Diagnostic Value of Sensitive Biomarkers for Early Kidney Damage in Diabetic Patients with Normoalbuminuria

Dong Zhang¹, Qiu-Xia Han², Ming-Hui Wu¹, Wan-Jun Shen¹, Xiao-Li Yang¹, Jia Guo², Shao-Kang Pan², Zhang-Suo Liu², Li Tang¹, Guang-Yan Cai¹, Xiang-Mei Chen³, Han-Yu Zhu¹

¹Department of Nephrology, Chinese People's Liberation Army General Hospital, Chinese People's Liberation Army Institute of Nephrology, State Key Laboratory of Kidney Diseases, National Clinical Research Center for Kidney Diseases, Beijing Key Laboratory of Kidney Diseases, Beijing 100853, China
²Department of Nephrology, The First Affiliated Hospital of Zhengzhou University, Research Institute of Nephrology in Zhengzhou University, Key Laboratory of Precision Diagnosis and Treatment for Chronic Kidney Disease in Henan Province, Zhengzhou, Henan 450052, China

Dong Zhang and Qiu-Xia Han contributed equally to this work.

To the Editor: Diabetic kidney disease (DKD) is the most common cause of end-stage renal disease (ESRD); however, the onset of DKD is difficult to detect.¹ When persistent microalbuminuria becomes detectable, DKD has already progressed to the third disease stage, and finding biomarkers that are more sensitive than microalbuminuria is therefore necessary to indicate kidney damage at an earlier stage of DKD.² Both glomerular and tubulointerstitial damages have been repeatedly demonstrated to be important factors in the pathophysiology of DKD.³ Therefore, we investigated the expression levels of six markers closely related to the glomerulus and renal tubule.

A total of 51 type 2 diabetic patients (diabetes duration >10 years) with normoalbuminuria and 27 healthy participants were enrolled in a cross-sectional study. The urine levels of the glomerular and tubular injury biomarkers such as podocalyxin, nephrin, podocin, neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule-1 (KIM-1), and tissue inhibitor of metalloproteinase-2 (TIMP-2) were measured by ELISA methods. Clinical data and biological parameters were assessed for all participants. Blood pressure and urine albumin-to-creatinine ratio data were the average results of three measurements.

We compared the ratios of six biomarkers to creatinine in urine between the two groups. The ratios of five biomarkers to creatinine were greater in the urine of diabetic patients with normoalbuminuria than in controls (P < 0.001 in podocalyxin, nephrin, and KIM-1; P < 0.010 in NGAL; and P < 0.050 in podocin). However, the ratio of TIMP-2 to creatinine in urine was not significantly different between the two groups (P = 0.130). We performed receiver operating characteristic (ROC) analyses of the five biomarkers that had statistically significantly higher concentrations in the group of diabetic patients with normoalbuminuria than those in the control group [Figure 1]. Four biomarkers had an area under the curve (AUC) >0.7; ranked from the best to the worst, these biomarkers were podocalyxin (0.879, 95% confidence interval [CI]: 0.806–0.952), nephrin (0.845, 95% CI: 0.762–0.929), KIM-1 (0.800, 95% CI: 0.700–0.900), and NGAL (0.727, 95% CI: 0.614–0.840). However, the AUC of podocin was low (0.658, 95% CI: 0.537–0.779).

Early identification of kidney damage in diabetic patients is crucial because it provides an opportunity to prevent the incidence of DKD or even slow the progression of ESRD attributed to DKD.

Figure 1: ROC curve analysis of podocalyxin, nephrin, podocin, NGAL, and KIM-1. The AUC values for these markers were 0.8791, 0.8453, 0.6576, 0.7269, and 0.7996, respectively. NGAL: Neutrophil gelatinase-associated lipocalin; KIM-1: Kidney injury molecule-1; AUC: Area under the ROC curve; ROC: Receiver operating characteristic.

Address for correspondence: Dr. Han-Yu Zhu, Department of Nephrology, Chinese People’s Liberation Army General Hospital, Chinese People’s Liberation Army Institute of Nephrology, State Key Laboratory of Kidney Diseases, National Clinical Research Center for Kidney Diseases, Beijing Key Laboratory of Kidney Diseases, Fuxing Road 29, Haidian District, Beijing 100853, China
E-Mail: hanyuzhu301@126.com

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

© 2018 Chinese Medical Journal | Produced by Wolters Kluwer - Medknow

Received: 01-08-2018 Edited by: Ning-Ning Wang
How to cite this article: Zhang D, Han QX, Wu MH, Shen WJ, Yang XL, Guo J, Pan SK, Liu ZS, Tang L, Cai GY, Chen XM, Zhu HY. Diagnostic Value of Sensitive Biomarkers for Early Kidney Damage in Diabetic Patients with Normoalbuminuria. Chin Med J 2018;131:2891-2.
diabetes. Currently, microalbuminuria is generally thought to be an early marker of DKD in clinical practice. However, a previous study revealed changes in the structure and decreased densities of podocytes in DKD patients with normoalbuminuria by using morphometric analysis based on kidney biopsy. Although biopsy is the gold standard for diagnosing DKD, it is not needed in most cases due to its invasive nature. In addition, biopsy has not been widely applied for routine use in clinical laboratories worldwide due to its technical complexity and need for experienced personnel. The ELISA method we used in the current study is safe, easy, quick, and sensitive. Our results clearly demonstrated that podocyte injury is present before the appearance of microalbuminuria in patients with diabetes. Elevated levels of podocyte-specific proteins, including podocalyxin, nephrin, and podocin, were observed in the diabetic normoalbuminuric patient group, and our results were consistent with those of previous studies. However, the mechanism underlying this phenomenon has not yet been fully elucidated. Some studies demonstrated that vesicles from the apical cell surface are shed into urine with podocyte biomarkers and provided evidence for this phenomenon in diabetic patients by demonstrating the presence of vesicles in urine using immunoelectron microscopy and immunofluorescence. Recent studies have demonstrated that the roles of both glomerular and tubular damages in the early stage of DKD are very important and our research supported this hypothesis. In addition to the markers of glomeruli, we also found some tubular markers, such as NGAL and KIM-1, whose levels were increased in diabetic patients with normoalbuminuria compared to those in the controls. Considering these data together, inflammatory stimuli and the hyperglycemic state likely cause glomerular as well as tubular damage in the early stage of DKD. ROC analysis showed that the AUC values for podocalyxin, nephrin, NGAL, and KIM-1 were >0.7. Thus, our findings indicate that these markers may have potential clinical applications in the early identification of high-risk individuals, thereby allowing the initiation of early treatment and the prevention or delay of DKD.

As a corollary, we have shown that a high percentage of DKD patients exhibit increased levels of these markers without microalbuminuria, suggesting their potential utility as early biomarkers. Further confirming these proteins to be biomarkers of preclinical DKD would promote our understanding of DKD pathogenesis and potentially provide new and earlier therapeutic targets. We recommend strict blood sugar control, more frequent follow-up, and appropriate drug applications to protect the kidneys of diabetic patients with early signs of DKD.

Declaration of patient consent
The authors certify that they have obtained all appropriate patient consent forms. In the forms, the patients/patients' guardians have given their consent for their images and other clinical information to be reported in the journal. The patients/patients' guardians understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

Financial support and sponsorship
This study was supported by the grants from the National Key R&D Program of China (No. 2016YFC1305500 and No. 2016YFC1305404), the National Natural Science Foundation of China (No. 61471399, No. 61671479, and No. 81670663), the Joint Funds of National Natural Science Foundation of China and Henan Province (No. U1604284), and the Special Research Project on Health Care of the Chinese People’s Liberation Army (No. 15BJZ35).

Conflicts of interest
There are no conflicts of interest.

References
1. Siddiqui K, Joy SS, Ilias S, Alzeer HS, Al-Rubeaan K. Urinary biomarkers reporting weakness and validation failure in type 2 diabetic nephropathy: Systematic review. Biomark Med 2018;12:487-99. doi: 10.2217/bmm-2017-0338.
2. Yang XL, Yu HJ, Zhu HY, Zheng Y, Han QX, Cai GY, et al. Potential value of Datura stramonium agglutinin-recognized glycopolymers in urine protein on differential diagnosis of diabetic nephropathy and nondiabetic renal disease. Chin Med J 2018;131:180-7. doi: 10.4103/0366-6999.222328.
3. Vijay S, Hamide A, Senthilkumar GP, Mehalingam V. Utility of urinary biomarkers as a diagnostic tool for early diabetic nephropathy in patients with type 2 diabetes mellitus. Diabetes Metab Syndr 2018;12:649-52. doi: 10.1016/j.dsx.2018.04.017.
4. Marshall CB. Rethinking glomerular basement membrane thickening in diabetic nephropathy: Adaptive or pathogenic? Am J Physiol Renal Physiol 2016;311:F831-43. doi: 10.1152/ajprenal.00313.2016.
5. Dalla Vestra M, Masiero A, Roiter AM, Saller A, Crepaldi G, Fioretto P, et al. Is podocyte injury relevant in diabetic nephropathy? Studies in patients with type 2 diabetes. Diabetes 2003;52:1031-5.
6. Hara M, Yamagata K, Tomino Y, Saito A, Hirayama Y, Ogasawara S, et al. Urinary podocalyxin is an early marker for podocyte injury in patients with diabetes: Establishment of a highly sensitive ELISA to detect urinary podocalyxin. Diabetologia 2012;55:2913-9. doi: 10.1007/s00125-012-2661-7.