Research in Health Science
ISSN 2470-6205 (Print) ISSN 2470-6213 (Online)
Vol. 9, No. 4, 2024
www.scholink.org/ojs/index.php/rhs

Original Paper

Progress in the Study of Diabetic Nephropathy Markers

Lesheng Wang[1,2], Yanjiao Zhang[2], Yongyue Hu[2], Rui Su[2], Jiangli Yan[2], Chuanmei Sun[2], Lin Luo[2], Shengjun Liu[2], Jinzhi Lu[1*]

[1] The First Affiliated Hospital of Yangtze University, Hubei Province 434000, China

[2] Department of Clinical Laboratory, Traditional Chinese Medicine Hospital of Meishan, Si'chuan Province 620000, China

[*] Corresponding author: Jinzhi Lu, The First Affiliated Hospital of Yangtze University, Hubei Province 434000, China

Received: October 9, 2024 Accepted: November 2, 2024 Online Published: November 5, 2024

doi:10.22158/rhs.v9n4p41 URL: http://dx.doi.org/10.22158/rhs.v9n4p41

Abstract

Diabetic nephropathy (DN) is the principal consideration of end-stage diabetic nephropathy in China, second only to chronic glomerulonephritis. It is the most common complication of diabetes and the cause of death in diabetic patients. The clinical manifestations of DN are proteinuria, edema, hypertension, and progressive renal damage, and its continuous development can turn into nephrotic syndrome and uremia. It has been reported that urinary microalbumin, cystatin C and homocysteine are related to the diagnosis of renal diseases. In addition, pathological examination of renal biopsy, as the golden standard, plays a vital role in clinical diagnosis of DN. In the meantime, with the rise of biomarkers research, such as Genetic Markers, miRNAs and AGEs, we can evaluate the renal function of diabetic patients by detecting these markers as early as possible. Taking into account the above information, we focus on research advances in the association of microalbuminuria, urinary inhibitory C, serum homocysteine, renal biopsy and biomarkers with DN.

Keywords

Diabetic nephropathy, microalbuminuria, cystatin C, homocysteine, renal biopsy, biomarkers

Introduction

Diabetic nephropathy (DN) is one of the common causes of End-stage renaldisease (ESRD), which can lead to the death of diabetic patients. Its clinical manifestation is characterized by persistent proteinuria and/or progressive decrease of glomerular filtration rate (GFR) [1]. According to a survey by the Chinese Diabetes Association in 2020, 20% to 40% of the total number of diabetic patients in China are complicated with DN, and the number of cases is still rising [2-4]. In 2021, the latest edition of the

Global Diabetes Map shows that there are currently about 140 million people with diabetes in China, and the number of people with diabetes is expected to increase by about 34 million compared to 30 years ago by the middle of the 21st century [5]. Hemodialysis is a common treatment for DN patients who develop to renal failure, while long-term dialysis treatment increases the psychological pressure and economic burden of patients [6]. At present, the five recognized clinical stages of renal function and structural lesion progression in diabetic patients are as follows: (1) Glomerular hyperfiltration period (Initial clinical stage), (2) Normal albuminuria stage (preclinical), (3) Early stage of diabetic nephropathy (Early diabetic nephropathy stage), (4) Clinical diabetic nephropathy stage or overt diabetic nephropathy stage (Clinical diabetic nephropathy stage), (5) Kidney failure (uremia stage). Previous studies have reported that urinary microalbumin, serum cystatin C, homocysteine and other detection indicators have a positive effect on the early detection of renal damage in diabetic patients [7-9]. Therefore, we will elaborate on the role and clinical significance of urinary microalbumin, serum cystatin C and homocysteine in the diagnosis of DN.

Urinary Microalbumin

Albumin is one of the critical proteins in plasma. Typically, most of the albumin membrane does not cross the glomerular basement membrane and contains less than 20 mg/L of albumin in the urine of healthy humans. Pathological conditions, when the glomerular basement membrane damage to make its permeability changes, albumin in urine presents proteinuria [10]. Previously, most studies agreed that the early laboratory diagnosis of DN was defined as the presence of persistent urinary albumin and impaired glucose tolerance, which could be diagnosed at 20 μg/min or 30mg/24h in the absence of other renal or urinary tract diseases by clinicians and laboratory tests [7]. Therefore, the determination of urinary microalbumin has a great early warning function in reflecting early kidney disease and kidney injury.

Researches have reported that early detection of urinary microalbumin in diabetic patients is beneficial to prevent or delay the progression of the disease [11]. Another study shows that the positive rate of urine trace albumin with longer duration of diabetes and the deterioration [12]. Typically, random urine is tested in clinical practice, which makes it easier for patients to sample and obtain test results quickly. Furthermore, the ratio of urinary albumin to urinary creatinine (UACR) was used to determine the status of patients [13]. According to the latest research, although the UACR of patients with DN is generally higher than that of patients with simple diabetes [14], in a sense, random urine samples are easily affected by patients' diet, drugs and their own physical conditions, which will interfere with the test results to a certain extent [15]. Meanwhile, due to the difference in individual physical condition, about 30% of the patients with DN in the urine trace albumin detection rate is extremely low [16]. Given the above, there are limitations in using urine microalbumin or UACR alone to determine a patient's status.

Cystatin C

Cystatin C (Cys C) is a protein composed of 122 amino acid residues with a molecular weight of 13.3 KD [17]. It can cross the glomerular basement membrane without being reabsorbed and secreted by renal tubular epithelial cells, making it an ideal endogenous marker of changes in glomerular filtration rate

(GFR). It is generally believed that Cys C is one of the most sensitive indicators for the detection of renal function, which is not affected by sample hemolysis, jaundice, and blood lipids. Due to its characteristics, the amount of Cys C in the serum is completely dependent on the filtration capacity of the kidney, so it can reflect the degree of renal damage in diabetic patients and is an independent risk factor for DN.

It has been reported that serum Cys C changes earlier than serum creatinine, urea nitrogen, and urinary microalbumin at the initial diagnosis of DN [18]. The sensitivity of serum Cys C, β2-microglobulin and urinary microalbumin for early diagnosis of DN was 79.7%, 50.6% and 75.9% respectively [19]. A study showed [20] that in 150 patients with type 2 diabetic nephropathy, the content of Cys C in patients with pure diabetes, early diabetic nephropathy and the middle and advanced diabetic nephropathy was higher than that in the control group. In different diabetic nephropathy groups, the level of Cys C in the diabetic nephropathy group was higher than that in the pure diabetic nephropathy group, and the middle and late diabetic nephropathy group was higher than that in the early diabetic nephropathy group. As a result, rapid determination of serum Cys C can reflect the severity of renal function changes in the course of diabetes earlier and more accurately.

Homocystein

Homocysteine, as a sulfur-containing amino acid, is an important intermediate in the metabolism of methionine and L-cysteine. There are two metabolic pathways of re-methylation and sulfur conversion in the human body, among which the sulfur conversion mainly occurs in the kidney[21]. A variety of enzymes such as methionine synthase, cystathionine beta synthase (CBS), vitamin B12, folate, and vitamin B6 are involved in the metabolism of homocysteine. In general, the rapid conversion of Hcy to glutathione and S-adenosyl-methionine in vivo is associated with liver detoxification, free radical clearance, and DNA repair. Therefore, an increase in Hcy means a decrease in the production of two substances, glutathione and S-adenosylmethionine, which can be seriously harmful to health. So, homocysteine is an indicator of our health. Serum homocysteine level greater than 15 umol/L can be diagnosed as "hyperhomocysteinemia " [22], which is a risk factor for cardiovascular and cerebrovascular diseases and DN. Currently, high performance liquid chromatography (HPLC), enzyme-linked immunosorbent assay (ELISA) and fluorescence quantitative method are widely used in clinic to detect homocysteine [23], but these methods are easily affected by age, gender, constitution and lifestyle of patients. Although there are many influencing factors in the detection of homocysteine, it is still an important detection method for the clinical diagnosis of DN, cardiovascular diseases and tumors due to its important pathological role [24].

Recent studies domestic and overseas have shown that the possible pathogenic mechanisms of Hcy in DN include: (1) damage to endothelial and mesangial cells by inducing oxidative stress and cytotoxic effects [25]; (2) induce the proliferation of renal vascular smooth muscle cells and promote vascular sclerosis; (3) strengthen the function of blood coagulation, platelet aggregation and thrombosis, affect the coagulation and fibrinolytic system, and further lead to renal microcirculation disorders. The interaction of the above pathogenic mechanisms causes microvascular lesions in the kidneys of diabetic

patients [26]. Homocysteine is more sensitive than serum creatinine and cysteine in reflecting the degree of renal damage [27-29]. The level of homocysteine in diabetic patients is higher than that in healthy populations, and more than 35% of patients with type 2 diabetes suffer from hyperhomocysteinemia. With the occurrence of diabetic microangiopathy complications (such as DN and retinopathy), the content of homocysteine in serum is also increasing. Therefore, early detection of homocysteine level and timely intervention by drugs or other conditions can effectively slow down the progress of diabetic microangiopathy.

Pathoiogical Examination of Renal Biopsy

In the early 1950s, Danish doctors Iversen and Bran took the lead in implementing percutaneous nephrolithotomy in patients with kidney diseases, ushering in a new era in the pathological diagnosis of kidney biopsy [30]. With the development of medical technology, especially the emergence of commercial antibody reagents used to detect various immunoglobulins, complement, fibrinogen and other components (such as hepatitis B virus antigen and type III collagen) deposited in renal tissue, as well as the great improvement of the safety of renal puncture technology, percutaneous renal puncture technology and renal biopsy have become an important means of clinical diagnosis of renal diseases. It has been reported that diffuse mesangial dilatation lesions can be observed in the kidneys of patients during the first 5 years of diabetes onset through ordinary light microscopy [31]. With the progress of the disease, 25% of patients with advanced diabetic nephropathy may have glomerular basement membrane thickening and matrix hyperplasia nodules, the latter also known as Kimmelstiel-Wilson nodules [32]. Compared with the diffuse mesangial dilatation of the kidney, patients with Kimmelstiel-Wilson nodules have more impaired renal function [33], which means that the patient has a worse prognosis. In addition, it should be noted that less than 10% of diabetic patients will not develop DN [34]. According to the differentiation of renal pathological morphology, the renal pathological changes of patients with diabetic non-diabetic nephropathy (NDRD) can be divided into IgA nephropathy, membranous nephropathy, mesangial proliferative glomerulonephritis, hypertensive nephrosclerosis, renal damage, minimal change disease, regional glomerulosclerosis and crescentic glomerulonephritis. IgA nephropathy is the most common pathological change in NDRD patients in China [35].

The pathological changes in each stage of diabetic nephropathy are as follows: (1) prominent features are glomerular ultrafiltration and glomerular afferent arteriole dilatation. (2) glomerular capillary basement membrane thickened and mesangial matrix slightly widened. (3) The basement membrane thickened, the mesangial matrix widened obviously, and the arterioles became glassy. (4) Glomerulopathy is more serious, with partial glomerulosclerosis and tubular atrophy. (5) most nephron atresia, often accompanied by retinopathy. Pathological examination of renal biopsy is the standard for diagnosis of diabetic renal injury at present. However, this is a traumatic operation, and patients should be cautious when they are complicated with hypertension, renal insufficiency or bleeding risk [36]. To some extent, it restricts the further development of the technology. However, renal biopsies must be

performed when the following clinical criteria are met :(1) short-term type 1 diabetes, (2) diagnosis of autoimmune disease, (3) mild or no retinopathy, (4) presence of red cell tubule in urine (active urinary deposition), (5) significant and persistent proteinuria [37, 38], and (6) a family history of non-diabetic nephropathy [39].

Genetic Markers

Genetic studies of DN have identified specific genes that may predispose individuals to the disease. By analyzing 297 samples (including 157 patients with confirmed DN), Xu CW et al. studied the correlation between activity-related variants of TAFI coding genes (505A/G,1040C/T) and DN. In the end, they confirmed that on the one hand, at 1040 c/G, T allele frequency and part in the general population who are closely associated with an increased risk of DN, on the other hand, in 505 a/G, almost no statistically significant differences between patients and the control group [40]. In a study of 207 polymorphisms in paraoxonase 1 (PON1), L55M and Q192R appeared to be genetic markers involved in the development of DN in type 1 diabetes mellitus (T1D) [41]. In addition, it has been reported that elevated CYP2E1 gene expression and protein levels in peripheral lymphocytes are associated with prolonged diabetes and chronic renal failure, and it is recommended that lymphocyte CYP2E1 levels can be used as an early risk predictor of diabetic nephropathy [42].

Urine Micro-RNAs (miRNAs)

In recent years, a large number of studies have been conducted worldwide on the role of micrornas in the pathogenesis of DN. Based on the survey, Yang Yeyi et al. [43] found through investigation that miRNA-377, miRNA-192, miRNA-216/217 and miRNA-144 were increased in body fluids of DN patients, while miRNA-21 and miRNA-375 were decreased. Thus, they believe that it is completely reasonable: urine specific mirna can be used as one of the novel biomarkers for early diagnosis of diabetic nephropathy. In another study, researchers found lower levels of miR-23b in the peripheral blood of diabetic patients and in the kidneys of type 1 or type 2 diabetic animals compared with non-diabetic patients. Therefore targeted miR - 23 b as a new potential therapeutic targets will be good for early treatment of patients [44].

Advanced Glycation End Products (AGEs)

Protein glycosylation and advanced glycosylation end products (AGEs) are formed by the reaction of reducing sugars with the amino groups of proteins, lipids and nucleic acids and play an important role in the pathogenesis of diabetic complications such as kidney disease [45]. Studies have shown that accumulation of AGEs, enhanced generation of reactive oxygen species (ROS) and activation of protein kinase C (PKC) can induce glomerular hypertrophy and podocyte apoptosis, thereby promoting the occurrence and progression of DN [46].

Summary

DN is one of the most dangerous complications of diabetes, a serious threat to the patient's life. With the deepening of research, its pathogenesis is being elucidated. The onset of DN is insidious, and it is difficult to diagnose at the early stage. So once DN is diagnosed clinically, most patients have already

suffered from end-stage renal disease, which brings serious economic pressure and mental burden to patients and their families. The sensitivity and specificity of serum Cys C and homocysteine in the diagnosis of early renal disease are higher than those of creatinine and urea nitrogen, which can be used as markers for early screening of renal disease. At the same time, urinary microalbumin can be used as a routine screening program because of its flexibility and specificity. In recent years, due to the continuous development of clinical laboratory technology, multi-item joint detection has played a positive role in the diagnosis of DN [47], such as urinary albumin creatinine ratio, homocysteine, serum high-sensitivity C-reactive protein, etc. In addition, although renal pathological biopsy plays a vital role as the gold standard for the diagnosis of renal injury, the risk of surgical trauma, stress and bleeding should be considered in practical work. At the same time, with the rise of biomarkers such as Genetic Markers, miRNAs, and AGEs, we can detect these markers early to evaluate kidney function in patients with diabetes for early prevention and intervention.

In the process of clinical treatment decision-making, we should fully evaluate the patient's physical condition and the severity of the disease, and finally choose the appropriate diagnosis and treatment means. In short, with the continuous development of medical technology and the improvement of the sensitivity of detection systems, these test markers will play an increasingly important role in the early diagnosis of DN!

References

[1] J.A.j.o.k.d.t.o.j.o.t.N.K.F., KDOQI Clinical Practice Guidelines and Clinical Practice Recommendations for Diabetes and Chronic Kidney Disease, *49*(2007) S12-154. https://doi.org/10.1053/j.ajkd.2006.12.005.

[2] M. Wakisaka, M. Kamouchi, T.J.I.j.o.m.s. Kitazono, Lessons from the Trials for the Desirable Effects of Sodium Glucose Co-Transporter 2 Inhibitors on Diabetic Cardiovascular Events and Renal Dysfunction, *20*(22), (2019). https://doi.org/10.3390/ijms20225668.

[3] D. Zhu, C.D. Society, Guideline for the prevention and treatment of type 2 diabetes mellitus in China (2020 edition), (2021). https://doi.org/10.3760/cma.j.cn311282-20210304-00142.

[4] X.X. Zhang, J. Kong, K.J.J.o.D.R. Yun, Prevalence of Diabetic Nephropathy among Patients with Type 2 Diabetes Mellitus in China: A Meta-Analysis of Observational Studies, *2020*(1), (2020) 1-11. https://doi.org/10.1155/2020/2315607.

[5] H. Sun, P. Saeedi, S. Karuranga, et al., IDF Diabetes Atlas: Global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045, Diabetes research and clinical practice, *183*(2022), 109119. https://doi.org/10.1016/j.diabres.2021.109119.

[6] R. Saran, B. Robinson, K.C. Abbott, et al., US Renal Data System 2017 Annual Data Report: Epidemiology of Kidney Disease in the United States, American journal of kidney diseases : the official journal of the National Kidney Foundation, *71*(3 Suppl 1), (2018) A7. https://doi.org/10.1053/j.ajkd.2018.01.002.

[7] I. de Boer, T. Rue, Y. Hall, et al., Temporal trends in the prevalence of diabetic kidney disease in the United States, *305*(24), (2011) 2532-9. https://doi.org/10.1001/jama.2011.861.

[8] N.H. Kim, J.S. Hyeon, N.H. Kim, et al., Metabolic changes in urine and serum during progression of diabetic kidney disease in a mouse model, Archives of biochemistry and biophysics, *646*(2018) 90-97. https://doi.org/10.1016/j.abb.2018.03.042.

[9] D.A. de Luis, N. Fernandez, M.L. Arranz, et al., Total homocysteine levels relation with chronic complications of diabetes, body composition, and other cardiovascular risk factors in a population of patients with diabetes mellitus type 2, Journal of diabetes and its complications, *19*(1), (2005) 42-6. https://doi.org/10.1016/j.jdiacomp.2003.12.003.

[10] M. Bhattarai, B.J.J.o.N.M.C. Bhandari, Microalbuminaria in hypertension: a review, *1*(2), (2012). https://doi.org/10.3126/jonmc.v1i2.7292.

[11] J. Verhave, R. Gansevoort, H. Hillege, et al., An elevated urinary albumin excretion predicts de novo development of renal function impairment in the general population, (92), (2004) S18-21. https://doi.org/10.1111/j.1523-1755.2004.09205.x.

[12] N. Pasko, F. Toti, A. Strakosha, et al., Prevalence of microalbuminuria and risk factor analysis in type 2 diabetes patients in Albania: the need for accurate and early diagnosis of diabetic nephropathy, *17*(4), (2013) 337-41. https://doi.org/10.1002/14651858.CD004029.pub4.

[13] Y. Watanabe, S. Mitomo, T. Naganuma, et al., The Impact of Diabetes Mellitus on Clinical Outcomes after Percutaneous Coronary Intervention with Drug-Eluting Stents for Left Main Distal Bifurcation Lesions in Patients with Chronic Kidney Disease, (6), (2020) 10. https://doi.org/10.1159/000508465.

[14] Y. Yang, C. Zeng, K. Yang, et al., Genome-wide Analysis Reflects Novel 5-Hydroxymethylcytosines Implicated in Diabetic Nephropathy and the Biomarker Potential, *3*(1) (2022) 49-60. https://doi.org/10.20517/evcna.2022.03.

[15] Dagher, A.J.C.J.o. Diabetes, Is Obesity Caused by Food Addiction?, *37*(supp_S2), (2013) S235-S236. https://doi.org/10.1016/j.jcjd.2013.03.122.

[16] J.H. An, Y.M. Cho, H.G. Yu, et al., The clinical characteristics of normoalbuminuric renal insufficiency in Korean type 2 diabetic patients: a possible early stage renal complication, Journal of Korean medical science, *24 Suppl*(Suppl 1), (2009) S75-81. https://doi.org/10.3346/jkms.2009.24.S1.S75.

[17] H.M. Hamed, S.A. El-Sherbini, N.A. Barakat, et al., Serum cystatin C is a poor biomarker for diagnosing acute kidney injury in critically-ill children, Indian journal of critical care medicine : peer-reviewed, official publication of Indian Society of Critical Care Medicine, *17*(2), (2013) 92-8. https://doi.org/10.4103/0972-5229.114829.

[18] M.S. Elsayed, A.E. Badawy, A.E. Ahmed, et al., Serum cystatin C as an indicator for early detection of diabetic nephropathy in type 2 diabetes mellitus, (1), (2019). https://doi.org/10.1016/j.dsx.2018.08.017.

[19] C. Shunyi, C. Huiyi, Z. Lili, et al., Diagnostic value of serum cystatin C, β2 microglobulin and urinary microalbumin in early diabetic nephropathy, Journal of practical medicine, *027*(009), (2011) 1678-1680. https://doi.org/10.3969/j.issn.1006-5725.2011.09.065.

[20] W. Limei, Diagnostic value of homocysteine, cystatin C and urinary microalbumin to creatinineratio in diabetic nephropathy, Chinese Journal of Modern Drug Application, *17*(08), (2023) 64-67. https://doi.org/10.14164/j.cnki.cn11-5581/r.2023.08.018.

[21] E. Muzurovi, I. Kraljevi, M. Solak, et al., Homocysteine and diabetes: Role in macrovascular and microvascular complications, *35*(3), (2021) 107834-. https://doi.org/10.1016/j.jdiacomp.2020.107834.

[22] C.H.L. Writing Group of Chinese Guidelines for the Management of Hypertension, Chinese Society of Cardiology, Chinese Medical Doctor Association Hypertension Committee, Hypertension Branch of China International Exchange, P.A.f. Medical, H.B.o.C.G.M.A. Health Care, 2018 Chinese guidelines for the management of hypertension, Chinese Journal of Cardiovascular Medicine, *24*(01), (2019) 24-56. https://doi.org/CNKI:SUN:YLYS.0.2019-05-084.

[23] W. Yu., Common methods and influencing factors of plasma homocysteine in clinical practice, Laboratory Medicine and Clinic, *7*(24), (2010) 3. https://doi.org/10.3969/j.issn.1672-9455.2010.24.083.

[24] L. Dong, C. Gangqiang, The clinical application value of homocysteine, International Journal of Laboratory Medicine, *33*(2), (2012) 3. https://doi.org/10.3969/j.issn.1673-4130.2012.02.030.

[25] Z. Jinqing, J. Ruhan, Z. Shenghao, et al., The Role of Plasma Homocysteine in Diabetic Renal Injury, Journal of Chinese Microcirculation, (5), (2009) 5. https://doi.org/CNKI:SUN:ZWXH.0.2009-05-020.

[26] S. Mao, W. Xiang, S. Huang, et al., Association between homocysteine status and the risk of nephropathy in type 2 diabetes mellitus %J Clinica Chimica Acta, *431*(2014) 206-210. https://doi.org/10.1016/j.cca.2014.02.007.

[27] Q. Lili, L.D. e, Relationship between Serum Levels of Homocysteine and Type 2 Diabetic Nephropathy, Chinese Journal of General Practice, *9*(6), (2011) 2. https://doi.org/CNKI:SUN:SYQY.0.2011-06-026.

[28] W. Kaiming, Clinical significance of combined detection of homocysteine and c-reactive protein in type 2 diabetic nephropathy patients, Experimental and Laboratory Medicine, *31*(4), (2013) 2. https://doi.org/10.3969/j.issn.1674-1129.2013.04.026.

[29] Z. Tengda, K. Xiaohu, C. Lirong, et al., The expression of homocysteine, cystatin C, and triglyceride to high-densitylipoprotein cholesterol ratio in diabetic nephropathy patients, China Modern Medicine, *23*(11), (2016) 4. https://doi.org/CNKI:SUN:ZGUD.0.2016-11-010.

[30] Y. Xiulian, M. Lijuan, W. Xiaoyan, Clinical significance of homocysteine detection in diabetic nephropathy, Ningxia Medical Journal, *32*(01), (2010) 67-68. https://doi.org/10.13621/j.1001-5949.2010.01.022.

[31] Z. ting, D. wei, L. ruizhao, et al., Progress in research on correlation between pathological characteristics and prognosisof diabetic kidney disease, Journal of Clinical Nephrology, *20*(9), (2020) 4. https://doi.org/10.3969/j.issn.1671-2390.2020.09.014.

[32] T.W. Tervaert, A.L. Mooyaart, K. Amann, et al., Pathologic classification of diabetic nephropathy, Journal of the American Society of Nephrology : JASN, *21*(4), (2010) 556-63. https://doi.org/10.1681/asn.2010010010.

[33] D. Hong, T. Zheng, S. Jia-qing, et al., Nodular glomerular lesion: a later stage of diabetic nephropathy?, Diabetes research and clinical practice, *78*(2), (2007) 189-95. https://doi.org/10.1016/j.diabres.2007.03.024.

[34] R.Z. Alicic, M.T. Rooney, K.R. Tuttle, Diabetic Kidney Disease: Challenges, Progress, and Possibilities, Clinical journal of the American Society of Nephrology : CJASN, *12*(12), (2017) 2032-2045. https://doi.org/10.2215/cjn.11491116.

[35] M. Fiorentino, D. Bolignano, V. Tesar, et al., Renal Biopsy in 2015--From Epidemiology to Evidence-Based Indications, American journal of nephrology, *43*(1), (2016) 1-19. https://doi.org/10.1159/000444026.

[36] Eugenia, Espinel, Irene, et al., Renal Biopsy in Type 2 Diabetic Patients, (2015). https://doi.org/10.3390/jcm4050998.

[37] S.M. Doshi, A.N. Friedman, Diagnosis and Management of Type 2 Diabetic Kidney Disease, Clinical journal of the American Society of Nephrology : CJASN, *12*(8), (2017) 1366-1373. https://doi.org/10.2215/cjn.11111016.

[38] A.R. Gosmanov, B.M. Wall, E.O.J.A.J.o.t.M.S. Gosmanova, Diagnosis and Treatment of Diabetic Kidney Disease, *347*(5), (2014) 406-413. https://doi.org/10.1097/MAJ.0000000000000185.

[39] N.M. Selby, M.W.J.D. Taal, Obesity, Metabolism, An updated overview of diabetic nephropathy: Diagnosis, prognosis, treatment goals and latest guidelines, *22* (2020). https://doi.org/10.1111/dom.14007.

[40] C.W. Xu, X.B. Wu, X.L. Ma, et al., Genetic variation in thrombin-activatable fibrinolysis inhibitor is associated with the risk of diabetic nephropathy, Journal of Endocrinological Investigation, *35*(7) (2012) 620-624. https://doi.org/10.1007/bf03345800.

[41] O. Fekih, S. Triki, J. Rejeb, et al., Paraoxonase 1 polymorphisms (L55M and Q192R) as a genetic marker of diabetic nephropathy in youths with type 1 diabetes, Endokrynologia Polska, *68*(1), (2017) 35-41. https://doi.org/10.5603/EP.a2016.0027.

[42] G.Y. Christina, A. Zaini, I. Othman, CYP2E1 level in peripheral lymphocytes correlates with early pathogenesis of diabetic nephropathy, Biomedical Research-India, *24*(1), (2013) 153-163. https://doi.org/10.1155/2013/578781.

[43] Y.Y. Yang, L. Xiao, J. Li, et al., Urine miRNAs: Potential biomarkers for monitoring progression of early stages of diabetic nephropathy, Medical Hypotheses, *81*(2), (2013) 274-278. https://doi.org/10.1016/j.mehy.2013.04.031.

[44] B.H. Zhao, H.Z. Li, J.T. Liu, et al., MicroRNA-23b Targets Ras GTPase-Activating Protein SH3 Domain-Binding Protein 2 to Alleviate Fibrosis and Albuminuria in Diabetic Nephropathy, Journal of the American Society of Nephrology, *27*(9), (2016) 2597-2608. https://doi.org/10.1681/asn.2015030300.

[45] V.P. Singh, A. Bali, N. Singh, et al., Advanced Glycation End Products and Diabetic Complications, Korean Journal of Physiology & Pharmacology, *18*(1), (2014) 1-14. https://doi.org/10.4196/kjpp.2014.18.1.1.

[46] K. Parwani, P. Mandal, Role of advanced glycation end products and insulin resistance in diabetic nephropathy, Archives of Physiology and Biochemistry, *129*(1), (2023) 95-107. https://doi.org/10.1080/13813455.2020.1797106.

[47] L. Wenping, Z. Qun, Z. Haiyan, Diagnostic application of the joint detection of serum Cys C, RBP Hcy and hs-CRP inpatients with diabetic nephropathy, China Modern Doctor, *52*(23), (2014) 3. https://doi.org/CNKI:SUN:ZDYS.0.2014-23-020.