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The Nature of Tubulointerestital Biomarkers in Diabetic Nephropathy

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Abstract: As an indicator of tubulointerstitial renal damage in urine of patients with diabetic nephropathy, a study of monocyte chemoattractant protein-1 and liver-type fatty acid binding protein is proposed. he significance of studying zinc-alpha-2-glycoprotein in the urine of patients with diabetic nephropathy as an indicator of the initial stage of disease progression instead of microalbuminuria was revealed. The study demonstrated the significance of inflammatory mediators IL-18 and IL-6 in the urine of patients with diabetic nephropathy as a test indicating the development and progression of the disease.

Keywords: Diabetic nephropathy, zinc-alpha-2-glycoprotein, biomarkers, monocyte chemoattractant protein-1 (MCP-19), Urinary liver-type fatty acid binding protein (L-FABP) (mcg/g creatinine).

Introduction

Attention to tubulointerstitial pathologies of the kidneys increases annually, due to its high proportion among diseases of the excretory system, the difficulty of its detection, as well as a number of difficulties in its treatment [1,2,3,4,7].

The basis for diagnosing tubulointerstitial renal disease is the summation of information from the examination of the general clinical type of the patient and the results of paraclinical studies, on the basis of which it is possible to clinically and morphofunctionally implement algorithms for verifying the diagnosis, establish the primary disease of other organs and systems, and exclude uropathy [13,15,18].

Recently, it has been increasingly reported that the renal tubulointerstitium plays an integral role in the pathogenesis of diabetic nephropathy.

It has been proven that the accuracy of diagnostics of tubulointerstitial renal damage and early diagnostics of the etiology of formation and pathogenetic mechanisms of pathologies, which underlie effective therapy.

Elements of the diabetic environment are known to induce MCP-1 mRNA synthesis and protein secretion by cultured renal parenchymal cells, suggesting that the onset of diabetes may trigger the recruitment of renal macrophages[9,10].

Elements of the diabetic environment induce renal parenchymal cells to secrete MCP-1, which attracts monocytes to the kidney and stimulates myofibroblast-like properties in mesangial cells[16,17].

Further exposure of renal macrophages to MCP-1 and the diabetic environment promotes macrophage activation, resulting in the release of reactive oxygen species (ROS),

proinflammatory cytokines (e.g., IL-1, TNF-α, MCP-1), and profibrotic growth factors (e.g., PDGF, TGF-β) [12,8].

The self-amplifying inflammatory response causes damage and death of parenchymal cells, and the fibrotic reaction causes proliferation of myofibroblasts and increased production of extracellular matrix by fibroblasts and mesangial cells. Together, these reactions contribute to the progression of diabetic nephropathy, leading to renal failure[11,14].

High glucose levels have been shown to stimulate MCP-1 production by human mesangial cells via a pathway that involves PKC activation, elevated levels of oxidative stress, and activation/nuclear translocation of the transcription factor nuclear factor-κB (NF-κB).

Renal epithelial cells, including glomerular podocytes and tubular cells, also produce MCP-1 in response to high glucose and advanced glycation end products. However, urinary MCP-1 levels closely reflect renal MCP-1 production and correlate significantly with levels of albuminuria, serum glycated albumin, and urinary N-acetylglucosaminidase (NAG).

The pupose of the study is to assess the diagnostic value of urine proteomic markers in the early diagnosis of diabetic nephropathy and to evaluate the significance of biomarkers in predicting this disease.

Materials and Methods

To solve the set tasks, we examined 58 patients with diabetes mellitus type 2, the study was carried out in a multidisciplinary clinic of the Tashkent Medical Academy together with endocrinologists, as well as nephrologists. The control group consisted of 18 healthy individuals, without any somatic pathology.

To determine the amount of liver-type fatty acid binding protein (L-FABP), urine was determined using a two-step enzyme-linked immunosorbent assay (ELISA) procedure in urine sandwich ELISA using the kits from HUMAN. Mindray MR 96A device. Detection range: 0.313-20 ng/ml, Sensitivity: 0.188 ng/ml, end ELISA kits from the company "BCM DIAGNOSTIC" allocated by ZAO BioKhimMak were used.

Results and Discussions

It is known that As can be seen from the presented study results (Table 1), elevated levels of urinary MCP-1 were found in the urine sample of patients with diabetic nephropathy 6.87 ± 0.59 pg/mg creatinine, compared to healthy subjects 1.14 ± 0.13 pg/mg creatinine. It should be noted that in 7% of patients with microalbuminuria, urinary MCP-1 levels remained low and averaged 1.43 ± 0.11 pg/mg creatinine.

In patients with pathological proteinuria (12%), the values were significantly elevated and the level of MCP-1 in urine averaged 18.63 ± 2.58 pg/mg creatinine, compared with healthy individuals.

The results are presented in Table 1 below.

These results indicate that urinary MCP-1 levels may have significant diagnostic value in assessing the renal inflammatory response in patients with diabetic nephropathy. Determination of the level of profibrogenic cytokines MCP-1 and TGF-β1 in urine is an informative non-invasive method for assessing the kidneys against the background of DN.

Table 1. Content of biochemical parameters of urine in patients with diabetic nephropathy M±m

№	Indicators	Control group n=18	Patients with DN n=58
1	Monocyte chemoattractant protein-1 (MCP-1 (pg/mg creatinine)	1,14 ± 0,13	6,87 ± 0,59*

2	Urinary liver-type fatty acid binding protein (L-FABP) (mcg/g creatinine)	1,86 ± 0,17	8,4 ± 0,78*
3	TGF-β1, пг/мл	1,76± 0,13	2,03±0,17

Note: * - reliability of differences (* - p<0.05) when comparing indicators of a group of healthy individuals.

The level of liver-type fatty acid binding protein in urine (L-FABP (mcg/g creatinine) in the urine of diabetic nephropathy is 8.4 ± 0.78 mcg/l higher than in the comparison group. 1.86 ± 0.17 mcg/g (p<0.05)

Based on the results of the study, the researchers indicated that the level of hL-FABP in urine reflects the severity of tubulointerstitial injury in kidney biopsies from patients with chronic kidney disease.

However, the dynamics of hL-FABP levels in the kidneys and changes in hL-FABP levels in urine in diabetic nephropathy, especially in tubulointerstitial damage, have not been studied.

In our studies, we attributed the revealed facts of increased hL-FABP protein in urine to increased excretion of hL-FABP in urine from proximal tubules of patients with DN. The expression levels of hL-FABP protein both in the kidneys and in urine significantly correlated with the degree of tubulointerstitial damage, macrophage infiltration, and type I collagen deposition.

Recently, urinary excretion of liver-type fatty acid binding protein (L-FABP) has been clinically recognized as a useful biomarker for monitoring chronic kidney disease and early detection of acute kidney injury.

In our studies, we considered that the increase in urinary hL-FABP excretion was caused by the increase in urinary hL-FABP excretion from the proximal tubule. One of the causes of renal hL-FABP expression is oxidative stress, which promotes severe tubulointerstitial injury.

It is known that with DN, there is depletion of interstitial capillaries and tissue hypoxia, which increases production. In the examined individuals with diabetic nephropathy, the concentration of L-FABP in the urine increased with the progression of diabetic nephropathy, which reflected the severity of DN.

Apparently, one of the reasons for the high urinary protein values is the disturbance of renal microcirculation caused by anemia, which is a factor in the progression of DN. Another reason for this result is that urinary L-FABP concentrations were closely related to urinary albumin concentrations.

It should be noted that the high value of L-FABP in urine was due to a higher risk factor for the progression of diabetic nephropathy than the presence of albuminuria at the start of the study.

These results suggest that urinary hL-FABP levels accurately reflect the extent of tubulointerstitial injury and may be useful as a real-time indicator of tubulointerstitial injury.

Thus, our results indicate that MCP-1 may be involved in the pathogenesis of common interstitial lesions of diabetic nephropathy as well as inflammatory kidney diseases through the recruitment and activation of inflammatory cells such as monocytes/macrophages (Mphs).

It has been found that MCP-1 production by the kidneys leads to the progression of diabetic nephropathy. At the same time, the level of MCP-1 in urine can monitor diabetic kidney inflammation, and this may be an important diagnostic value in assessing the effectiveness of new or combined treatments.

Urinary L-FABP measurement reflects the degree of tubulointerstitial damage and is a biomarker for early detection and monitoring of diabetic nephropathy progression in clinical practice.

In the experiment, urine markers became the basis for practical application for monitoring disease activity, assessing its prognosis and justifying therapy.

This raises the possibility that the increase in IL-18 in diabetic nephropathy may precede the observed increase in IL-6. IL-18 is constitutively expressed in renal tubular epithelium, and infiltrating monocytes, macrophages, and proximal tubular cells have been identified as potential sources of IL-18 production.

Table 2. Content of biochemical parameters of urine in patients with diabetic nephropathy M±m

As can be seen from the presented results of the study (Table 2), the content of IL-18 increases 352.74 ± 23.25 pg/ml in serum and urine significantly higher compared to healthy control individuals 252.68 ± 16.43 pg/ml.

№	Indicators	Control group n=18	Patients with DN n=58
1.	Serum interleukin-18 (pg/ml)	252,68 ± 16,43	352,74 ± 23,25
2.	Interleukin-18 in urine (pg/ml)	598,47± 26,43	709,58± 32,63
3.	Interleukin-18 IL-18/Level creatinine(mg/g)	7,24± 0,69	12,89± 1,14*
4.	Interleukin-6 serum (pg/ml)	0,85± 0,05	3,64± 0,27*

Note: * – differences relative to the comparison group are significant (* – p<0.05).

That elevated levels of interleukin-6 lead to the formation of superoxide radicals and oxidative stress, which in turn negatively affects the efficiency of metabolism of the latter fatty acids.

As can be seen from the results of the study, high levels of interleukin-6 3.64±0.27 pg/ml in blood serum and urine are often associated with pronounced excretion of albumin in urine in individuals with diabetic nephropathy; however, serum and urine levels do not correlate with each other.

Analysis of the obtained results of the study showed a significant increase in the level of IL-6 in the urine of patients with DN P<0.05.

Cytokines, in turn, can have a negative impact on the protein barrier glomerular permeability, which may lead to changes in renal cell hemodynamic factors such as thickening of the glomerular basement membrane, expansion of renal mesangial cells (extraglomerular or intraglomerular cells) and hyperplasia of extraglomerular cells, a matrix that plays a crucial role in diabetic nephropathy in people with long-standing type 2 diabetes.

In this regard, it is advisable to conduct a comprehensive study of parameters, including the analysis of such markers as albumin, IL-6; IL-18, and CAG, Monocyte chemoattractant protein-1 (MCP-1 (pg / mg creatinine), as well as an assessment of liver-type fatty acid binding protein in urine (L-FABP mcg / g creatinine).

This will allow us to identify early violations of the functional state of the kidneys and identify groups of patients who need particularly effective therapy.

Conclusion

As a result, biomarkers of diabetic kidney pathology, identified on the basis of proteomics studies, prove a direct relationship between oxidative stress and inflammatory reactions in individuals with diabetes, which by regulating urinary inflammatory biomarkers in this patient population indicates their diagnostic value and improves therapeutic options for diabetic nephropathy.

Therefore, the detection of the above-mentioned markers in the acute period of the disease requires dynamic monitoring of the functional state of the kidneys and the implementation of nephroprotective therapy.

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