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ORIGINAL ARTICLE

Hemorheological Approach for Early Detection of Chronic Kidney Disease and Diabetic Nephropathy in Type 2 Diabetes

Seohui Lee, MD,[1] Min Young Lee, MD,[1] Ji Sun Nam, MD, PhD,[1,2] Shinae Kang, MD, PhD,[1,2] Jong Suk Park, MD, PhD,[1,2] Sehyun Shin, PhD,[3] Chul Woo Ahn, MD, PhD,[1,2] and Kyung Rae Kim, MD, PhD[1]

Abstract

Background: Hemorheologic alterations or changes in blood viscosity have been suggested to play a role in the pathogenesis of microvascular complications in diabetes. We measured various hemorheologic parameters in type 2 diabetes patients at different stages of chronic kidney disease (CKD) and assessed their possible role as early markers of diabetic nephropathy and renal insufficiency.

Subjects and Methods: One hundred-five patients with type 2 diabetes were divided into four groups according to glomerular filtration rate (GFR), which represents the kidney function. Hemorheologic parameters, including erythrocyte deformability, fibrinogen/elongation index (EI), and aggregation index (AI) were measured using a microfluidic hemorheometer, and critical shear stress (CSS) was measured using a microfluidic technique. Various metabolic parameters were assessed from fasting blood samples, and the urine albumin-to-creatinine ratio (ACR) was calculated from first morning voided urine.

Results: There were significant differences in red blood cell (RBC) deformability, AI, CSS, fibrinogen/EI, and ACR among patients in different stages of CKD (all $P < 0.05$). RBC deformability and fibrinogen/EI significantly differed between normal (GFR >90 mL/min/1.73 m^2) and CKD stage 2 (GFR 60–90 mL/min/1.73 m^2) patients, whereas there was no such difference in ACR. In multiple regression analysis, fibrinogen/EI under a moderate shear stress of 3 Pa was an independent predictor of GFR ($\beta = -0.328$, $P < 0.05$). Also, AI, CSS, and fibrinogen/EI were significantly different among patients at different stages of diabetic nephropathy, with a significant difference in fibrinogen/EI between normal and microalbuminuric patients (all $P < 0.05$).

Conclusions: RBC deformability and fibrinogen/EI are sensitive parameters measured via point-of-care testing for detecting erythrocyte alterations in early CKD and nephropathy in patients with type 2 diabetes. Further studies are warranted to verify their use as screening tools for diabetic nephropathy and renal impairment.

Introduction

The worldwide pandemic of diabetes and the subsequent complications present major threats to the community health. Diabetes complications consist mainly of vascular diseases, such as microangiopathy and macroangiopathy, and they have been shown to be closely associated with hemorheological abnormalities such as elevated whole blood viscosity.[1] Hemorheological alterations were reported in patients with new-onset diabetes,[2,3] and other studies suggested that it may precede the development of diabetic microangiopathy.[2,4] Therefore, hemorheological disturbances seem to occur in the early phase of disease progression.[3,5]

Among factors determining blood viscosity, including hematocrit, plasma proteins such as fibrinogen, red blood cell (RBC) deformability, and RBC aggregation,[1,6,7] previous research was mainly focused on the correlations between diabetic nephropathy (DN) and erythrocyte deformability and aggregation.[8] According to a recent study, all of these factors are intimately connected to one another, and whole blood

[1]Division of Endocrinology and Metabolism, Department of Internal Medicine, Yonsei University College of Medicine, Seoul, Korea.
[2]Severance Institute for Vascular and Metabolic Research, Yonsei University College of Medicine, Seoul, Korea.
[3]Department of Engineering, Korea University College of Medicine, Seoul, Korea.

viscosity is determined not only by these individual factors, but also by their interactions.[9] These changes in whole blood viscosity may in turn play important roles in the pathogenesis of vascular complications associated with diabetes mellitus.[2,4]

In order to prevent the progression of diabetes complications, an early detection is crucial. In particular, when patients at an early phase of DN receive optimal therapy, the progression to chronic kidney disease (CKD) can be slowed or prevented.[10] CKD is graded based on glomerular filtration rate (GFR) and albuminuria. Normal kidney function refers to a GFR of >90 mL/min/1.73 m^2 without proteinuria, whereas Grade 1 CKD refers to a GFR of >90 mL/min/1.73 m^2 with proteinuria or other kidney damage, Grade 2 to GFR between 60 and 89 mL/min/1.73 m^2, Grade 3 to GFR between 30 and 59 mL/min/1.73 m^2, Grade 4 to GFR between 15 and 29 mL/min/1.73 m^2, and Grade 4 to less than 15 mL/min/1.73 m^2.[11] Albuminuria categories are as following: A1 when the albumin-to-creatinine-ratio (ACR) is <30, A2 (microalbuminuria or moderately increased albuminuria) when ACR is between 30 and 300 mg/g, and A3 (macroalbuminuria or severely increased albuminuria) when ACR is >300 mg/g.[11] Moderately increased albuminuria precedes the development of severely increased albuminuria and is considered to be an important predictor for future nephropathy.[12]

Currently, diabetes patients are encouraged to undergo regular urinary analyses, ultrasonography, and blood examinations to screen for DN. However, this requires high costs and demands patient compliance, and thus regular checkups for diabetes complications are difficult in clinical settings. For example, in many primary care clinics, especially in rural areas in Korea, blood samplings or urine analysis is rarely done, and diabetes patients are monitored by checking the glucose level from the glucometer at each visit. Moreover, although an increased excretion of urinary albumin is considered a gold standard for detecting the onset and progression of DN,[11] it has several limitations. Although spot urine ACR has been shown to be as accurate as 24-h urine collection and is recommended as a screening tool for DN,[13] it may be affected by exercise within 24 h, infection, fever, heart failure, hyperglycemia, or marked hypertension, and it can vary up to 40% from one day to another.[13,14] Moreover, in a large-scale prospective study, the 6-year cumulative incidence of remission, defined as the decrease in albumin excretion rate to <30 mg/24 h, was about 50%, whereas the risk of progression to proteinuria was only 15%,[15] and the degree of albuminuria was not necessarily linked to disease progression in patients with DN in a cohort study conducted with 79 new-onset microalbuminuric, normal renal function, type 1 diabetes patients as a part of the Joslin Kidney Study.[16] Therefore, more simple and stable biomarkers with higher sensitivity and specificity are needed for early prediction of the onset and monitoring of the progression of DN.[17]

In our previous study, RBC deformability and its index were assessed by point-of-care testing with <500 μL of whole blood and requiring complex multistep processes to prepared blood samples. The whole blood is simply mixed with a high viscous liquid and placed in ektacytometer to measure RBC deformability and calculate elongation index (EI) from it.[18] There were significant differences in the degree of RBC deformability when comparing diabetes patients with healthy controls, as well as also between diabetes patients with chronic renal disease or end-stage renal disease and those with normal renal function.[18] Because hemorheologic alteration have been shown to occur at a very early stage of diabetes and even before diabetes complications,[2–4] in this study we assessed these known hemorheologic parameters, including erythrocyte deformability, EI, aggregation index (AI), and critical shear stress (CSS) as well as a novel hemorheologic index, fibrinogen/EI at 3 Pa, in diabetes patients at varying degrees of CKD in terms of GFR and albuminuria. In this study we planned to assess the conventional and novel markers of hemorheologic disturbances, including erythrocyte deformability, EI, AI, CSS, and fibrinogen/EI at 3 Pa, and determine if they can be used as sensitive markers for early CKD or DN.

Research Design and Methods

Subjects

One hundred five type 2 diabetes mellitus patients were enrolled in this study. Diabetes was defined as both previously diagnosed and undiagnosed type 2 diabetes mellitus. Diagnosed diabetes mellitus was based on self-reported responses. The diagnosis of diabetes was based on the following American Diabetes Association criteria: fasting plasma glucose level of ≥126 mg/dL (7.0 mmol/L); symptoms of diabetes plus a casual plasma glucose level of ≥200 mg/dL (11.1 mmol/L); 2-h postload glucose level of ≥200 mg/dL (11.1 mmol/L) during a 75-mg oral glucose tolerance test; or glycated hemoglobin level of ≥6.5% (47.5 mmol/mol).

The subjects were divided into four groups according to their GFR, calculated using the Modification of Diet in Renal Disease (MDRD) formula as follows: $186 \times (\text{creatinine})^{-1.154} \times (\text{age})^{-0.203} \times (0.742 \text{ if female}) \times (1.210 \text{ if black})$. Seventy-three subjects were classified as diabetes patients with normal renal function (GFR >90 mL/min/1.73 m^2 without proteinuria), 19 patients had mild CKD (CKD stage 2; GFR, 60–89 mL/min/1.73 m^2), 10 subjects had moderate CKD (stage 3; GFR, 30–59 mL/min/1.73 m^2), and three subjects had severe CKD (stages 4 and 5; GFR, 0–29 mL/min/1.73 m^2).

Women who were pregnant or lactating, as well as subjects with anemia, acute illness, cancer, abnormal liver function, or other conditions, including any RBC disorders, not suitable for study entry according to the researchers' judgment, were excluded from the study.

All participants gave informed consent, and the study was approved by the Institutional Review Board of Gangnam Severance Hospital (Seoul, Korea).

Design

This study is a cross-sectional study, in which all data and samples were collected at a baseline visit, and includes patients from the Gangnam Severance Hospital, an 800-bed tertiary-care center. Patient information was obtained from all patients with diabetes by a standardized questionnaire.

Measurements

Anthropometric and biochemical measurements. Body weight and height were measured in the morning with light clothing without shoes. Body mass index was calculated as body weight (in kg) divided by height in meters squared (in kg/m^2). Blood pressure was measured twice in the sitting position, and a mean value was calculated. Fasting blood

samples were drawn and analyzed for glycated hemoglobin, serum glucose, total cholesterol, triglycerides, high-density lipoprotein and low-density lipoprotein cholesterol, blood urea nitrogen, creatinine, fibrinogen, hemoglobin, and the erythrocyte sedimentation rate (ESR). The urine ACR was determined centrally from the first voided morning urine specimen in all patients. Renal function was calculated using the MDRD formula as described above.

Sample preparation and measurement techniques for hemorheologic parameters. Blood samples were drawn from the antecubital vein into EDTA-containing Vacutainer® (BD, Franklin Lakes, NJ) devices. All analyses were completed within 8 h of fasting blood collection.

The erythrocyte deformability and aggregation were measured by using a microfluidic hemorheometer (RheoScan-AnD300; RheoMeditech, Seoul). For deformability measurements, 60 μL of whole blood was mixed with a solution of 0.14 m*M* polyvinypyrrolidone (molecular mass, 360,000; Sigma, St. Louis, MO) at the optimal hematocrit of 0.5. The differential pressure drove the erythrocyte suspension through the microchannel ($0.2 \times 4 \times 40$ mm) of the disposable kit, and the waste was collected in a waste chamber. A laser beam (wavelength, 635 nm) from a 1.5-mW laser diode passed through the diluted erythrocyte suspension during the flow. The diffraction pattern of the moving erythrocytes at plural shear stresses was projected onto a screen, and the images were captured by a charge-coupled device video camera every 0.5 s. The images were analyzed using an ellipse-fitting computer program.[19] The EI of the erythrocytes was defined as $(L - W)/(L + W)$, where L and W are the major and minor axes of the ellipse, respectively. Also, the EI in RBCs exposed to moderate shear stress at 3 Pa was used to assess deformability.[18] The full analysis took 100 s. Next, to assess erythrocyte aggregation, 80 μL of whole blood sample was perfused to a microchip, and a magnetic rotating mechanism was used to stir the suspension in order to induce shear flow to disaggregate the RBCs. A light signal transmitted through the blood sample was detected by a photodiode, and all data were analyzed by a computer in 120 s.

CSS was measured using native whole blood without adjusting for hematocrit. CSS was defined as the minimum shear stress required to disperse RBC aggregates. For the CSS measurement, a transient microfluidic technique was adopted with optical detection. When 500 μL of whole blood sample stored in a reservoir chamber was driven by a pressure differential through a narrow microchannel (model K-01; RheoMeditech), the pressure differential exponentially decreased with time, and the flow ceased asymptotically. During the process, the time-varying backscattered light intensity and pressure data were recorded in a computer data file and analyzed within 50 s. When the backscattered light yielded a maximum, the corresponding time and shear stress were determined as critical time and CSS, respectively. Further details of this technique are provided elsewhere.[20]

Statistical analysis

Statistical analysis was performed using SPSS version 20 software (SPSS Inc., Chicago, IL). The baseline characteristics were expressed as mean ± standard deviation values for

Table 1. Baseline Characteristics of the Patients ($N = 105$)

	GFR				
	> 90 mL/min/ 1.73 m² (n = *73*)	*60–90 mL/min/ 1.73 m²* (n = *19*)	*30–60 mL/min/ 1.73 m²* (n = *10*)	*< 30 mL/min/ 1.73 m²* (n = *3*)	P *value*
Age (years)	57.8	62.5	56.7	56.3	0.124
Sex female (%)	41.1	47.4	50	0	0.44
BMI (kg/m²)	24.24	25.4	25.79	27.2	0.124
Blood pressure (mm Hg)					
Systolic	122	123	131.5[b]	147.6[ce]	< 0.001
Diastolic	73	74	75.9	80.3	0.287
HbA1c [% (mmol/mol)]	7.4 (57)	9.2 (77)	7.9 (53)	8.9 (63)	0.22
FPG (mg/dL)	149	216.95	151	212	0.23
Urine ACR (mg/mmol)	9.86	272.63	2,231.2[bd]	3,474[cef]	< 0.001
Total cholesterol (mg/dL)	121.99	171.16	206.9	153	0.134
Triglycerides (mg/dL)	121.9	173.0[a]	152.40	187.6[c]	0.026
HDL (mg/dL)	51	44.3	49.3	35.3	0.758
LDL (mg/dL)	103.3	90.5	116.6	75.6	0.268
Hb (g/dL)	14.08	13.53	12.4[b]	13[c]	0.007
Serum creatinine (mg/dL)	0.90	0.99	1.30[bd]	3.0[cef]	< 0.001
BUN (mg/dL)	14.33	19.1[a]	24.3[bd]	35.3[cef]	< 0.001

The *P* value represents the analysis of variance *P* for the baseline measures among the groups.
[a] $P < 0.05$ between glomerular filtration rate (GFR) >90 and GFR 60–90 mL/min/1.73 m².
[b] $P < 0.05$ between GFR >90 and GFR 30–60 mL/min/1.73 m².
[c] $P < 0.05$ between GFR >90 and GFR <30 mL/min/1.73 m².
[d] $P < 0.05$ between GFR 60–90 and GFR 30–60 mL/min/1.73 m².
[e] $P < 0.05$ between GFR 60–90 and GFR <30 mL/min/1.73 m².
[f] $P < 0.05$ between GFR 30–60 and GFR <30 mL/min/1.73 m².
ACR, albumin/creatinine ratio; BMI, body mass index; BUN, blood urea nitrogen; FPG, fasting plasma glucose; Hb, hemoglobin; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

Table 2. Hemorheologic Parameters in Different Stages of Chronic Kidney Disease

	GFR				
	> 90 mL/min/ 1.73 m² (n = *73*)	*60–90 mL/min/ 1.73 m²* (n = *19*)	*30–60 mL/min/ 1.73 m²* (n = *10*)	*< 30 mL/ min/1.73 m²* (n = *3*)	P *value*
RBC deformability (EI at 3 Pa) (%)	0.320 ± 0.002	0.314 ± 0.006[a]	0.313 ± 0.007[b]	0.313 ± 0.012	0.047
Aggregation index (%)	39.76 ± 5.61	41.94 ± 5.74	44.38 ± 7.17[b]	46.05 ± 2.67[c]	0.027
Critical shear stress (Pa)	267.97 ± 121.12	298.66 ± 74.20	411.27 ± 199.87[b]	344.87 ± 92.66	0.008
Fibrinogen/EI at 3 Pa (mg/dL%)	851.07 ± 196.87	1,034.32 ± 316.59[a]	1,203.08 ± 245.29[b]	1,363.44 ± 273.11[c]	< 0.001
ESR (mm/h)	14.96 ± 14.85	26.58 ± 23.72[a]	47.8 ± 21.36[bd]	49 ± 35.08	< 0.001
Fibrinogen (mg/dL)	271.5 ± 57.7	315 ± 103.3	374 ± 69.09[b]	424 ± 60.6[c]	< 0.001

Data are mean ± SD values. Comparison among the groups according to glomerular filtration rate (GFR) was done using analysis of variance and post hoc analysis. The *P* value represents the analysis of variance *P* for the baseline measures among the groups.
[a]$P < 0.05$ between GFR >90 and GFR 60–90 mL/min/1.73 m².
[b]$P < 0.05$ between GFR >90 and GFR 30–60 mL/min/1.73 m².
[c]$P < 0.05$ between GFR >90 and GFR <30 mL/min/1.73 m².
[d]$P < 0.05$ between GFR 30–60 and GFR <30 mL/min/1.73 m².
RBC, red blood cell; EI, elongation index; ESR, erythrocyte sedimentation rate.

continuous variables and as frequencies for categorical variables. The statistical significance of differences in categorical variables between groups was tested using the χ^2 test. Statistical differences among the four groups according to GFR and urinary ACR were assessed using one-way analysis of variance, and post hoc analysis was done for intergroup analysis. Multiple linear regression analysis was used to assess the influence of hemorheologic parameters on GFR after adjustment for confounding factors. Levels of statistical significance were set at $P < 0.05$.

Results

Baseline characteristics

The participants were divided into four groups according to GFR. For comparing the baseline characteristics of the groups, clinical data were obtained and analyzed. There were no significant differences among the groups in terms of sex, diastolic blood pressure, glycated hemoglobin, fasting plasma glucose, body mass index, and total, low-density lipoprotein, and high-density lipoprotein cholesterol. Urine ACR, hemoglobin, triglyceride, systolic blood pressure, blood urea nitrogen, and serum creatinine were significantly different among the groups (Table 1).

Correlations between hemorheologic parameters and GFR

Participants were divided into four groups according to their GFR for comparison of the hemorheologic parameters. The EI, which indicates the RBC deformability, showed a gradual decrease with the deterioration of GFR (Table 2), and a significant reduction in erythrocyte deformability was observed between the mild CKD (CKD stage 2) and normal GFR groups (0.314 ± 0.006 vs. 0.320 ± 0.002; $P < 0.05$) (Table 2). The AI, which indicates the RBC aggregation, tended to show an overall inverse relationship with GFR and with a statistical significance between the normal GFR and moderate CKD groups (CKD stage 3) (Table 2). CSS also showed a significant difference between the normal GFR and moderate CKD groups (267.97 ± 121.12 vs. 411.27 ± 199.87; $P < 0.05$) (Table 2). ESR was significantly different between the normal GFR and mild CKD groups as well as between the normal GFR and moderate CKD groups. Furthermore, there was a significant difference in fibrinogen/EI at 3 Pa between the normal GFR and mild CKD groups as well as between the normal and both moderate and severe CKD (CKD stage 4) groups (all $P < 0.05$) (Table 2). In multiple regression analysis, fibrinogen/EI at 3 Pa was an independent predictor of GFR in a model adjusted with body mass index, hemoglobin, ESR, and age ($\beta = -0.328$, $P < 0.05$) (Table 3).

Also, there are several factors associated with hemorheologic parameters, including fibrinogen, that affected the AI and showed significant inverse correlations with GFR ($P < 0.001$) (Table 1). Fibrinogens and AI showed similar tendencies, with fibrinogen significantly differing between moderate CKD patients and those with normal GFR (Table 2).

Correlations between urinary ACR and hemorheologic parameters

ACR showed a significant ($P < 0.001$) inverse correlation with GFR (Table 1). ACR differed significantly between the moderate CKD and normal GFR group ($P < 0.05$) but not between the mild CKD and normal GFR groups (Table 1). Significant correlations were observed between various hemorheologic parameters, including fibrinogen/EI at 3 Pa, AI,

Table 3. Multiple Regression Analysis for Glomerular Filtration Rate

Independent variable	*GFR*				
	B	*SE*	*β*	T	P *value*
Constant	111.5	20.637		5.403	0.000
BMI	−1.371	0.555	−0.198	−2.468	0.015
ESR	−0.172	0.148	−0.175	−1.163	0.248
Hb	2.640	1.252	0.204	2.108	0.038
Fibrinogen/EI at 3 Pa	−0.025	0.010	−0.316	−2.363	0.020

B, unstandardized regression coefficient; *β*, standardized *β*; BMI, body mass index; EI, elongation index; ESR, erythrocyte sedimentation rate; GFR, glomerular filtration rate; Hb, hemoglobin; SE, unstandardized SE.

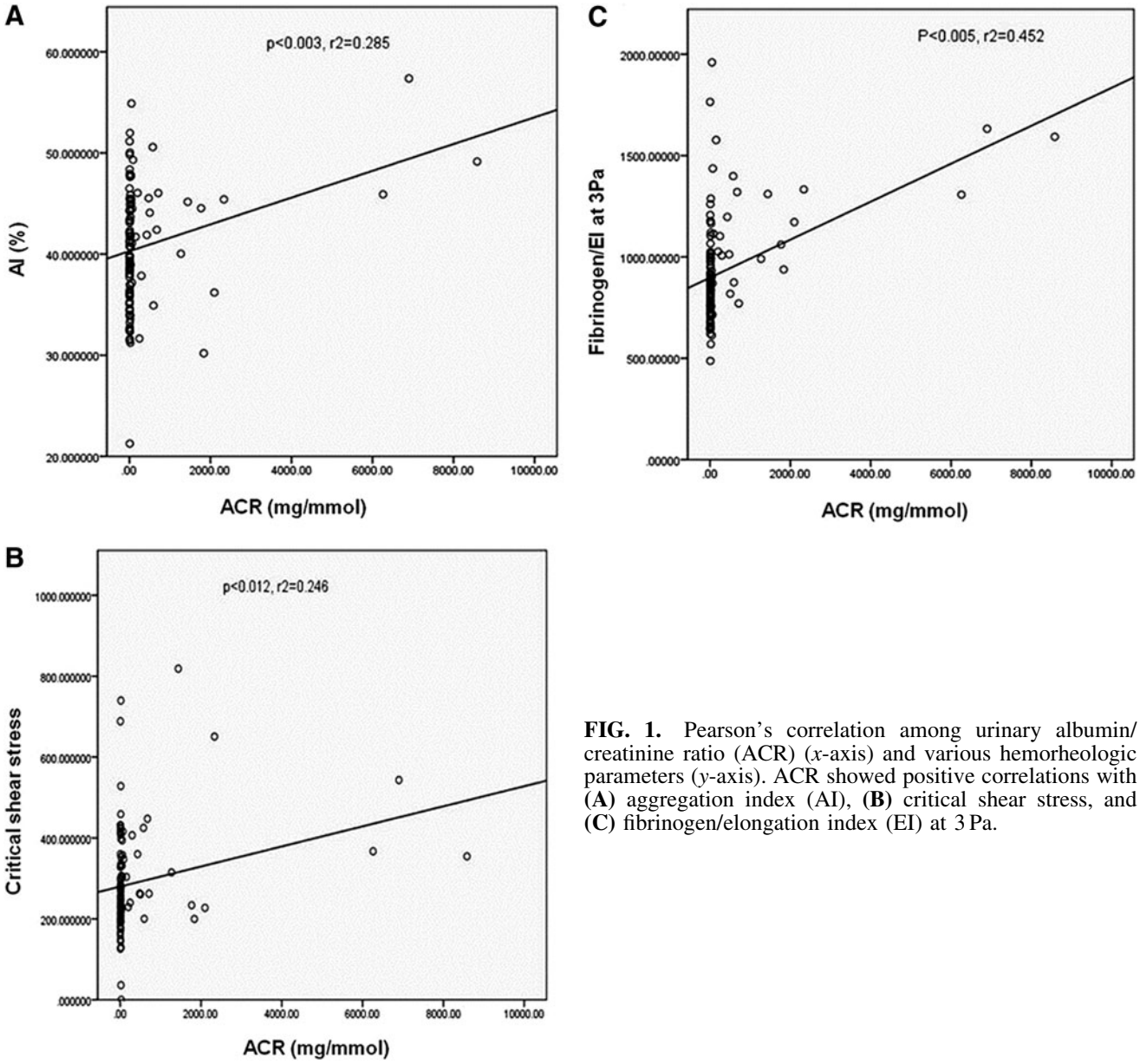

FIG. 1. Pearson's correlation among urinary albumin/creatinine ratio (ACR) (*x*-axis) and various hemorheologic parameters (*y*-axis). ACR showed positive correlations with **(A)** aggregation index (AI), **(B)** critical shear stress, and **(C)** fibrinogen/elongation index (EI) at 3 Pa.

CSS, and urine ACR ($r^2 = 0.452$, 0.285, and 0.246, respectively; $P < 0.005$, 0.003, and 0.012, respectively) (Fig. 1), and they remained significant after adjusting for hemoglobin, ESR, glucose, and body mass index. Serum fibrinogen was also in a positive correlation with urine ACR ($r^2 = 0.426$, $P < 0.001$).

When subjects were divided into normal (ACR <30 mg/g), microalbuminuria (ACR between 30 and 300 mg/g), and macroalbuminuria (ACR >300 mg/g) groups according to ACR, there was a significant difference in fibrinogen/EI at 3 Pa between the normal and microalbuminuria groups ($P < 0.05$) (Table 4).

Discussion

In the present study, we demonstrated significant differences in several hemorheologic parameters in different stages of CKD and DN in patients with type 2 diabetes. In particular, RBC deformability, measured by EI at 3 Pa and fibrinogen/EI at 3 Pa, were able to differentiate patients with mild CKD from those with normal renal function as well as microalbuminuria from normoalbuminuria (Table 4). In addition, AI, CSS, and fibrinogen/EI at 3 Pa showed close linear relationships to urinary ACR (Table 4).

About 50 years have passed so far since Skovborg et al.[21] first studied blood viscosity in diabetes patients. Since then, many researchers have reported that blood viscosity is significantly increased in diabetes patients, and there have been numerous studies on the correlations between erythrocyte deformability and diabetic micro- and macroangiopathies.[2,4,8,22] For example, Brown et al.[8] reported an association between reduced erythrocyte deformability and DN. They demonstrated greater impairment of erythrocyte deformability in patients with diabetes with normal renal function compared with controls without diabetes. Subsequently, more severe impairment in erythrocyte deformability was noted in patients with DN.

Table 4. Hemorheologic Parameters in Different Stages of Diabetic Nephropathy

	ACR <30 mg/mmol (n = 77)	*ACR 30–300 mg/mmol (n = 12)*	*ACR >300 mg/mmol (n = 16)*	P *value*
AI (%)	39.73 ± 5.51	43.53 ± 6.15[a]	43.71 ± 6.41[b]	0.010
Critical shear stress (Pa)	266.96 ± 188.24	322.26 ± 66.05	370.65 ± 173.99[b]	0.008
RBC deformability (EI at 3 Pa) (%)	0.318 ± 0.021	0.308 ± 0.027	0.316 ± 0.02	0.23
Fibrinogen/EI at 3 Pa (mg/dL%)	856.01 ± 195.89	1104.89 ± 381.13[a]	1170.65 ± 261.63[c]	< 0.001

Data are mean ± SD values. Comparison among the groups according to glomerular filtration rate was done using analysis of variance and post hoc analysis. The *P* value represents analysis of variance *P* for the baseline measures among the groups.
[a]$P < 0.05$ between albumin/creatinine ratio (ACR) <30 mg/mmol and ACR 30–300 mg/mmol.
[b]$P < 0.05$ between ACR <30 mg/mmol and ACR >300 mg/mmol.
[c]$P < 0.05$ between ACR 30–300 mg/mmol and ACR >300 mg/mmol.
EI, elongation index.

Elevated blood viscosity in patients with diabetes can be explained by an increase in erythrocyte aggregation (hyperaggregation) and a decrease in erythrocyte deformability. Hyperaggregation of erythrocytes increases low-shear viscosity, whereas decreased cell deformability increases high-shear viscosity.[18] Generally, hyperglycemia leads to membrane glycation, which stiffens the erythrocyte membranes. In turn, the stiffened membranes reduce cellular deformability, which is frequently observed in local hypoxia and microangiopathies, potentially resulting in the development of DN.[15]

Currently, DN is diagnosed, and its progression can be estimated by urinary analysis (ACR and protein-to-creatinine ratio), blood sampling (GFR, creatinine), and renal sonography. ACR is a very convenient tool for early detection of DN, and in the early microalbuminuria stage, the urinary ACR can be reversed or regressed if the proper management is started in time, emphasizing the importance of early detection and diagnosis of this disorder.[23] However, there are several shortcomings of using ACR as described earlier, including up to 40% day-to-day variation from variable factors,[16] and also the inability to predict the deterioration of renal function in patients with nonalbuminuric DN.[12] Therefore, there is a need for more stable marker that can detect microalbuminuria as well as early renal impairment, and we assessed the efficacy of point-of-care testing that can detect the early stages of DN.

In our study, the patients were classified according to their estimated GFR based on MDRD equation, which does not represent the actual renal function but an estimate. The GFR, which is the volume of fluid filtered from the renal glomerular capillaries into the Bowman's capsule per unit time, is clinically important because it represents an accurate measurement of renal function.[24] Our study results showed that renal function was associated not only with RBC deformability, AI, and critical stress, but also with ESR and fibrinogen (Tables 1 and 2). ESR is known to be frequently elevated in patients with renal insufficiency, and accordingly we found that the elevation of ESR was higher when the renal insufficiency was more severe.[25–27] Also, our study results are in line with the previous study showing close associations between fibrinogen and coronary artery disease and nephropathy in patients with diabetes.[28]

Among the hemorheologic parameters, analysis of variance showed significant differences in RBC deformability, AI, CSS, and fibrinogen/EI at 3 Pa among different CKD stage groups. When post hoc analysis was done, CSS and fibrinogen values were not significantly different between the mild CKD and normal GFR groups, and significantly lower erythrocyte deformability (EI at 3 Pa) was observed in the mild CKD group compared with the normal GFR group (Table 2). These findings suggest that the change in erythrocyte deformability may occur at an earlier stage CKD compared with changes in CSS and fibrinogen alone. Furthermore, the fibrinogen/EI at 3 Pa significantly ($P < 0.05$) differed between the moderate and mild CKD groups, as well as between the mild CKD and normal GFR groups (Table 2), suggesting that erythrocyte deformability and fibrinogen/EI at 3 Pa could potentially be used clinically for identifying patients at risk of developing renal impairment.

In terms of urine ACR, it was negatively correlated with GFR, and a significant ($P < 0.05$) difference was observed between the normal GFR and mild CKD groups as well as between the mild and moderate CKD groups (Table 1). When subjects were divided according to ACR, there was a significant difference in fibrinogen/EI at 3 Pa between the normal and microalbuminuria group, which suggests its role in detecting early DN (Table 4). This is in accordance with a previous study showing the early impairment in RBC deformability in diabetes patients with normal renal function.[18] Other hemorheologic parameters, including AI and CSS, showed a significant difference between the normal and macroalbuminuria groups but not between the normal and microalbuminuria groups.

Patients with diabetes are required to undergo regular checkups, including urinary and blood analyses and ultrasonography, to detect potential complications. However, this requires high patient compliance and is associated with high costs to the patients. Thus, regular checkups for microvascular complications of diabetes, including nephropathy, are difficult in a clinical setting. In terms of nephropathy, in order to measure urine ACR, we need to send the specimen to a clinical laboratory, and it takes several hours to even days depending on the availability of the lab. For hemorheological parameters, in the presence of a microfluidic hemorheometer, the result can be obtained within seconds. If we can develop accurate clinical hemorheologic markers, it would be possible to detect early deterioration of renal function and DN using 60–500 μL of blood within 50–120 s per parameter, which would result in increased compliance and enable more patients to detect their diabetes complications earlier. Among various parameters, fibrinogen/EI at 3 Pa was the most sensitive parameter for the detection of early DN.

The present study has several limitations. First, it is a relatively small study with only 105 participants and very few advanced CKD patients. Second, although we were able to

observe the hemorheologic changes in early CKD or microalbuminuric patients, we could not provide a cutoff value for the diagnosis, again, because of the small sample size. Third, hemorheologic parameters are known to be affected by blood glucose level, hemoglobin, platelet, albumin, fibrinogen, and electrolyte balance.[9] Although some of the factors, including hemoglobin, fibrinogen, and glucose levels, were adjusted, other possible confounding factors were considered when analyzing the results. Therefore, further large-scale studies are hence needed on the topic before any firm conclusions can be drawn. With a larger patient sample, the exact tendencies of the hemorheologic factors in the groups can be analyzed, resulting in the identification of parameters that will enable us to estimate nephropathy. Nevertheless, a major strength of the present study is that this is the first study on the association of DN and hemorheology in comparison with ACR. In addition, CKD patients with anemia were excluded because it is a major contributing factor to hemorheologic alterations. However, it might have caused some selection bias, and a future larger study is warranted. Also, because this is a cross-sectional study, it does not confer a causal relationship between hemorheologic alterations and the development of DN. A prospective study is warranted to see how these parameters change along the progression of CKD or DN.

In summary, examination of hemorheological factors, especially fibrinogen/EI at 3 Pa, was comparable to ACR as an early marker of renal impairment and DN in type 2 diabetes patients. Fibrinogen/EI at 3 Pa may be used to alert patients and doctors for further evaluation in primary care centers where a laboratory exam is inconvenient. With the accumulation of clinical data and the establishment of appropriate reference values, hemorheologic parameters may be used as one of the screening tools for renal dysfunction and DN.

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Author Disclosure Statement

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Address correspondence to:
Ji Sun Nam, MD, PhD
Division of Endocrinology and Metabolism
Department for Internal Medicine
Gangnam Severance Hospital
146-92 Dogok-dong
Gangnam-gu, Seoul 120-752, Korea

E-mail: jisunn@yuhs.ac