JMS 2022; 5(1):20-27
UDC: 616.633:[616.379-008.64-06:616.61

SODIUM DODECYL SULFATE-POLYACRYLAMIDE GEL ELECTROPHORESIS AS A USEFUL TOOL FOR ASSESSING THE SEVERITY OF DIABETIC NEPHROPATHY

Irena Kostovska[1], Danica Labudovic[1], Katerina Tosheska Trajkovska[1], Jasna Bogdanska[1], Sonja Topuzovska[1], Julijana Brezovska Kavrakova[1], Hristina Ampova[1], Svetlana Cekovska[1]
[1]Department of Medical and Experimental Biochemistry, Faculty of Medicine, Ss.Cyril and Methodius University in Skopje, R. North Macedonia

Abstract

Proteinuria is a hallmark of diabetic nephropathy (DN). Qualitative analysis of proteinuria plays an important role in the diagnosis and prognosis of DN. SDS-PAGE is the most frequently used method for qualitative analysis of proteinuria.

The aim of this study was to test the significance of SDS-PAGE of urinary proteins in assessing the severity of kidney disease in DN patients by comparing the results of kidney laboratory tests among different proteinuric DN patients.

This study included 92 confirmed cases with DN. Urinary protein separation was performed using ultrathin horizontal gradient linear (4-22%) SDS-PAGE. According to the type of proteinuria, all patients were categorized into three groups: patients with glomerular, patients with tubular, and patients with mixed proteinuria.

Urinary microalbumin to creatinine ratio (UMCR) and glomerular filtration rate (GFR) were estimated in all patients.

Blood serum was used for biochemical analyses. SDS-PAGE analysis showed that 50% of studied DN patients had a mixed type of proteinuria. UMCR and serum creatinine were significantly increased, while eGFR was significantly decreased in patients with mixed proteinuria compared to patients with glomerular or tubular proteinuria ($p<0.001$).

We found a significant difference between patients with mixed proteinuria and patients with glomerular or tubular proteinuria regarding the duration of disease and glycaemic control ($p<0.001$). DN patients with mixed proteinuria have a significant decline in kidney function than those who excrete glomerular or tubular proteins only. SDS PAGE is a good noninvasive tool for assessing the severity of DN.

Keywords: SDS-PAGE, diabetic nephropathy, proteinuria.

Introduction

Diabetic nephropathy (DN) is the major cause of chronic kidney disease (CKD) and end-stage renal disease (ESRD) worldwide [1]. Laboratory tests play an important role in the diagnosis, management, and prognosis of DN patients.

Several laboratory tests are utilized to determine the degree of kidney failure and to monitor the course of the disease.

The most widely used laboratory tests are glomerular filtration rate (GFR) estimation, a measure of kidney function, and proteinuria detection, a measure of kidney injury [2].

Only clinical and laboratory results do not predict pathological kidney damage and cannot play a decisive role, so the diagnosis of DN involves a kidney biopsy, which is an invasive procedure with potentially significant complications. At present, a kidney biopsy is reserved only for diabetic patients who are suspected of having kidney disease other than DN [3].

Proteinuria is the main characteristic of DN. In addition to quantitative urinary protein analysis, qualitative proteinuria analysis plays a significant role in diagnosis and prognosis of DN.

Generally, a small amount of albumin is excreted in the urine, because it is almost entirely reabsorbed in the renal tubules. Damaged kidneys allow more albumin to cross the glomerular filtration barrier (GFB) into the urine, exceeding the capacity of the tubules to reabsorb [4].

DN is characteristically associated with increased urinary excretion of albumin [5].

The presence of high-molecular-weight (HMW) proteins such as albumin (69-kilo Daltons-kDa), transferrin (78 kDa), and immunoglobulin G (IgG -150 kDa) characterizes the damage of the GFB.

Proximal tubular damage is characterized by the presence of low-molecular-weight (LMW) proteins, for example retinol-binding protein (RBP-21 kDa), β2-microglobulin (11.8 kDa) and α1-microglobulin (27 kDa) [6,7]. In certain kidney diseases including DN, mixed, simultaneously glomerular and tubular proteinuria is detected [8].

The most frequently used method of separation of urinary proteins for clinical purposes is sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

The separation of urinary proteins in SDS PAGE depends on their molecular weight, therefore the urinary protein patterns reflect the location of kidney lesions.

All health professionals must recognize the significance of laboratory kidney tests to better define the severity of disease in DN patients, guide therapy, and predict outcomes.

The aim of study was to test the significance of SDS-PAGE of urinary proteins in assessing the severity of kidney disease in DN patients by comparing the results of kidney laboratory tests among different types of proteinuric DN patients.

Material and methods

In this cross-sectional study were included 92 confirmed cases of DN. Before entering the study, all DN patients referred to SDS-PAGE and other laboratory investigations signed an informed consent agreement, consistent with the ethical standards of the current Helsinki Declaration.

The study was conducted at the Department of Medical and Experimental biochemistry, Faculty of Medicine in Skopje, North Macedonia. Patients with DN were recruited from the University Clinic for Nephrology, Skopje, North Macedonia.

Inclusion criteria for DN patients were clinically proven DN, which was characterized by albuminuria (>300 mg/24 h, or a urinary microalbumin to creatinine ratio (UMCR) >300 mg/g) confirmed in two of the three tests within 3-6 months period, a continuous reduction in glomerular filtration rate (GFR), and progressive increase in blood pressure [9].

All patients were categorized into three groups by the type of proteinuria determined by SDS-PAGE: patients with glomerular, patients with tubular, and patients with mixed (glomerular and tubular) proteinuria.

Baseline demographic characteristics, medical history, and clinical data of patients were obtained from the filled questionnaire surveys.

We used spot urine samples and venous blood for analyses. Urine samples (10 ml) were collected in disposable plastic clean tubes. Blood samples were obtained in Eppendorf tubes and serum was separated by centrifugation for 10 min at 3000 rounds per minute.

Blood sera were analyzed for different biochemical parameters: glucose, urea, creatinine, total cholesterol, triglycerides, high-density lipoproteins (HDL), low-density lipoproteins (LDL), total proteins, and albumin.

Urinary microalbumin was measured using the immunoturbidimetric method, while urine creatinine was measured with a modified kinetic Jaffe reaction on biochemical analyzer ChemWell (2910 Awareness Technology, Inc.).

In spot urine samples we calculated the ratio of urinary microalbumin and urinary creatinine concentration (UMCR), since it corresponds well with urine albumin excretion in a 24-hour urine.

UMCR results were reported as mg albumin/g creatinine and are equal to the excretion of mg albumin/day [10]. The Cocroft and Gault equation was used to estimate the glomerular filtration rate (eGFR) [11].

Urinary protein separation was performed by SDS-PAGE according to Görg [12].

SDS-PAGE method was performed through several steps: preparation of gradient linear polyacrylamide gel (4-22%), preparation of urine samples (urine samples with sample buffer (1.5 M Tris / HCl, 10% SDS, pH 8.8) in ratio 1:10 were boiled for 3 minutes), electrophoresis at 5°C on Multiphor II Unit, LKB (Brown, Sweden) for 2 hours, fixation of gel, at the end staining of a gel with Coomassie blue R250.

The identification of the separated protein fractions was performed using commercially standards - a mixture of proteins with well-known molecular weight (SDS 6H Kit by Sigma, Aldrich Steinheim, Germany).

The stained gels were analyzed visually, and the findings were categorized into three groups: 1. glomerular proteinuria – albumin fraction and HMW proteins fractions; 2. tubular proteinuria - albumin and one or more LMW proteins fractions (10-69 kDa); and 3. mixed proteinuria - albumin, and both HMW and LMW proteins fractions (Figure 1).

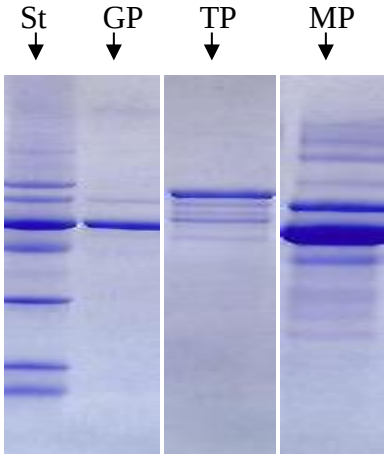

Figure 1. Different electrophoretic patterns in DN patients (St - standard, GP - glomerular pattern, TP - tubular pattern, MP - mixed pattern)

Statistical analysis

Statistical analysis was performed using MedCalc statistical package for Windows, version 15.0 (MedCalc App, Ostend, Belgium). Quantitative data are presented as mean ± SD.

ANOVA test was used to compare the differences between multiple groups. Values of $p < 0.05$ were considered to be statistically significant.

Results

Frequency of proteinuria type detected by SDS-PAGE in DN patients

The type of proteinuria in evaluated DN patients was determined by SDS-PAGE. We found that the most common type of proteinuria in DN patients was mixed proteinuria.

Among the 92 examined patients with DN, 27% of them showed a glomerular proteinuria, 23% had a tubular proteinuria, and 50% had a mixed proteinuria. The percentage of type of proteinuria in examined DN patients is presented in Figure 2.

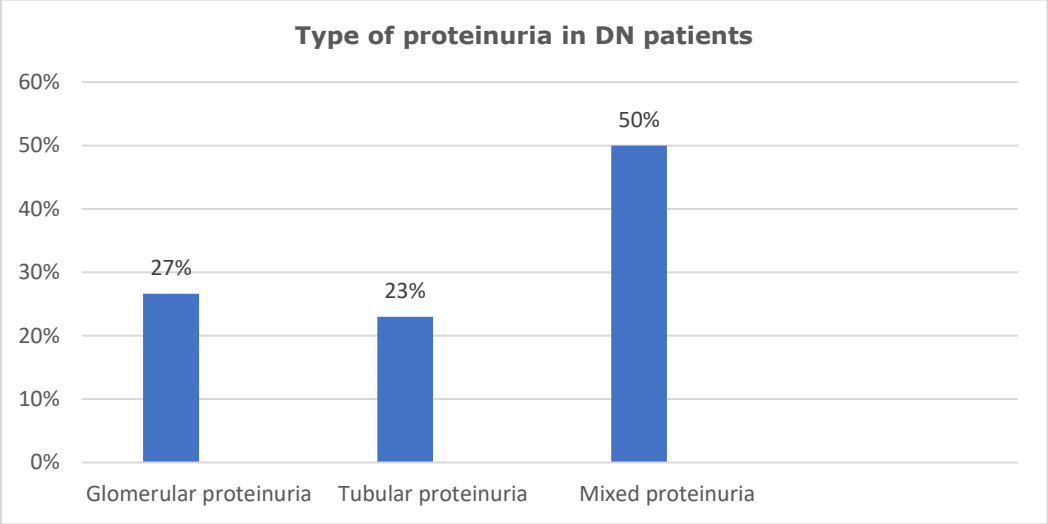

Figure 2. Frequency of type of proteinuria in DN patients

Comparison of clinical and biochemical data in DN patients with different type of proteinuria

The comparison of clinical and biochemical data in groups of DN patients categorized according to the type of proteinuria is illustrated in Table 1.

Table 1. Clinical and biochemical data in DN patients with different type of proteinuria

	Glomerular proteinuria n=25	**Tubular proteinuria n=21**	**Mixed proteinuria n=46**	**P value**
Age (years)	55.6±9.7	53.1±8.7	58.9±9.1	0.02
Duration of disease (y)	9.2±7.5	5.1±1.9	13.1±5.9	<0.001
Glucose (mmol/L)	7.2±2.7	8.5±2.7	8.9±6.3	0.03
UM/CR (mg/g)	157.8±132.8	129.6±57.9	235±121.6	<0.001
HbA1c (%)	9.1±3.1	7.5±2.5	9.5±3.8	<0.001
Total proteins (g/L)	62.1±8.1	64.8±7.4	62.9±17.5	0.028
Albumin (g/L)	37.2±7.3	34.2±7.2	33.5±6.5	0.035
Urea (mmol/L)	7.7±1.2	6.9±1.4	7.9±1.5	0.02
Creatinine (μmol/L)	85.2±10.3	81.2±15.1	102.1±16.8	<0.001
eGFR (ml min^{-1} 1.73 m^{-2})	64.1±17.1	80.1±21.9	48.8±10.9	<0.001
Total cholesterol (mmol/L)	5.9±1.5	6.5±1.6	7.5±10.5	0.048
Triglycerides (mmol/L)	2.2±1.5	2.5±1.6	2.5±1.5	0.031
HDL (mmol/L)	1.2±1.5	0.9±1.6	0.5±1.5	0.046
LDL (mmol/L)	4.2±1.3	3.5±1.9	4.5±1.5	0.025

Results are shown as mean±SD. Abbreviations: UMCR - urinary microalbumin to creatinine ratio, HbA1c - glycated hemoglobin A1c, eGFR - (estimated Glomerular Filtration Rate), LDL – low-density lipoproteins, HDL – high-density lipoproteins, y -years.

In DN patients with mixed proteinuria, UM/CR and serum creatinine were significantly elevated, while eGFR was significantly decreased compared to DN patients with glomerular and tubular proteinuria.

The disease duration and glycemic regulation in patients with DN were strongly associated with the progression of kidney disease.

Discussion

DN is characterized by persistent proteinuria accompanied by a severe decrease in kidney function. Proteinuria is an important feature of DN. Microalbuminuria is an early sign of DN and gradual progression to macroalbuminuria or proteinuria indicates a development of an overt nephropathy.

A gradual progression of proteinuria contributes to a gradual reduction in kidney function [13, 14]. Increased glomerular permeability and damage in the structure of the GFB, podocytes, glomerular basement membrane, and glomerular capillary endothelium are defined as a key feature of glomerular injury in DN.

Glomerular damage results in appearance of HMW proteins in urine, such as albumin, transferrin, and IgG. Although the significance of glomerular injury in DN proteinuria development is well established, it has been suggested that the rate of decrease in kidney function correlates better with the degree of tubular injury and tubulointerstitial fibrosis.

Tubular injury results in presence of LMW proteins in urine, such as α1-microglobulin**,** β2-microglobulin, and RBP [15].

SDS-PAGE is a well-established, reliable, non-invasive test, commonly used for screening protein abnormalities in urine and other biological fluids in routine clinical and laboratory practice. Before applying for much more expensive (molecular genetic testing) and/or invasive (kidney biopsy) diagnostics, this laboratory test may assist the clinicians in the diagnostic process, evaluating the stage and severity of DN [16, 17].

In our study, we performed SDS-PAGE to detect the type of proteinuria in patients with clinically proven DN and to assess the kidney function in different proteinuric DN patients using UMCR and eGFR as a laboratory kidney function test.

We found that the most frequent type of proteinuria in patients with DN was mixed (glomerular and tubular) proteinuria.

It has also been reported that DN could involve both glomerular and tubular lesions [7].

Patients with mixed proteinuria have a significant decline in renal function observed by increased UMCR and decreased eGFR values than patients who excreted only glomerular or tubular proteins. Both tests showed a significantly increased risk of progression to ESRD [18].

Our results demonstrated that the progression of proteinuria and kidney function impairment in DN patients were strongly associated with the duration of disease and glycemic control.

The study of Kyung Jin Yun also found that glycemic control affected the progression of DN [19], while the study of Alwakeel revealed that duration of disease more than 10 years could predict the progression of DN [20].

Early screening of kidney dysfunction is particularly important in DN patients.

Our observation is that in an early stage of DN when the kidney function tests are slightly unsettled, glomerular, or tubular protein excretion is only frequently detected, while in the stage of overt nephropathy a mixed proteinuria is observed.

A major limitation of the current study is its cross-sectional design, and a small number of studied patients.

We recommend a larger longitudinal prospective study for early identification of the type of proteinuria and monitoring the progression of proteinuria in DN patients.

Conclusion

The most common type of proteinuria in DN patients detected by SDS-PAGE is mixed proteinuria, indicating both, impaired glomerular permeability and tubular function.

The laboratory kidney tests indicate that patients with mixed proteinuria have a significant decline in kidney function than those who excrete glomerular or tubular proteins only.

We can conclude that the SDS PAGE is a good non-invasive tool for assessing the severity of DN.

References

1. Umanath K, Lewis JB. Update on Diabetic Nephropathy: Core Curriculum 2018. Am J Kidney Dis 2018;71(6):884-895.
2. Gounden V, Bhatt H, Jialal I. Renal Function Tests. [Updated 2020 Jul 20]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing 2020.
3. Gonzalez Suarez, ML, Thomas DB, Barisoni L, Fornoni A. Diabetic nephropathy: Is it time yet for routine kidney biopsy?. World J Diabetes 2013;4(6):245-255.
4. Julian BA, Suzuki H, Suzuki Y, Tomino Y, Spasovski G, Novak J. Sources of Urinary Proteins and their Analysis by Urinary Proteomics for the Detection of Biomarkers of Disease. Proteomics Clin Appl 2009;3(9):1029-1043.
5. Narva AS, Bilous RW. Laboratory Assessment of Diabetic Kidney Disease. Diabetes Spectr 2015;28(3):162-166.
6. Fiseha T, Tamir Z. Urinary Markers of Tubular Injury in Early Diabetic Nephropathy. Int J Nephrol 2016;2016:4647685.
7. Koliakos G, Papachristou F, Papadopoulou M, Trachana V, Gaitatzi M, Sotiriou I. Electrophoretic analysis of urinary proteins in diabetic adolescents. J Clin Lab Anal 2001;15(4):178-183.
8. D'Amico G, Bazzi C. Pathophysiology of proteinuria. Kidney Int 2003 Mar;63(3):809-25.
9. Persson F, Rossing P. Diagnosis of diabetic kidney disease: state of the art and future perspective. Kidney Int Suppl (2011) 2018;8(1):2-7.
10. Huan L, Yuezhong L, Chao W, HaiTao T. The urine albumin-to-creatinine ratio is a reliable indicator for evaluating complications of chronic kidney disease and progression in IgA nephropathy in China. Clinics (Sao Paulo) 2016;71(5):243-50.
11. Cocroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. Nephron 1976;16:31-41.
12. Koliakos G, Papachristou F, Papadopoulou M, Trachana V, Gaitatzi M, Sotiriou I. Horizontal SDS electrophoresis in ultra thin pore-gradient gels for the analysis of urinary proteins. Science Tools 1985;32:5–9.
13. Williams ME. Diabetic nephropathy: the proteinuria hypothesis. Am J Nephrol 2005;25(2):77-94.
14. Varghese RT, Jialal I. Diabetic Nephropathy. [Updated 2020 Jul 26]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing 2020.
15. Lee SY, Choi ME. Urinary biomarkers for early diabetic nephropathy: beyond albuminuria. Pediatr Nephrol 2015;30(7):1063-1075.
16. Roden AC, Lockington KS, Tostrud LJ, Katzmann JA. Urine protein electrophoresis and immunoelectrophoresis using unconcentrated or minimally concentrated urine samples. Am J Clin Pathol 2008;130(1):141-5.
17. Bökenkamp A. Proteinuria-take a closer look! Pediatr Nephrol 2020;35(4):533-541.
18. Polkinghorne KR. Estimated Glomerular Filtration Rate versus Albuminuria in the Assessment of Kidney Function: What's More Important?. Clin Biochem Rev 2014;35(2):67-73.
19. Yun KJ, Kim HJ, Kim MK, Kwon HS, Baek KH, Roh YJ, Song KH. Risk Factors for the Development and Progression of Diabetic Kidney Disease in Patients with Type 2 Diabetes Mellitus and Advanced Diabetic Retinopathy. Diabetes Metab J 2016 Dec;40(6):473-481.

20. Alwakeel JS, Isnani AC, Alsuwaida A, Alharbi A, Shaffi SA, Almohaya S, Al Ghonaim M. Factors affecting the progression of diabetic nephropathy and its complications: a single-center experience in Saudi Arabia. Ann Saudi Med 2011;31(3):236-242.