently occurs in conjunction with an increase in blood pressure, and persistent albuminuria (or albuminuria rate >300 mg/day or 200 μg/min) measured at least twice within a three- to six-month interval are the hallmarks of diabetic nephropathy. These factors ultimately lead to the end stage of chronic kidney disease (5).

Clinic biomarkers of acute kidney injury must be easily accessible, quickly assayed, highly sensitive, specific, and so on to be considered clinically relevant. There isn't a single "optimal" acute kidney injury marker that can be used in every clinical scenario, but can objectively

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evaluate the majority of kidney pathophysiological processes thanks to certain substances' analytical qualities (6).

EXCRETORY DYSFUNCTION KIDNEY MARKERS

Over the past 40 years, the most often studied indicator of renal function has been *serum/plasma creatinine*. It comes from muscle tissue and enters the bloodstream at a rate that is specific to each plasma molecule. Age, gender, and muscle mass all affect the relatively constant concentrations of creatinine (7). Although it is not bound to plasma proteins, creatinine is freely filtered in the glomeruli, nearly never reabsorbed by the proximal tubules, and secreted in trace amounts into the urine. Patients with a moderate and pronounced decrease in glomerular filtration rate (GFR; (<50 ml/min)) may have a false overestimation of their GFR in the Rehberg test due to an increase in tubular secretion of creatinine as its concentration in the plasma rises. A GFR range of 120 to 50-60 ml/min is regarded as "blind" to creatinine due to the non-linear relationship between the concentration of plasma creatinine and the value of GFR, which prevents us from detecting a slight decrease in the filtration processes in the glomeruli (8). Due to the significant "inertia" of this indicator, a significant increase in its concentration might not be seen for at least 24 hours following acute kidney injury. In addition, it is regarded as one of the most trustworthy methods for identifying chronic glomerular filtration disorders (9).

Cystatin C is a 13 kDa protein that belongs to the cysteine proteinase inhibitor family. It is synthesized by body cells, constantly enters the bloodstream, is freely filtered in the glomeruli of the kidneys, is completely metabolized in the proximal tubules, and is not secreted there. Cystatin C has a high diagnostic significance as a measure of renal excretory function in adult patients, according to numerous studies (7). Compared to creatinine, cystatin C has an advantage for use as an early indicator of kidney damage due to its low plasma level dependence on age, sex, and muscle mass, as well as its near-complete absence of tubular reabsorption and secretion. When determining the presence of kidney damage, cystatin C has an 86% clinical sensitivity and 82% specificity (7). Compared to serum creatinine concentration, the determination of plasma/serum cystatin C concentration in acute tubular necrosis allows a more accurate prediction of the need for renal replacement therapy. The rate of glomerular filtration is determined by taking the blood plasma's cystatin C concentration and adding creatinine to it (10).

STRUCTURAL KIDNEY DAMAGE MARKERS

Inflammation can be accompanied by decreased local blood flow in the renal outer medulla, which can harm tubular function and viability. Simultaneously, renal tubular cells produce and secrete pro-inflammatory cytokines when they are under "stress". Damage to all tissues and organs, including the kidneys, is accompanied by an increase in their blood plasma concentration. However, cytokines are not routine laboratory markers of cellular and tissue damage, in part because of their short half-lives, significant inter individual variability in levels, and tissue and organ non-specificity. Nonetheless, certain cytokines are thought to be potential indicators of renal damage.

Interleukin-18 (IL-18) is secreted and almost completely degraded in the proximal tubules of the kidney. In the damaged kidneys, IL-18 can be found in urine. Urinary IL-18 levels are significantly elevated in patients with established renal impairment, as opposed to urinary tract infection, chronic kidney disease, nephrotic syndrome, or prerenal azotemia. The increase in the level of IL-18 in urine allows us to detect the development of kidney damage 24 hours before the rise in serum creatinine levels and to predict the need for renal replacement therapy (11).

Kidney Injury Molecule-1 (KIM-1) is a 90 kDa transmembrane glycoprotein that is highly expressed in proximal tubular cells following toxic or ischemic injury. The rise in urea and creatinine levels is preceded by an increase in the serum concentration of KIM-1. Even in cases of mild kidney damage, the increase in KIM-1 in the urine enables early kidney damage diagnosis (12). The plasma KIM-1 levels predict future decline in glomerular function in nonproteinuric diabetic individuals. Higher tissue expression of KIM-1 is associated with a rapid decline in eGFR in patients with diabetic nephropathy.

β2-microglobulin (β2-MG) is a 12 kDa protein, and is part of the light chain of membrane-bound HLA-antigens. Because of its small size, β2-MG can pass through the glomerular membrane, and is almost completely absorbed

in the proximal tubule. In glomerular pathology, the filtration rate of β2-MG slows down, as a result, its concentration increases in the blood and decreases in the urine. When the tubules are damaged, the amount of reabsorbed β2-MG decreases, its level in the urine increases, and in the blood decreases (13).

CCN1 (cysteine-rich protein 61; Cyr61) is an extracellular matrix-associated protein that plays an important role in the regulation of cell adhesion, gene expression, migration, proliferation, differentiation, and cell survival after injury. Synthesis of CYR61 in the proximal tubule begins within one hour after an episode of ischemia, reaches a maximum after 4–8 hrs, and remains elevated for at least 24 hrs. Subsequently, despite the progression of renal damage, urinary CYR61 levels decrease (14).

The dimeric glycoprotein *clusterin* may be a biomarker for tubular injury. Clusterin contributes to cell aggregation and inhibits apoptosis. Kidneys in good health do not contain any clusterin mRNA (15). Following a nephrectomy, unilateral ureteral obstruction, renal ischemia-reperfusion injury, and exposure to nephrotoxic substances, its expression is elevated. Urinary clusterin and KIM-1 levels remain elevated during renal structure regeneration. Urinary clusterin excretion measurement helps monitor chronic kidney damage later on (during the recovery period) (15).

Albumin in the urine Microalbuminuria is not specific to DN and has several drawbacks, including increased variability, low sensitivity, and inability to predict the outcome. Three stages make up the development of diabetic kidney disease: macroalbuminuria, microalbuminuria, and normoalbuminuria (16). Damage to the kidneys glomerular basement membrane was the only factor that led to the development of albuminuria. According to recent experimental research, albumin in rats is effectively absorbed in the proximal tubule after passing through the intact glomerular basement membrane. This suggests that damage to the proximal tubule, rather than the glomerulus, is what causes albuminuria. The significance of albuminuria in the diagnosis of proximal tubular injury has also been shown to be comparable to that of glutathione S-transferase and clusterin. Albuminuria can also be a response to various physiological and pathological conditions, such as significant exercise, fever, dehydration, diabetes mellitus, or hypertension. Albumin excretion in the urine can be observed even without damage to the proximal tubules - simply by inhibiting protein uptake by the cells of the latter, which limits the use of albuminuria as a biomarker of kidney damage (17). It is thought that glomerular hyperfiltration is a sign of an impending illness. Albuminuria is characterized by a progressive decline in the glomerular filtration rate (GFR), which is correlated with blood pressure, glycemia, and albumin excretion rate. Other factors, such as female gender, obesity, and the presence of hypertriglyceridemia, may also contribute to the decrease in GFR, which would explain why the heterogeneity of DN phenotypically is not closely related to typical histological lesions (16-18).

INFLAMMATION AND OXIDATIVE STRESS MARKERS

Increased intraglomerular pressure, activation of the renin-angiotensin system (RAS), oxidative stress, and fibrotic alterations are among the functional abnormalities associated with the pathophysiology of diabetes mellitus (DN), which are layered on top of an innate genetic

Increased ROS generation is not the only process in hyperglycemia. It is also accompanied by changes in the activities of antioxidant enzymes - superoxide dismutase, catalase, glutathione peroxidase, and glutathione. Persistent OS is indicated by reduced Cu-Zn SOD activity and low glutathione. Considerable research is still being done to determine how ROS and AGEs affect the development of micro- and macrovascular disorders (22, 23). Endothelial dysfunction is linked to both factors and maintaining appropriate vascular tone is one of the most important mechanisms for regulating the activity of endothelial cells. Vasodilators and vasoconstrictors are primarily synthesized and released to accomplish this. Irritation of the endothelium with DN by the bloodstream is the primary mechanism that triggers the release of vasodilators such as nitric oxide, prostacyclin (PGI2), and endothelial-derived hyperpolarizing factor. Endothelin-1 is the most well-known vasoconstrictor (24). The actions of

thromboxane A2 and prostaglandin PgF2α are comparable. Angiotensin II and superoxide anion also narrow blood vessels. The endothelium's indicated properties are altered in diabetes and a few other pathological conditions, including arterial hypertension, atherosclerosis, etc. "Endothelial dysfunction" describes an imbalance between substances that promote or retain growth, thrombotic and fibrinolytic mediators, and vasodilators and constrictors (25). Endothelial dysfunction is manifested biochemically as a deficiency in the formation of bradykinin, prostacyclin, nitric oxide, and tissue plasminogen activator with the simultaneous appearance of endothelial prostaglandin endoperoxide, peroxynitrite, superoxide, and tissue inhibitor of plasminogen activator. This imbalance between local endothelial protective and aggressive factors leads to an increase in the levels of the main pro-inflammatory mediators (including interleukins, C-reactive protein), and also activates the vascular pro-inflammatory signaling systems of MAP-kinases, NFkB, etc. As a result of this damage, narrowing of the arteries occurs, also caused by: altered proliferation of smooth muscle cells; increased adhesion of leukocytes (granulocytes, monocytes) and platelets; predisposition to deposition of atheromas; altered permeability of vessels; enhanced expression and increased secretion of proteins of the intercellular matrix, etc (25).

In contrast to nuclear DNA, the DNA found in mitochondria is kept in open chromatin. A DNA characteristic as an open structure is its heightened susceptibility to ROS damage. Exposure to hyperglycemia impairs the electron transport chain's ability to produce mitochondrial ATP, which facilitates the transfer of electrons to molecules like oxygen (O_2) and the creation of superoxide ($\bullet O_2^-$) anion radicals. The functions of mitochondrial proteins, such as those in the electron transport chain, can be interfered with by ROS. Peroxynitrite (ONOO-) and hydrogen peroxide (H_2O_2) can penetrate membranes and damage molecules in various regions of the cell (26). The activation of poly (ADP-ribose) polymerase, or PARP, is brought on by hyperglycemia. This activation can then suppress sirtuin 1 (SIRT1), thereby creating a potential feedback loop that increases the production of ROS in the mitochondria and exacerbates the effects of OS (26).

In summary, ROS generated in hyperglycemia impair mitochondrial functions and consequently lead to an increase in their production. The end product of the reaction between peroxynitrite and free tyrosine or proteins containing tyrosine is 3-nitrotyrosine, which is created when nitrogen radicals, such as peroxynitrite ($ONOO^-$) and nitrogen dioxide (NO_2), react with tyrosine residues and add a nitro group to the tyrosine residues (26). Oxidative stress is one of the main pathophysiological processes involved in the formation of the complications of diabetes mellitus and disrupting the basic functions of the cell. Disturbances persist for a long time even after normalization of carbohydrate metabolism parameters, which reflects the phenomenon of metabolic memory during the development of diabetes complications.

Advanced glycation end products (AGEs) are formed at Maillard pathway when carbohydrates react non-enzymatic with the amino group found in proteins, lipids, and nucleic acids. This process starts with the transformation of reversible Schiff base adducts into more stable, covalently linked rearrangement products under conditions of hyperglycemia and oxidative stress. Even after reaching euglycemia, AGEs are present, break down slowly, and stay in the vessels for a considerable amount of time (27). Long-lived proteins like collagen are directly cross-linked by AGEs, which accelerates the growth of micro- and macrovascular problems. Diabetes type I AGEs are members of the immunoglobulin superfamily and have a receptor (RAGE) that is found on the surface of cells both in a free and bound state. In addition, modified AGE proteins cause numerous organs and systems to malfunction, and promote the synthesis of peroxynitrite while suppressing the expression of endothelial nitric oxide synthase in endothelial cells (28).

It has been demonstrated that AGEs are essential to the pathophysiology of diabetic nephropathy. The initial hyperperfusion and hyperfiltration of the kidneys are linked to protein kinase C activating phospholipase A2. However, extracellular matrix deposition and renal vasoconstriction brought on by advanced diabetes increase the risk of nephrosclerosis and systemic hypertension. Information on oxidative profiles to the simultaneous measurement of several distinct biomarkers is generally lacking. One of the primary causes of

end-stage renal failure is diabetic nephropathy, whose incidence is on the rise despite mounting evidence that suggests it is, at least theoretically, preventable with prompt intervention.

Previously, it was believed that patients with type 2 diabetes mellitus (T2DM) had a much better prognosis for their kidneys than those with type 1 diabetes mellitus (T1DM) and that the risk of end-stage renal disease was only slightly higher in T2DM patients than in the general population (5). Only a small percentage of T2DM patients (<5%) developed end-stage renal disease, so this view has given rise to the myth that T2DM particularly in older adults is a relatively benign condition with minimal effects on the kidneys. These days, this viewpoint has been entirely rewritten. Patients with type 1 and type 2 diabetes mellitus were found to have similar cumulative risks of proteinuria and renal failure with significant proteinuria in both forms of the disease (25).

Patients with T2DM experience a similar degree of glomerular filtration rate reduction as those with T1DM in cases of advanced nephropathy. In addition, individuals with type 2 diabetes were primarily found to have micro albuminuria and macro albuminuria. High blood pressure and a high mean HbA1c during the follow-up period were found to be risk factors for the development of nephropathy symptoms. After controlling for blood pressure, glycemic control, sex, smoking, and diabetes, patients with type 2 diabetes mellitus, tended to have an increased risk of renal involvement compared to those with type 1 diabetes mellitus. In the first ten years after the onset of the disease, diabetics are still not immune against developing kidney disease, even with current treatment and consistent blood sugar management (23-25).

CONCLUSION

Controlled antioxidant therapy combined with a science-based therapeutic protocol is made possible by a thorough understanding of the mechanisms underlying oxidative stress in type 2 diabetes mellitus and the damage it causes. In order to achieve optimal results with minimal risk, efforts will be focused on finding a new approach to the treatment of diabetes that affects the body's antioxidant defense in addition to stabilizing the metabolism of carbohydrates. This will help to restore the body's antioxidant status and minimize oxidative damage. by reactive molecules that cause damage, such as ROS.

ACKNOWLEDGMENT

This research was funded by the scientific project No.15/2023 Medical Faculty, Trakia University, Bulgaria.

REFERENCES

1. Dilworth, L., Facey, A., Omoruyi, F. Diabetes mellitus and its metabolic complications: the role of adipose tissues. *Intern J Mol Sci*, *22*(14): 7644, 2021.
2. Hoogeveen, E. K. The epidemiology of diabetic kidney disease. *Kidney and Dialysis*, *2*(3): 433-442, 2022.
3. Trifonova, O.P., Maslov, D.L., Balashova, E.E., Lichtenberg, S., Lokhov, P.G. Potential plasma metabolite biomarkers of diabetic nephropathy: untargeted metabolomics study. *J Person Med*, *12*(11):1889, 2022.
4. Piwkowska, A., Zdrojewski, Ł., Heleniak, Z., Dębska-Ślizień, A. Novel markers in diabetic kidney disease—current state and perspectives. *Diagnostics*, *12*(5):1205, 2022.
5. Pafundi, P. C., Garofalo, C., Galiero, R., Borrelli, S., Caturano, A., Rinaldi, L., Provenzano, M., Salvatore, T., De Nicola, L., Minutolo, R., & Sasso, F. C. Role of albuminuria in detecting cardio-renal risk and outcome in diabetic subjects. *Diagnostics*, *11*(2): 290, 2021.
6. Xiao ,Z., Huang, Q., Yang, Y., Liu, M., Chen, Q., Huang, J., Xiang, Y., Long, X., Zhao, T., Wang, X., Zhu, X., Tu, S., Ai, K. Emerging early diagnostic methods for acute kidney injury. *Theranostics*. 21, 12(6):2963-2986, 2022
7. Haines, R. W., Fowler, A. J., Liang, K., Pearse, R. M., Larsson, A. O., Puthucheary, Z., and Prowle, J. R. Comparison of cystatin C and creatinine in the assessment of measured kidney function during critical illness. *Clin J Am Soc Nephrol*, *18*(8): 997-1005, 2023.
8. Benoit, S.W., Ciccia, E.A., Devarajan, P. Cystatin C as a biomarker of chronic kidney disease: latest developments. *Expert Rev Mol Diagn.* 20(10):1019-1026, 2020
9. Gembillo, G., Ingrasciotta, Y., Crisafulli, S., Luxi, N., Siligato, R., Santoro, D., Trifirò, G. Kidney disease in diabetic patients: from pathophysiology to pharmacological aspects with a focus on therapeutic inertia. *Intern J Mol Sci*, *22*(9): 4824, 2021.
10. Jaques, D.A., Spahr, L., Berra, G., Poffet, V., Lescuyer, P., Gerstel, E., ... and Ponte, B. Biomarkers for acute kidney injury in

decompensated cirrhosis: a prospective study. *Nephrol*, *24*(2): 170-180, 2019.
11. Yasuda, K., Nakanishi, K., and Tsutsui, H. Interleukin-18 in health and disease. *Internat j mol sci*, *20*(3), 649, 2019.
12. Naber, T., and Purohit, S. Chronic kidney disease: role of diet for a reduction in the severity of the disease. *Nutrients*, *13*(9): 3277, 2021.
13. Argyropoulos, C.P., Chen, S.S., Ng, Y.H., Roumelioti, M.E., Shaffi, K., Singh, P.P., Tzamaloukas, A.H. Rediscovering Beta-2 microglobulin as a biomarker across the spectrum of kidney diseases. *Front Med (Lausanne)*. 15(4):73, 2017.
14. Panayotova, M. Oxidative stress and inflammatory bowel disease in pediatrics. *Trakia J Sci*, *21*(4): 375, 2023.
15. Iłżecka, J., Iłżecki, M., Grabarska, A., Dave, S., Feldo, M., and Zubilewicz, T. Clusterin as a potential marker of brain ischemia-reperfusion injury in patients undergoing carotid endarterectomy. *Upsala J Med Sci*, *124*(3): 193-198, 2019.
16. Gheith, O., Farouk, N., Nampoory, N., Halim, M. A., and Al-Otaibi, T. Diabetic kidney disease: worldwide difference of prevalence and risk factors. *J Nephropharm*, *5*(1): 49, 2015.
17. Panayotova, M., Penkova, M. Measurement of oxidative stress-related markers in gastro-intestinal damages in Bulgarian pediatric patients. *Bulg Chem Commun*, 56(SI):142-147, 2024
18. Gburek, J., Konopska, B., and Gołąb, K. Renal handling of albumin—from early findings to current concepts. *Internat J Mol Sci*, *22*(11): 5809, 2021.
19. Anders, H.J., Huber, T.B., Isermann, B., and Schiffer, M. CKD in diabetes: diabetic kidney disease versus nondiabetic kidney disease. *Nat Rev Nephrol*, *14*(6): 361-377, 2018.
20. Cole, J. B., Florez, J. C. Genetics of diabetes mellitus and diabetes complications. *Nature reviews nephrology*, *16*(7): 377-390, 2020.
21. Giacco, F., Brownlee, M. Oxidative stress and diabetic complications. *Circulat res*, 107 (9): 1058–1070, 2010.
22. Mengozzi, A., Pugliese, N. R., Chiriaco, M., Masi, S., Virdis, A., and Taddei, S. Microvascular ageing links metabolic disease to age-related disorders: the role of oxidative stress and inflammation in promoting microvascular dysfunction. *J Cardiovasc Pharmacol*, 78: S78-S87, 2021.
23. Koulis, C., Watson, A. M. D., Gray, S. P., and Jandeleit-Dahm, K. A. Linking RAGE and Nox in diabetic micro-and macrovascular complications. *Diabetes & metabolism*, *41*(4): 272-281, 2015.
24. Mangana, C., Lorigo, M., & Cairrao, E. Implications of endothelial cell-mediated dysfunctions in vasomotor tone regulation. *Biologics*, *1*(2), 231-251, 2021.
25. Kolluru, G. K., Bir, S.C., Kevil, C.G. Endothelial dysfunction and diabetes: effects on angiogenesis, vascular remodeling, and wound healing. *Intern J Vasc Med*, 2012(1): 918267, 2012
26. Sifuentes-Franco, S., Padilla-Tejeda, D. E., Carrillo-Ibarra, S., Miranda-Díaz, A.G. Oxidative stress, apoptosis, and mitochondrial function in diabetic nephropathy. *Intern J Endocrinol*, 2018(1): 1875870, 2018.
27. Twarda-Clapa, A., Olczak, A., Białkowska, A. M., Koziołkiewicz, M. Advanced glycation end-products (AGEs): Formation, chemistry, classification, receptors, and diseases related to AGEs. *Cells*, 11(8): 1312, 2022.
28. Förstermann, U., & Sessa, W.C. Nitric oxide synthases: regulation and function. *Europ Heart J*, 33(7): 829-837, 2012.