ORIGINAL RESEARCH

Correlation of Asymmetric Dimethylarginine With Podocytopathy Markers in Diabetic Kidney Disease Patients

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Background: Diabetic kidney disease (DKD) is the leading cause of end-stage kidney disease, and podocytopathy is an early manifestation of DKD characterized by the urinary excretion of podocyte-specific proteins, such as nephrin and podocin. Asymmetric dimethylarginine (ADMA)—a biomarker of endothelial dysfunction—is associated with progressive kidney dysfunction. However, the mechanism of endothelial dysfunction in DKD progression is unclear. The aim of this study was to investigate the correlations of ADMA levels with nephrin, podocin, and the podocin:nephrin ratio (PNR) in DKD patients.

Methods: A cross-sectional study of 41 DKD outpatients was performed in two hospitals in Jakarta from April–June 2023. The collected data included the subjects' characteristics, histories of disease and medication, and relevant laboratory data. Serum ADMA was measured using liquid chromatography, while urinary podocin and nephrin were measured using the enzyme-linked immunosorbent assay (ELISA) method. A correlation analysis was performed to evaluate the correlation of ADMA with nephrin, podocin, and PNR. Regression analysis was performed to determine confounding factors.

Results: The mean value of ADMA was 70.2 (SD 17.2) ng/mL, the median for nephrin was 65 (20–283 ng/mL), and the median of podocin was 0.505 (0.433–0.622) ng/mL. ADMA correlated significantly with nephrin ($r = 0.353$, $p = 0.024$) and PNR ($r = −0.360$, $p = 0.021$), but no correlation was found between ADMA and podocin ($r = 0.133$, $p = 0.409$). The multivariate analysis showed that body mass index was a confounding factor.

Conclusion: This study revealed weak positive correlations between ADMA and urinary nephrin and between ADMA and PNR. No correlation was found between ADMA and urinary podocin.

Keywords: diabetic kidney disease, ADMA, nephrin, podocin, podocin:nephrin ratio

Introduction

Diabetes mellitus (DM) is a major global health concern, since the prevalence is predicted to rise by 45% to 537 million by 2045. In 2021, 90 million South-East Asians were diagnosed with DM, with Indonesia alone reporting 19 million cases, affecting one in nine adults.[1] Diabetic kidney disease (DKD) is a microvascular complication of DM characterized by persistently elevated urinary albumin excretion (urinary albumin:creatinine ratio (UACR) >30 mg/g) and/or a low estimated glomerular filtration rates (eGFR < 60 mL/min/1.73 m²) in people with diabetes.[2] DKD is a common cause of end-stage kidney disease,[3] accounting for 50% of cases worldwide[4] and 28% in Indonesia, hence imposing a significant global burden. In addition to renal risk, DKD poses increased risks of infection and cardiovascular events, significantly

reducing quality of life of people with DM and burdening health-care systems. Therefore, early detection and intervention are vital for effectively managing DKD.

DKD risk factors are diverse, with chronic, subclinical, and nonresolving inflammation playing an important role in its onset and progression.[5,6] Podocytes contribute to the integrity of the glomerular filtration barrier, and an early, key event in the development of DKD is the loss of podocyte function in the kidney glomerulus.[7] Podocytes and their foot processes support the outer layer of the kidney ultrafiltration barrier, forming the glomerular slit diaphragm in an actin cytoskeleton-dependent manner.[7] Animal studies have revealed that endothelial nitric oxide synthase (eNOS) deficiency and hyperglycemia may predispose to podocyte and renal injury.[8] Increased dimethylarginine—an eNOS inhibitor—may compromise nitric oxide (NO) bioactivity, increasing kidney, cardiovascular, and metabolic disorders.[9]

Asymmetric dimethylarginine (ADMA) is a proteolysis residue of arginine methylated protein that inhibits NO synthesis and the maintenance of vascular tone and structure.[10] Approximately 80% of ADMA is metabolized by dimethylarginine dimethylaminohydrolase (DDAH), predominantly found in the kidneys. Oxidative stress renders DDAH activity ineffective, resulting in ADMA accumulation[11] ADMA is a predisposing factor of endothelial dysfunction, increasing systemic vascular resistance and blood pressure, and resulting in reduced cardiac output.[12] Increased ADMA is associated with worsening kidney function in diabetic and nondiabetic subjects. However, some studies have reported no association between ADMA, albuminuria, and decreased GFR, implying that various factors influence DKD pathogenesis.

Podocytopathy is markedly observed in DKD, evidenced by diaphragm slit changes, foot process architecture remodeling, fusion of filtration slits, and apical displacement. Podocytes detach from the glomerular basement membrane as damage progresses, resulting in the urinary excretion of nephrin and podocin.[13] Nephrin and podocin are key structural proteins of the slit diaphragm in podocytes, and their roles in podocyte function and injury differ in important ways. Nephrin, a transmembrane protein, is crucial for maintaining the integrity of the slit diaphragm, serving as a scaffold for signaling pathways that regulate the filtration barrier. A reduction in nephrin expression or function can lead to increased glomerular permeability and proteinuria, signifying podocyte dysfunction.[14] Podocin, on the other hand, is a membrane-associated protein that interacts with nephrin and other components of the slit diaphragm. It plays a key role in stabilizing the structural integrity of the filtration barrier and regulating cytoskeletal dynamics in podocytes.[15] Damage to podocin disrupts these interactions, leading to podocyte effacement and glomerular injury. While both nephrin and podocin are essential to the maintenance of podocyte structure and function, the distinct roles they play in the slit diaphragm suggest that their loss may contribute differently to the progression of podocyte damage and subsequent kidney disease.The podocin:nephrin ratio (PNR) may serve as a marker of podocyte damage and a prognostic marker for glomerulosclerosis.[16]

To date, no researchers have analyzed the correlation of endothelial dysfunction based on increased levels of ADMA in plasma with specific markers of podocytopathy, such as nephrin and podocin in urine. The objective of this study was to identify correlations between ADMA, nephrin, podocin, and PNRs in DKD patients. This study aims to lay the groundwork for research in resource-limited countries, where advanced diagnostics are often inaccessible. Given the high cost of mRNA testing for new markers like nephrin and podocin, if urinary levels of these markers prove reliable, they could offer a more cost-effective and practical diagnostic solution. This would enable broader application, improving early detection and disease monitoring in regions with limited resources, and ultimately advancing equitable healthcare.

Methods
Study Design, Settings, Participants, and Sample Size

A cross-sectional analytical study was conducted to assess the correlations among ADMA, nephrin, podocin, and PNR in 41 Indonesian DKD outpatients who visited internal medicine clinics at the Dr. Cipto Mangunkusumo and Pelni Hospitals. The inclusion criteria were adults aged 18–70 years with type 2 DM, UACR > 30 mg/g, and eGFR > 45 mL/min/1.73 m^2 who agreed to enroll in the study. Subjects were excluded if they suffered from other diseases that could cause proteinuria (eg, urinary tract infection, urolithiasis, or renal tuberculosis) based on their histories and previous examinations, had severe acute or chronic diseases (eg, diabetic complications, coronary heart disease,

cerebrovascular diseases, hypertensive crises, liver diseases, or systemic infection), were smokers or had previously smoked, or were pregnant/breastfeeding women. All patients provided informed consent, in accordance with the Declaration of Helsinki.

Ethics

The Ethical Committee of the Faculty of Medicine, Universitas Indonesia, approved this study (ref: 176/UN2.F1/ETIK/PPM.00.02/2022).

Variable and Data Sources

The subjects were consecutively recruited at the study sites. Their characteristics were collected from medical records, including demographic data, past medication histories (eg, antidiabetic drugs, lipid lowering agents, or antihypertensive agents), histories of comorbidity, physical examinations, and relevant laboratory data one month prior to primary blood and urine data collection. Approximately 5 mL of venous blood was collected from each participant in an Ethylenediaminetetraacetic acid (EDTA) container for blood analysis (complete blood count, HbA1c, urea, creatinine, eGFR, calcium, phosphate, and ADMA), and a random 15-mL midstream urine sample was collected for nephrin, podocin, and UACR measurements. ADMA was measured using liquid chromatography tandem mass spectrometry (LC-MS/MS). Nephrin and podocin were measured using a sandwich enzyme-linked immunosorbent assay (ELISA).

Statistical Analysis

The data were analyzed using SPSS® version 20. Data are presented with narration, tables, and/or figures as appropriate. Numerical data are shown as means and standard deviations if distributed normally, or medians with interquartile ranges (IQRs) otherwise. Categorical data are presented as percentages.

The relationships between ADMA, nephrin, podocin, and PNR were assessed using Pearson correlation for normally distributed data, or Spearman correlation for non-normally distributed data. To account for potential confounding variables, a multivariate linear regression was conducted. Variables included in the regression were age, gender, mean arterial pressure, BMI, HbA1c, LDL, triglycerides, GFR, ACR, and treatment with ACE inhibitors or ARBs. These variables were included if their p-values were less than 0.25 in the initial bivariate analysis. In the multivariate model, confounding variables were added stepwise, starting with those with the smallest p-values, and the adjusted R^2 values were reported to reflect the model's explanatory power.

Results

Forty-six patients with DKD were enrolled in this study. One patient withdrew; three subjects were suspected of suffering from other diseases that could cause proteinuria (2 hematuria, 1 nephrolithiasis); and one blood sample lysed in the laboratory; therefore, 41 subjects were retained for further analysis.

The participants' median age was 60 (IQR 55–63) years, 43% were aged below 60, and 43.9% were male. The median duration of diabetes was 10 (IQR 4.0–17.5) years. Hypertension was present in 80.5% of the subjects, and ACE inhibitors/ARBs were used by 58.5% of them. The median BMI was 25.95 kg/m² (IQR 21.31–29.19), and 53.7% of the participants were obese (BMI ≥ 25 kg/m²). The mean HbA1c was 7.07% (SD 0.62), and 63.4% of the subjects had HbA1c ≥ 7.0%. Moreover, 68.3% of the subjects had GFR ≥ 60 mL/min/1.73 m² and 75.6% of the subjects had ACR values < 300 mg/g. Table 1 shows the participants' demographic characteristics.

The mean ADMA concentration was 70.17 ng/mL (SD 17.22). The median value of nephrin and podocin concentrations were 65 ng/mL (IQR 20–283) and 0.505 ng/mL (IQR 0.433–0.622), respectively. The median PNR was very low at 0.0087 (IQR 0.0018–0.0267), and most of the participants (95.1%) had ADMA concentrations below 100 ng/mL.

Table I Participants' Demographic and Clinical Characteristics

Characteristics	N = 41
Age (year), median (IQR)	60 (55–63)
Duration of diabetes (years), median (IQR)	10 (4.0–17.5)
Gender, n (%)	
Male	18 (43.9)
Female	23 (56.1)
Comorbidity	
Hypertension, n (%)	33 (80.5)
Dyslipidemia, n (%)	22 (53.7)
Coronary heart disease, n (%)	11 (26.8)
ACE-i/ARB, n (%)	
Yes	24 (58.5)
No	17 (41.5)
OAD, n (%)	
Yes	40 (97.6)
No	1 (2.4)
Insulin, n (%)	
Yes	20 (48.8)
No	21 (51.2)
SBP, mmHg, mean (SD)	137.12 (SD 14.144)
DBP, mmHg, median (IQR)	81 (72.5–86.0)
Mean arterial pressure, mmHg, mean (SD)	98.03 (SD 10.200)
BMI, kg/m^2, median (IQR)	25.96 (21.31–29.19)
Hemoglobin, mmol/L, mean (SD)	7.93 (SD 1.12)
HbA1c, %, mean (SD)	7.07 (SD 0.62)
LDL, mmol/L, mean (SD)	2.97 (SD 0.87)
Triglyceride, mmol/L, median (IQR)	1.6 (1.24–2.36)
Calcium, mmol/L, mean (SD)	2.35 (SD 2.34)
Phosphate, mmol/L, mean (SD)	1.33 (SD 0.18)
Uric acid, μmol/L, mean (SD)	380.08 (SD 89.91)
Urea, mmol/L, median (IQR)	5.49 (3.57–7.12)
Blood creatinine, μmol/L, median (IQR)	79.58 (61.9–101.68)
GFR, mL/min/1.73 m^2, median (IQR)	74,8 (55.3–100.5)
ACR, mg/g, median (I`QR)	142 (55–299.5)
ADMA, ng/mL, mean (SD)	70.17 (SD 17.22)
Nephrin, ng/mL, median (IQR)	65 (20–283)
Podocin, ng/mL, median (IQR)	0.505 (0.433–0.622)
PNR	0.0087 (0.0018–0.0267)

Abbreviations: ACEi/ARB, angiotensin converting enzyme inhibitor/angiotensin receptor blocker; BMI, body mass index; DBP, M diastolic blood pressure; OAD, oral anti diabetic; HbA1c, hemoglobin A1c; LDL, low density lipoprotein; GFR, glomerular filtration rate; ACR, albumin:creatinine ratio; ADMA, asymmetric dimethylarginine; PNR, podocin:nephrin ratio; SBP, systolic blood pressure.

Correlations Between ADMA, Nephrin, Podocin, and PNR

Following the normality test, bivariate analysis was performed using the Spearman method to analyze the correlations among ADMA, nephrin, and PNR (Table 2). The podocin data distribution was normalized using the inverse transformation method. Pearson correlation analysis was then applied to analyze the correlation between ADMA and podocin. There was a weak positive correlation between ADMA and nephrin ($r = 0.353$, $p < 0.05$) as shown in Figure 1; However, as shown in Figure 2, there was no correlation between ADMA and podocin ($r = 0.133$, $p > 0.05$). We found a weak negative correlation between ADMA and PNR ($r = -0.360$, $p < 0.05$) as depicted in Figure 3.

Table 2 Correlations Between ADMA and Podocytopathy Markers

Variable	ADMA	
	r	p
Nephrin	**0.353**	**0.024***
Podocin†	0.133	0.409^
PNR	**−0.360**	**0.021***

Note: *Spearman correlation, † inverse transformation result, ^Pearson correlation. The bolded text highlights the study's significant findings, showing a correlation between ADMA, nephrin, and PNR. **Abbreviations**: ADMA, asymmetric dimethylarginine; PNR, podocin: nephrin ratio.

Multivariate Analysis

Linear regression showed a significant correlation between ADMA and nephrin after adjusting for BMI, GFR, ACR, and HbA1c (Table 2). BMI was the most significant variable, indicated by the R^2 increasing by more than 10%. Multivariate analysis revealed no correlations between ADMA, podocin, and PNR.

Correlation Between Nephrin, Podocin, and PNR Using the Confounding Variables

Bivariate analysis was performed to assess the correlations between the dependent variables (nephrin, podocin, and PNR) and the confounding factors. The numerical confounding factors are shown in Table 3. A Mann–Whitney U-test was performed, but no significant differences were found in the nephrin ($p = 0.655$), podocin ($p = 0.423$), and PNR ($p = 0.773$) values between males and females. No differences were found in the nephrin ($p = 0.615$), podocin ($p = 0.884$) and PNR ($p = 0.672$) values in patients who received or did not receive ACE inhibitors/ARBs.
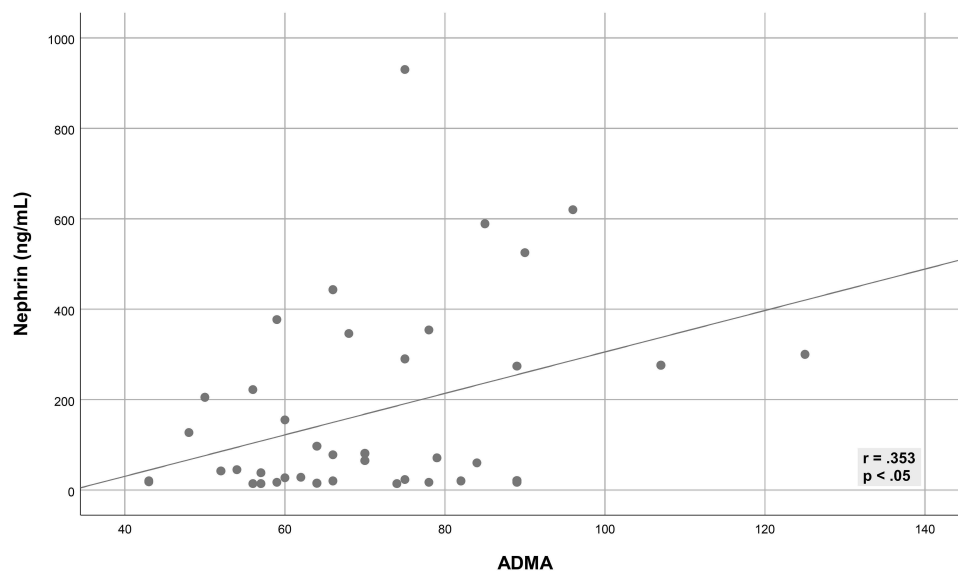

Figure 1 Scatter diagram showing correlations between ADMA and nephrin in DKD patients. ADMA and nephrin had a weak positive correlation.
Abbreviations: ADMA, asymmetric dimethylarginine; DKD, diabetic kidney disease.
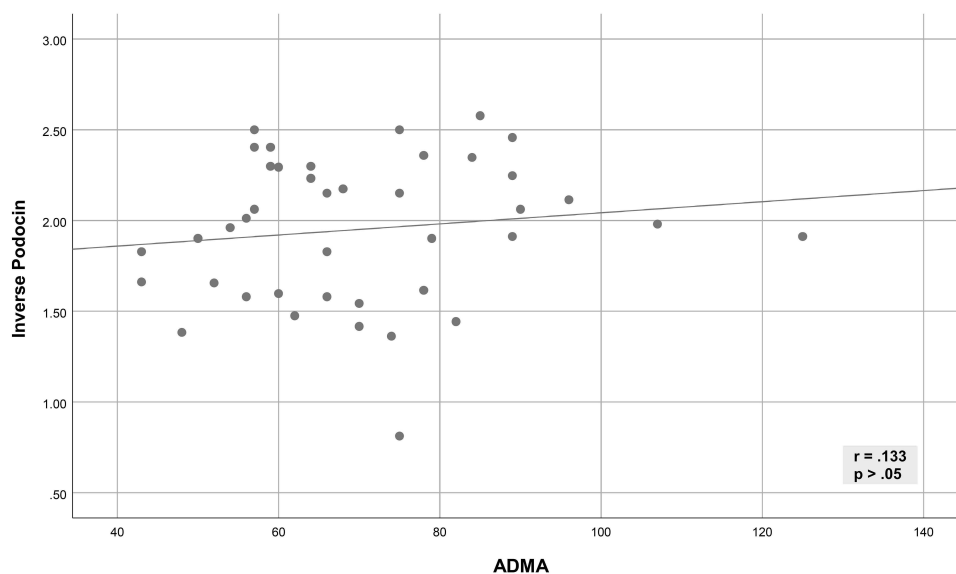
Figure 2 Scatter diagram showing correlations between ADMA and the inverse transformation for podocin in DKD patients. No correlation was found.
Abbreviations: ADMA, asymmetric dimethylarginine; DKD, diabetic kidney disease.
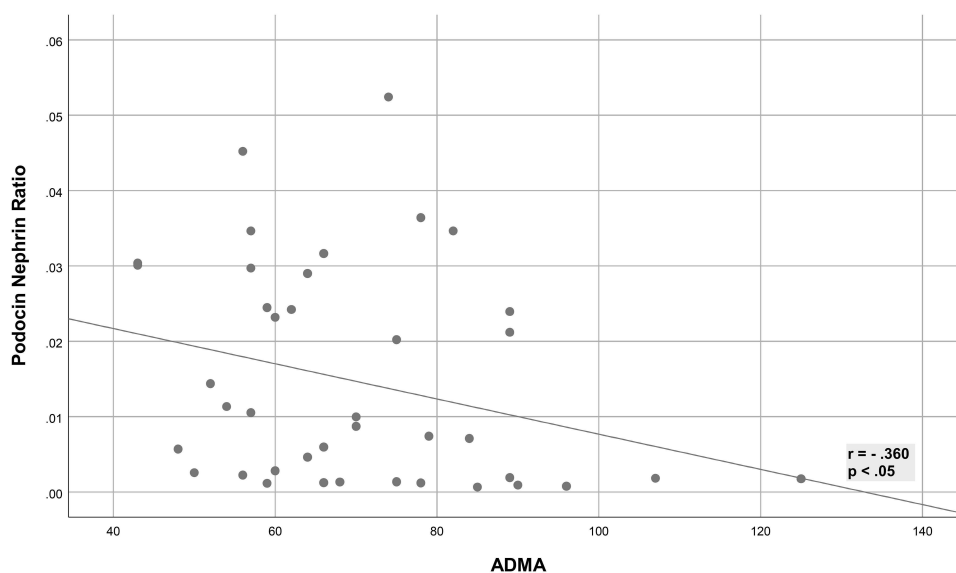

Figure 3 Scatter diagram showing correlations between ADMA and PNR in DKD patients. A weak negative correlation was observed.
Abbreviations: ADMA, asymmetric dimethylarginine; DKD, diabetic kidney disease; PNR, podocin nephrin ratio.

Linear regression was performed, and it revealed significant correlations between ADMA and nephrin after adjusting for BMI, GFR, ACR, and HbA1c (Table 4). BMI was the most significant confounding variable, indicated by an increase in R^2 of more than 10%. Multivariate analysis showed no significant correlations between ADMA, podocin, and PNR.

Discussion

In this study, we assessed the correlations between ADMA and podocytopathy markers (nephrin, podocin, and PNR) in DKD patients. Our subjects were 43.9% male to align with the 2018 Indonesian National Basic Health Survey data, which reported that diabetes was more prevalent in females (12.7%) than males (9.0%)[17] The age range of the

Table 3 Correlations of Nephrin, Podocin, and PRN Values with Age, MAP, BMI, HbA1c, Triglyceride, LDL, GFR, and UACR

Confounding Variable	Nephrin		Podocin^		PNR	
	r	*p*	*r*	*p*	*r*	*p*
Age	0.092	0.566	0.155	0.332	−0.090	0.575
MAP	0.072	0.654	−0.142	0.375	0.013	0.934
BMI	−0.396	0.010	0.008	0.105	0.387	0.012
HbA1c	−0.187	0.241	0.105	0.515	0.134	0.404
Tg	−0.020	0.494	0.078	0.630	0.021	0.895
LDL	0.057	0.786	0.130	0.537	−0.085	0.688
GFR	−0.196	0.245	−0.084	0.601	0.180	0.260
UACR	0.193	0.226	0.121	0.453	−0.223	0.161

Note: ^ inverse transformation for podocin.
Abbreviations: MAP, mean arterial pressure; BMI, body mass index; HbA1c, hemoglobin A1c; Tg, triglyceride; LDL, low density lipoprotein; GFR, glomerular filtration rate; UACR, urinary albumin:creatinine ratio; PNR, podocin nephrin ratio.

Table 4 Linear Regression of ADMA with Nephrin and Podocin

Variable	R^2	Adjusted R^2	*p*	Delta R^2
ADMA/Nephrin	0.139	0.117	0.016	
+BMI	0.280	0.242	0.015	50.3%
+GFR	0.310	0.254	0.018	9.67%
+UACR	0.322	0.247	0.037	3.73%
+HbA1c	0.322	0.226	0.044	0%
ADMA/Podocin	0.018	0.008	0.409	
+BMI	0.024	0.027	0.398	25%
ADMA/PNR	0.078	0.054	0.077	
+BMI	0.152	0.107	0.086	46.68%
+UACR	0.152	0.083	0.104	0%
+HbA1c	0.142	0.058	0.115	0%

Abbreviations: ADMA, asymmetric dimethylarginine; BMI, body mass index; GFR, glomerular filtration rate; UACR, urinary albumin:creatinine ratio; HbA1c, hemoglobin A1c; PNR, podocin:nephrin ratio.

subjects was 55–63 years, with a median of 60 years. Of all subjects, 75.6% had UACR < 300 mg/g and 31.7% had eGFR < 60 mL/min/1.73 m², indicating that the subjects were in the early DKD phase. The mean ADMA value was 70.2 (SD 17.2) ng/mL or 0.35 (SD 0.09) μmol/L—a lower value than that found by Abbasi et al, who reported a mean ADMA of 1.59 (0.22) μmol/L.[18] This could be explained by a population difference, since the participants in the previous study were predominantly male with higher BMIs and unreported GFR and albuminuria values.[18] Other studies also reported higher ADMA values, with differences in demographic characteristics and methods.[10,19,20] The ELISA method has been known to overestimate values compared to the liquid chromatography method. The mean value difference between our study and the previous studies can also be explained by genetic variations. Lind et al found genotype variations (single nucleotide polymorphisms) in the DDAH1 gene—an enzyme responsible for ADMA elimination—that affected serum ADMA values.[16]

Correlation of ADMA with Nephrin, Podocin, and PNR

Our study found a weak positive correlation between ADMA and urinary nephrin ($r = 0.353$, $p = 0.024$), indicating a link between endothelial dysfunction and podocyte damage. Multivariate analysis showed that ADMA had a 22.6% effect on

the variation of nephrin value. This aligns with previous studies regarding ADMA with markers of kidney damage in diabetic patients. However, these studies used albuminuria and GFR as outcomes.[10,21,22] An observational study by Hanai et al showed a positive correlation between ADMA and ACR ($r = 0.21$, $p = 0.002$) and a negative correlation between ADMA and GFR ($r = -0.15$, $p = 0.028$).[22] A multivariate analysis found that the adjusted hazard ratio increased by 1.3 (CI 95% 1.04–1.63) for every 0.01 μmol/L increase in ADMA.[22] A study by Assal et al, which included 90 subjects (15 healthy subjects and 15 with normoalbuminuric DM), revealed larger correlation coefficients between ADMA and ACR ($r = 0.60$, $p < 0.01$) than between ADMA and serum creatinine ($r = 0.77$, $p < 0.01$).[21] However, these two studies did not look for a causal relationship that could explain the role of ADMA in the pathophysiology of albuminuria or decreased glomerular filtration rate. Nephrinuria is also associated with albuminuria and decreased GFR.[23,24] Jim et al found a positive correlation between urinary nephrin, albuminuria ($r = 0.89$, $p < 0.001$), and serum creatinine ($r = 0.43$, $p = 0.0002$).[24]

Although podocin is a specific protein that can potentially be used as a marker for podocyte damage, this study showed no significant correlation between ADMA levels and urinary podocin ($r = 0.133$, $p = 0.409$). The results of this study do not align with other studies regarding the association of podocin with other clinical markers of kidney damage.[25,26] Zeng et al conducted a study on 118 patients with biopsy-proven DKD. Correlation was found between podocin value (ELISA) with GFR downslope ($r = 0.238$; $p = 0.01$). It is imperative to note that compared to our study population, this study enrolled people with later stage of DKD, with eGFR 41.4 mL/min/1.73m^2 (SD 31.3) and median proteinuria 2.5 (1.7–4.5) g/day.[26] A study by Nakatsue et al of mice with membranous nephropathy showed that nephrin was excreted in their urine in the early stages of nephritis, unlike podocin.[27] Nephrin and podocin will dissociate before released to the urine. Hence, we did not find the correlation between ADMA and podocin due to our subject being in the early phase of podocyte damage. One of the problems with using podocyte-specific proteins in urine as a clinical marker is the low concentration of the protein obtained. The small size of the podocin molecule (42 kDa) compared to other podocyte proteins can narrow the range of examination results. The differences in molecular size and kinetics between these two proteins could explain the differences in the correlation test results we obtained.

PNR values indicate stress in podocytes based on previous research that used the mRNA ratio with the reverse transcription polymerase chain reaction (RT-PCR) examination method. Fukuda et al reported that PNR values correlate with glomerular destruction at the histological level.[16] ADMA levels in this study had a weak negative correlation with PNR ($r = -0.360$, $p = 0.021$), and the correlation coefficient value was slightly higher than that for ADMA with nephrin. The direction of the negative correlation illustrates that the relationship occurred in the early DKD phase when nephrin expression was still quite high in damaged podocyte cells. The direction of this negative correlation also reflects the kinetics of the nephrin increase, which does not align with podocin. Although nephrin and podocin are dysregulated at the mRNA level during podocyte stress, nephrin is excreted in the urine earlier than podocin.[27] Thus, nephrin levels in urine may be higher than podocin levels in the early DKD phase. These findings appear to correspond with previous study reports. Although earlier studies were conducted using the ratio of podocin and nephrin mRNA. Petrica et al reported that nephrin mRNA correlated with urinary nephrin ($R^2 = 0.404$; $p < 0.05$).[28]

Multivariate Analysis

Linear regression was performed to analyze the effect of confounding variables on the correlations among ADMA, nephrin, podocin, and PNR. BMI was a confounding factor that affected the relationship between ADMA and nephrin and resulted in a determinant coefficient change of more than 10%.

Obesity has a structural and functional impact on the kidneys. Profibrotic signals released by adipocytes induce extracellular matrix deposition. Podocytes that are not able to fully close the enlargement will result in dysfunction and may even be released into the urine. Previous studies have demonstrated an association between obesity and endothelial dysfunction, with varying results. Eid et al conducted a cross-sectional study of 563 patients aged 64–76 years to examine the effect of plasma ADMA levels on several metabolic factors known to contribute to atherosclerosis.[29] This study revealed a weak positive correlation between BMI and ADMA ($r = 0.12$, $p = 0.006$). Tack et al conducted a double-blind randomized trial on 15 obese patients (with normal HbA1c) aged 25–50 years to examine the benefits of

administering troglitazone (increasing insulin sensitivity) for improving forearm blood flow after norepinephrine administration. This study reported no endothelial dysfunction despite insulin sensitivity being increased by troglitazone.[30]

ADMA and nephrin can be used to identify patients (particularly obese patients) with a high risk of worsening DKD. Further prospective cohort studies with stratified BMI classifications should be conducted to explain the impact of obesity on endothelial dysfunction and podocytopathy.

To the best of our knowledge, no other researchers have examined the relationship between endothelial and podocyte damage in DM patients. Thus, this study, using ADMA as a marker of endothelial damage and nephrin and podocin as markers of podocyte damage, serves as a preliminary study. Samples were taken consecutively to minimize selection bias. We also tried to rule out other possible causes of glomerular disorders by paying attention to anamnesis, physical examination, and supporting examination (including urinalysis) results.

However, several weaknesses of this study must be taken into account. The study cohort was considerably small, which could explain the weak statistical association. We could not consider all factors that might affect the results, such as inflammation and oxidative stress, due to limited time and resources. Subjects without albuminuria or deficient kidney function were not included because they were participating in a different study in our center. It was not possible to fully understand the correlations over a long period. Further research is needed to explore these associations in more depth.

Conclusion

Our study found a weak positive correlation between ADMA and urinary nephrin, a weak negative correlation between ADMA and PNR, and no correlation between ADMA and urinary podocin in DKD patients.

Funding

Disclosure

The authors have no conflicts of interest to declare.

References

1. International Diabetes Federation. Diabetes Around The World; 2021.
2. Alicic RZ, Rooney MT, Tuttle KR. Diabetic Kidney Disease. *Clin J Am Soc Nephrol*. 2017;12(12):2032–2045.
3. Perhimpunan Nefrologi Indonesia. 11th Report Of Indonesian Renal Registry. Jakarta; 2018.
4. Hoogeveen EK. The Epidemiology of Diabetic Kidney Disease. *Kidney and Dialysis*. 2022;2(3):433–442.
5. Hofherr A, Williams J, Gan LM, Söderberg M, Hansen PBL, Woollard KJ. Targeting inflammation for the treatment of Diabetic Kidney Disease: a five-compartment mechanistic model. *BMC Nephrol*. 2022;23(1):1–17.
6. Li L, Shen Q, Rao S. Association of Neutrophil-to-Lymphocyte Ratio and Platelet-to-Lymphocyte Ratio with Diabetic Kidney Disease in Chinese Patients with Type 2 Diabetes: a Cross-Sectional Study. *Ther Clin Risk Manag*. 2022;18:1157–1166.
7. Diez-Sampedro A, Lenz O, Fornoni A. Podocytopathy in Diabetes: a Metabolic and Endocrine Disorder. *Am J Kidney Dis*. 2011;58(4):637–646.
8. Yuen DA, Stead BE, Zhang Y, et al. eNOS deficiency predisposes podocytes to injury in diabetes. *J Am Soc Nephrol*. 2012;23(11):1810–1823.
9. Carlström M. Nitric oxide signalling in kidney regulation and cardiometabolic health. *Nat Rev Nephrol*. 2021;17(9):575–590.
10. Jayachandran I, Sundararajan S, Paramasivam P, et al. Association of circulatory asymmetric dimethylarginine (ADMA) with diabetic nephropathy in Asian Indians and its causative role in renal cell injury. *Clin Biochem*. 2017;50(15):835–842. doi:10.1016/j.clinbiochem.2017.05.007
11. Ichi YS, Ueda S, Nakamura K, Matsui T, Okuda S. Role of Asymmetric Dimethylarginine (ADMA) in Diabetic Vascular Complications. *Curr Pharm Des*. 2008;14(25):2613–2618. doi:10.2174/138161208786071326
12. Wieczór R, Wieczór AM, Kulwas A, Rość D. ADMA (asymmetric dimethylarginine) and angiogenic potential in patients with type 2 diabetes and prediabetes. *Exp Biol Med*. 2021;246(2):153. doi:10.1177/1535370220959738
13. Kandasamy Y, Smith R, Lumbers ER, Rudd D. Nephrin – a biomarker of early glomerular injury. *Biomark Res*. 2014;2(1). doi:10.1186/2050-7771-2-21
14. Verma R, Venkatareddy M, Kalinowski A, et al. Nephrin is necessary for podocyte recovery following injury in an adult mature glomerulus. *PLoS One*. 2018;13(6):e0198013. doi:10.1371/journal.pone.0198013
15. Blaine J, Dylewski J. Regulation of the Actin Cytoskeleton in Podocytes. *Cells*. 2020;9(7):1700. doi:10.3390/cells9071700
16. Fukuda A, Wickman LT, Venkatareddy MP, et al. Urine podocin:nephrin mRNA ratio (PNR) as a podocyte stress biomarker. *Nephrol Dial Transplant*. 2012;27(11):4079–4087.
17. Ministry of Health and National Institute of Health Research and Development. National report on basic health research, Riskesdas, 2018. Jakarta; 2018.
18. Abbasi F, Asagmi T, Cooke JP, et al. Plasma concentrations of asymmetric dimethylarginine are increased in patients with type 2 diabetes mellitus. *Am J Cardiol*. 2001;88(10):1201–1203.

19. Hsu CP, Hsu PF, Chung MY, Lin SJ, Lu TM. Asymmetric dimethylarginine and long-term adverse cardiovascular events in patients with type 2 diabetes: relation with the glycemic control. *Cardiovasc Diabetol*. 2014;13(1):156.

20. Krzyzanowska K, Mittermayer F, Shnawa N, et al. Asymmetrical dimethylarginine is related to renal function, chronic inflammation and macroangiopathy in patients with Type 2 diabetes and albuminuria. *Diabet Med*. 2007;24(1):81–86.

21. Assal HS, Younes K, Alsayed A, Hasan N, Ali AR. Asymmetric Dimethylarginine (ADMA) and Progression of Nephropathy in Patients with Type 2 Diabetes. *Kidney*. 2009;18(5):247–251.

22. Hanai K, Babazono T, Nyumura I, et al. Asymmetric dimethylarginine is closely associated with the development and progression of nephropathy in patients with type 2 diabetes. *Nephrol Dial Transplant*. 2009;24(6):1884–1888.

23. Kostovska I, Tosheska-Trajkovska K, Topuzovska S, et al. Urinary nephrin is earlier, more sensitive and specific marker of diabetic nephropathy than microalbuminuria. *J Med Biochem*. 2020;39(1):83–90.

24. Jim B, Ghanta M, Qipo A, et al. Dysregulated nephrin in diabetic nephropathy of type 2 diabetes: a cross sectional study. *PLoS One*. 2012;7(5): e36041.

25. ElShaarawy A, Behairy MA, Bawady SA, Abdelsattar HA, Shadad E. Urinary podocin level as a predictor of diabetic kidney disease. *J Nephropathol*. 2018;8(3):26.

26. Zeng L, Fung WWS, Chan GCK, Ng JKC, Chow KM, Szeto CC. Urinary and Kidney Podocalyxin and Podocin Levels in Diabetic Kidney Disease: a Kidney Biopsy Study. *Kidney Med*. 2023;5(1):100569.

27. Nakatsue T, Koike H, Han GD, et al. Nephrin and podocin dissociate at the onset of proteinuria in experimental membranous nephropathy. *Kidney Int*. 2005;67(6):2239–2253.

28. Petrica L, Ursoniu S, Gadalean F, et al. Urinary podocyte-associated mRNA levels correlate with proximal tubule dysfunction in early diabetic nephropathy of type 2 diabetes mellitus. *Diabetol Metab Syndr*. 2017;9(1):31.

29. Eid HMA, Arnesen H, Hjerkinn EM, Lyberg T, Seljeflot I. Relationship between obesity, smoking, and the endogenous nitric oxide synthase inhibitor, asymmetric dimethylarginine. *Metabolism*. 2004;53(12):1574–1579.

30. Tack CJ, Ong MK, Lutterman JA, Smits P. Insulin-induced vasodilatation and endothelial function in obesity/insulin resistance. Effects of troglitazone. *Diabetologia*. 1998;41(5):569–576.