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REVIEW ARTICLE

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Modern Markers of Renal Damage in Diabetes Mellitus

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Abstract

Diabetes mellitus (DM) is a public health problem worldwide. Despite the presence of various sugar-lowering drugs, correcting hyperglycemia and preventing late DM complications remain a difficult task. Unfortunately, DM complications are often detected at late stages, when there are irreversible changes and a global decrease in the function of damaged organs and systems. One of the most dangerous complications leading to early death of patients is diabetic nephropathy. However, the problem of finding early markers of renal damage that can detect the earliest renal damage in global clinical practice remains unresolved. The following are the modern markers that are likely to be able to timely reflect the preclinical manifestations of diabetic nephropathy (DN): Type-IV collagen, NGAL, b2MG, cystatin C, E-cadherin, podocalyxin, and nephrin. Heparan sulfate, mindin, TGF-β, ICAM-1, KIM-1, uromodulin, and LFABP are also being studied. The task of modern medicine is to find the most sensitive of these markers to diagnose DN in a timely manner. (**International Journal of Biomedicine. 2020;10(1):9-15.**)

Key Words: diabetic nephropathy • markers of renal damage • glomerular filtration rate • albuminuria

Abbreviations

β2MG, β2-microglobulin; **AngII**, Angiotensin II; **CysC**, cystatin C; **CKD**, chronic kidney disease; **DM**, diabetes mellitus; **DN**, diabetic nephropathy; **GFR**, glomerular filtration rate; **ICAM-1**, inter-cellular adhesion molecule-1; **KIM-1**, kidney injury molecule-1; **LFABP**, liver-type fatty-acid-binding proteins; **MAU**, microalbuminuria; **NAU**, normoalbuminuria; **NGAL**, neutrophil gelatinase-associated lipocalin; **TGF-β**, transforming growth factor-β; **T1DM**, type 1 diabetes mellitus; **T2DM**, type 2 diabetes mellitus

Worldwide, the prevalence of diabetes mellitus (DM) is growing every year, acquiring the scale of an epidemic.[1,2] Diabetic nephropathy (DN) is one of the most dangerous complications of DM that lead to patients` early death. Despite the available screening algorithms for this complication, timely detection of DN remains an urgent problem among DM patients.[2]

Hyperglycemia, intraglomerular hypertension, dyslipidemia and chronic inflammation play a role in the formation of this complication,[3,4] as does oxidative stress.[5-7] According to the current classification, there are 3 stages of DN: albuminuria, proteinuria, and renal failure.[3] The stages of proteinuria and renal failure are irreversible, since by the time proteinuria occurs, 50%-70% of the renal mass has already been sclerosed.[3,8] During the progression of nephropathy, the number of actively filtering nephrons continues to decrease, which leads to a drop in the GFR and to uremia formation, so diagnosing DN at an early preclinical stage is a major problem, the solution of which will protect patients from early disability and death. According to current data, 29.5% of patients with T1DM and 36.9% of patients with T2DM have DN in the albuminuria stage.[9] It is known that in the conditions of timely appointed nephron-protective therapy, this stage of DN is capable of regression (remission). These data are confirmed in several studies. In the Araki S. study, 216 patients with T2DM took part, among which the regression rate was 51%

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over the 6-year follow-up period.[10] In STENO-2, 151 patients with T2DM and MAU were studied for 7 years, and 46(30.4%) patients showed regression to NAU stage.[11] Two hundred and seventy-three patients with T2DM were involved in the Kashiwa study for 8 years. Among the studied patients, 94 exhibited DN at the MAU stage, and regression against the background of carbohydrate metabolism correction and blood pressure control was recorded in 44% of patients.[12] Three hundred and fifty-two T1DM patients participated in the EURODIAB study for 7 years, and MAU regression to NAU on the background of carbohydrate metabolism correction reached 50.6%.[13] In this study, patients were not compromised by an additional risk factor in the form of hypertension. In a study of 1513 DM patients, de Zeeuw et al.[14] found a relationship between the level of albuminuria and the prospective kidney condition—the higher the albumin excretion with the urine, the less likely that kidney function will return to NAU. However, the concept of "normoalbuminuric" nephropathy, which requires active study of early markers of renal damage in DM, is increasingly encountered in modern literature data.[15]

To date, determination of serum creatinine and albuminuria levels, GFR calculation, and determination of the albumin to creatinine ratio[3,4] are actively used to diagnose kidney damage in DM in clinical practice. However, these indicators are affected by various factors: The level of serum creatinine and albuminuria may increase transiently with ketoacidosis, a high-protein diet, and increased physical activity; and a decrease in GFR may be temporary in urinary infection and DM decompensation.[8,9] A persistent decrease in GFR occurs if a large mass of working nephrons is lost and renal dysfunction is irreversible. Currently, the well-known physiological reasons make the serum creatinine an imperfect GFR biomarker. Because the relationship between serum creatinine and glomerular filtration rate is hyperbolic, all known analytical limitations will impact not only the precision of serum creatinine but still more the precision of different creatinine-based equations, especially in low or normal-low creatinine levels (or high or normal-high glomerular filtration rate range).[8,16] The task of modern diabetology is to find more sensitive and specific markers of early preclinical kidney damage in order to be able to timely prescribe nephron-protective therapy in order to influence the formation of renal failure. In addition to glomerulosclerosis, tubulointerstitial kidney damage occurs in DN.[17] Tubular changes occur as a result of hyperglycemia and the subsequent cascade of inflammatory reactions with the activation of protein kinase C, which is a catalyst for stimulating collagen synthesis in the kidneys. In addition, when hyperglycemia and intraglomerular arterial hypertension are combined, when AngII levels increase, TFR-β is synthesized, which also leads to increased collagen synthesis, and AngII itself contributes to the death of tubulointerstitial cells.[18] Tubular dysfunction is formed, including in diabetic nephropathy, probably ahead of the glomerular apparatus damage.[19] An additional damaging factor of the tubules is enhanced filtration of plasma proteins, resulting in a "vicious circle," where tubulointerstitial damage is a process that provokes glomerular dysfunction, and against this background, the dysfunction of the tubular apparatus is aggravated.

Type IV collagen. Among the currently actively studied markers of renal damage that are proposed for consideration is type IV collagen (ColIV), which is one of the components of the glomerular mesangial matrix and provides mechanical stability of the membrane, forming a support network. The tendency to the increased excretion of ColIV is observed in DM patients who are already at the NAU stage and increases with further progression of DN. Thus, in the study by Bondar et al.,[19] the urinary ColIV level increased as albuminuria progressed. Among NAU patients, an increased level of ColIV was recorded in 39.1% of cases, which is comparable to the indicators in the control group of healthy individuals. It was noted that in NAU patients, ColIV increased among those who had hypertension. As albuminuria progresses,[20] the amount of ColIV in the urine increases (during the MAU formation): 53.6% of patients had an increased ColIV level, and at the stage of proteinuria – 100%. Similar data were obtained by Cawood et al.[21]: an increased ColIV level was found in 26% and 58% of patients with NAU and MAU, respectively, and at the stage of proteinuria – in 65% of patients. In T2DM 254 patients (185 with NAU and 69 with MAU), Araki et al.[22] revealed an increase in ColIV level in both groups. In a prospective follow-up of 8 years, 16 out of 185(8.6%) patients in the NAU group had elevated levels of ColIV, and 9 out of 69(13%) patients in the MAU group had DN progression. In the study, a clear inverse correlation was found in the relationship between the ColIV level and the GFR level. When observing patients in the NAU group, the annual decrease in GFR was more pronounced among the patients with elevated ColIV levels than among normal ColIV level patients. The same pattern was found in the group of patients with MAU. Morita et al.[23] also recorded data confirming the results in a study of 231 T1DM patients over a period of 7.4±1.3 years: the GFR reduction rate was higher in patients with elevated levels of ColIV in both the NAU and MAU groups. Thus, it is likely that the determination of ColIV excretion can serve for early DN diagnosis and have a prognostic value regarding changes in GFR.

Neutrophil Gelatinase-Associated Lipocalin. NGAL, which is expressed in small concentrations in many tissues, including the kidneys, being an indicator of the tubular apparatus condition, is also considered as one of the markers of kidney damage that may help in early DN diagnosis. In the study of the NGAL level as an early marker of renal damage, Lacquaniti et al.[15] found an increase of it in blood serum of T1DM patients up to 193.7 ng/ml at NAU, while in healthy individuals the median was 46.4ng/ml, in urine – 25.5 ng/ml and 6.5 ng/ml, respectively. Mahfouz et al.,[24] who studied NGAL levels in 150 T2DM patients, obtained similar data. The patients were divided into groups according to the albuminuria level. An increase in the NGAL level was recorded at the NAU stage, in comparison with the group of healthy individuals. The NGAL level increased progressively with the increase in albuminuria level: 46.46±8.56 ng/ml in healthy individuals, 55.6±16.95 ng/ml in DM patients with NAU, 97.8±10.97 ng/ml in DM patients with MAU, and 131.0±27.29 ng/ml in DM patients with proteinuria.

In several studies, NGAL has shown a relationship with creatinine and GFR levels: An increase in the level of

this marker in the urine has a positive correlation with an increase in blood creatinine levels, while in DM patients, the NGAL level increased earlier than did creatinine.[25] A negative correlation with GFR level was found in a study by Woo et al.:[26] In patients with GFR<60 ml/min, the NGAL level was 96.0 [2.7–975.2] ng/ml, and in the control group of healthy individuals with normal GFR - 18.8 [1.3–81.9] ng/ml. An increase in NGAL levels at normal serum creatinine concentrations is a sign of subclinical acute renal damage associated with the risk of rapid progression of this condition to the clinical stage. Nielsen et al.,[27] studying the relationship between NGAL and GFR in 177 T2DM patients, found that a higher NGAL level was associated with a faster decrease in GFR. Similar data were obtained in a study by Zylka et al.:[28] the increased NGAL level was inversely correlated with GFR in 80 T2DM patients at the NAU and MAU stages. Currently, NGAL can probably be considered as a marker of acute renal damage. Its timely detection should make it possible to start treatment earlier in order to prevent the progression and chronization of the renal failure process.[29]

Cystatin C. CysC, a protein with a molecular weight of 13 kDa, is offered as a marker that can participate in a more reliable GFR calculation than can creatinine. Studying the CysC level in 58 T2DM patients, Klimontov et al. compared the GFR levels calculated using CysC and creatinine levels. It was found that in 14(24.1%) patients the difference between these indicators was 11-19ml/min/1.73m^2, and in 12(20.7%) patients the difference was more than 20 ml/min/1.73m^2.[30] Waheed et al.[31] described an earlier decrease in GFR calculated using CysC levels compared with GFR calculated by creatinine levels in patients at the NAU stage. The high sensitivity of CysC in comparison with creatinine was also confirmed by Inker et al.,[32] who analyzed 13 studies involving 5352 DM patients. When recalculating GFR by CusC level, CKD was further reclassified in 19.4% of patients with GFR>60 ml/min/1.73m^2 and 16.9% of patients with GFR in the range of 45-59 ml/min/1.73m^2. This fact is extremely important for establishing the stage of CKD, especially in patients with NAU. It is also interesting to determine the excretion of this marker with the urine.[33] Given that CysC is normally metabolized in the renal tubules, an increase in its level in the urine is an indicator of damage to the tubular apparatus, which precedes MAU. Early detection of an increase in the CysC level in the urine makes it possible to identify patients with the maximum risk of renal complications.[34] The relationship between the patients' body weight and the blood level of this protein is that the higher the proportion of the fat component in the body weight, the lower the GFR calculated by the CysC level.[30] CysC is more sensitive than creatinine for determining GFR, but in some patients (morbid obesity, elderly patients), additional research is required.[30]

β2-microglobulin (β2MG) is a protein with a molecular weight of 11.8 kDa, which is synthesized in all cells of the body that have nuclei; its amount in the blood reflects both the process of cell synthesis and the level of cell decay. β2MG is filtered by glomerular capsules, followed by reabsorption and metabolism in the proximal tubules, so the amount of β2MG in the urine is minimal in normal kidney function. Therefore, an increase in the plasma β2MG level may be evidence of a dysfunction in renal glomerulus capsules, and in the urine the concentration of this marker increases when the renal tubules are disturbed, indicating a pathology of renal filtration.[34] One of the factors that allows β2MG to penetrate the filtration barrier earlier than albumin is the small molecular weight compared to the mass of albumin (69 kDa). Available studies have shown that the level of β2MG excretion in DM patients is higher than in people without DM.(34) This was confirmed by Monteiro et al.[35] in 51 patients with T1DM, compared to healthy individuals. In addition, β2MG has a positive correlation with the levels of creatinine and CysC,[36] and a negative correlation with GFR. In a study by Kim et al.,[37] an increased serum β2MG level was found in T2DM patients with higher MAU. Zeng et al.[38] confirmed the relationship of β2MG level with tubal lesions during subsequent renal biopsy in 46 patients: 30 patients had increased β2MG level, and tubal changes were found in 100% of cases during biopsy. Given the presence of tubular damage in DN formation, it is likely that β2MG can be identified as a marker of renal damage.

E-cadherin. E-cadherin, previously known as an oncomarker for lesions of the colon, breast, etc., is being studied as another suspected indicator of kidney damage in DM. According to available data, the ratio of the E-cadherin fragment to creatinine (80 kDa) in the urine increases in patients with CD at MAU (2751.5±164 mcg/g) and macroalbuminuria (5839.6±428 mcg/g), compared with the control group of patients(652.7±87 mcg/g), and also increases in patients with NAU (721.9±93 mcg/kg).[39] Taking into account the change in the expression of this protein with excessive caloric intake, it is suggested that E-cadherin is involved in the formation of cancer in DN with T2DM,[40] but still, the question of its role in the formation of fibrosis in DN remains controversial to date. Further research is needed to answer this question.

Podocalyxin. Another process that occurs when the glomerulus is damaged is a decrease in the adhesion of podocytes to the basal membrane, which increases the number of podocytes in the urine, which includes both cells that have already undergone apoptosis and viable ones. Podocalyxin, a protein whose amount correlates with the level of glycated hemoglobin and albumin in the urine, is expressed on the surface of podocytes.[41] When studying this marker, it was found that in DM patients at the NAU stage, its number increases in 53.8% of patients, in the MAU stage – in 64.7% of patients, and in the proteinuria stage – in 66.7% of patients, but there was no correlation between the podocalixin level and GFR.[42] A deeper study of this marker is required to accurately answer the question whether its increase can be a predictor of renal damage in DM.

Nephrin. Diagnosis of podocyte damage is an interesting direction in diagnosis of nephropathy. The nephrin protein can also act as a DN marker.[43,44] Normally, nephrin is part of the podocytes involved in the formation of the filtration barrier. An increase in the amount of nephrin in the urine is a consequence of the destruction of podocytes when podocytopathies of various geneses occur. When studying the nephrin level in 381 DM patients, it was found that nephrinuria is closely associated with a decrease in GFR and the ratio of albumin to

creatinine;[43] similar indicators were found by Tai et al.[44] in a study of 70 DM patients. Jim et al.[45] identified the presence of this marker in the urine of 54% of patients with NAU and 100% of patients with micro- and macroalbuminuria. Similar results were recorded by do Nascimento et al.[46]: nephrin was detected in the urine at the NAU stage in 53% of DM patients with DM, at the MAU stage – in 71% of DM patients, and in 90% of DM patients with proteinuria. Another important point is the increase of nephrin in the urine before the level of GFR decreases. In the above study, the average GFR was 85 ml/min/1.73m^2, meaning that the level of this protein increases even before the glomerular function decreases, and nephrin can act as an early marker of tubular damage in DN.

Mindin. Another protein specific for podocyte damage is mindin. Murakoshi et al.,[47] studying the mindin level in mice with DM, found an increase in this marker, compared to a group of healthy animals. In addition, an increased excretion of mindin was found in T2DM patients, in comparison with the control healthy group, and a correlation was established between the mindin level and the ratio of albumin to creatinine.[48] Determining markers of podocyte damage, such as nephrin and mindin, is probably one of the promising areas, but the question of specificity of changes in these indicators remains open.

Heparan sulfate. The glomerular basal membrane contains various types of glycosaminoglycans, one of them is heparan sulfate, the amount of which increases in the urine when the glomerular apparatus of the kidneys is damaged, including in patients with T1DM.[49] However, the question of the specificity of this indicator remains unresolved.

In several studies, the above-described TGF-β has been shown as the marker whose excretion is increased during the formation of a fibroporous process in renal glomeruli[50] and in the formation of micro- and macroalbuminuria in DM patients, compared to healthy individuals.[51]

ICAM-1. A number of studies have shown the relationship between changes in the blood levels of ICAM-1 and the levels of ICAM-1 expression in the kidneys.[52] A study of 63 patients with T1DM revealed a tendency to an increase in ICAM-1 in MAU formation, and a significant increase in this marker was recorded in patients with proteinuria, but there were no significant differences between patients with NAU and healthy individuals.[52]

KIM-1. As an early marker of nephropathy, KIM-1 was studied in several papers. EL-Attar et al. found an increase in the ratio of KIM-1 to creatinine (KIM-1/Cr) in T2DM patients with MAU and proteinuria, and the KIM-1/Cr ratio was a more sensitive method for determining renal damage than determining only the KIM-1 level. The KIM-1/Cr ratio was positively correlated with the AU/Cr ratio.[53]

Uromodulin. Uromodulin is the most abundant protein secreted in urine. Chang et al. recorded a progressive decrease in uromodulin excretion in 62.5% of DM patients with renal insufficiency, compared to 20% of individuals with non-diabetic nephropathy.[54]

L-FABP. *L-FABP* is a protein with a molecular weight of 15 kDa. According to modern concepts, L-FABP can be used as a promising marker for assessing tubule damage. Kamijo-Ikemori et al. examined 142 patients with T2DM, compared to a control group of healthy individuals. During the study, the progression of nephropathy was considered as an increase in albuminuria, the formation of end-stage renal failure, and the initiation of renal replacement therapy (program hemodialysis). According to the study results, a progressive increase in L-FABP was found in DM patients: the level of urinary L-FABP increased according to the stage of nephropathy. The concentration of urinary l-FABP was higher in patients with DM and NAU compared to the control group (P<0.05).[55]

The question whether the indicators of the lipid peroxidation system-antioxidant protection can autonomously act as additional markers of DN remains open. Their role in the formation of vascular complications is known;[5,6,56-60] there are also data on the increase in the level of diene conjugates, malondialdehyde, and ketodienes directly in DN,[61] but whether it is possible to predict the formation or outcome of DN based on changes in their level is a task that modern medicine has yet to solve.

Thus, currently, the main early marker of kidney damage in diabetic patients due to hyperglycemia is albuminuria; at the same time, there is a need to expand the range of markers that allow high accuracy in diagnosing DN in DM at an early preclinical stage with preserved kidney function and laboratory NAU. Probably, a more promising direction in the search for early diagnosis is the study of tubular damage markers, the level of which increases earlier than laboratory indicators of glomerular dysfunction. The direction of studying proteins with a small molecular weight is also seen as promising, so that they penetrate the filtration barrier earlier than albumin. The question about reduction in the level of markers that are likely to participate in the earlier diagnosis of DN remains insufficiently studied— whether the existing renal lesions in DM undergo complete regression or are still irreversible. If there is a decrease in them, then under what circumstances: whether the existing classical nephron-protective therapy and correction of carbohydrate metabolism and dyslipidemia are sufficient or additional methods of influence are required, and whether markers behave the same in patients with T1DM and T2DM, or genetic factors in T1DM, age and body weight in T2DM and additional aspects of pathogenesis will require modern medicine to develop different corrective approaches. The presence of a high level of tubular and glomerular damage markers in DM patients in the absence of a high level of albuminuria increasingly leads to talk about the presence of normoalbuminuric nephropathy in some patients, which may require adjustments to the modern classification of DN.

Competing Interests

The authors declare that they have no competing interests.

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