HEMOGLOBIN A1c LEVEL HIGHER THAN 9.05% CAUSES A SIGNIFICANT IMPAIRMENT OF ERYTHROCYTE DEFORMABILITY IN DIABETES MELLITUS

Q. Li¹, L.Z. Yang^{1,*}

Shanghai Ninth People's Hospital affiliated Shanghai Jiaotong University School of Medicine, Division of Endocrinology and Metabolism, Shanghai, China

Abstract

Context. Clinical studies demonstrated erythrocyte deformability (ED) is impaired in diabetic patients and described the correlations between HbA1c and ED. Few studies further investigated what an exact elevated HbA1c level linked to the impairment of ED in diabetes.

Objective. This study was to determine a cut-off point of HbA1c level leading to the impairment of ED in patients with diabetes.

Design. This was a retrospective observational study. ROC curve analysis was used to determine an optimal cut-off value of HbA1c for the increasing HSRV.

Subjects and Methods. In this study, 300 type 2 diabetic patients were enrolled. The whole blood viscosity was measured. High shear reductive viscosity (HSRV) was used to indirectly estimate ED. Based on the obtained cut-off value and glycemic control criteria for HbA1c, we divided all the cases into different groups to further confirm the accuracy of the cut-off value.

Results. In 300 patients, ROC curve illustrated that 9.05% was the optimal cut-off value as a predictor of the increasing HSRV. And higher odds ratio (OR) for significant decrease in ED was seen in the patients with HbA1c >9.05% compared to those with HbA1c ≤9.05% (OR: 3.78, 95% CI: 2.08-6.87). HSRV increased significantly in patients with HbA1c level >9.05% in comparison to patients with HbA1c levels <6.5% between 6.5 and 8.0% and between 8.0 and 9.05%.

Conclusion. ED decreased significantly in diabetic patients as soon as HbA1c level was higher than 9.05%.

Key words: Erythrocyte deformability, Type 2 diabetes mellitus, High shear reductive viscosity, HbA1c.

INTRODUCTION

Diabetes Mellitus (DM) characterized by chronic hyperglycemia influences the rheological behavior, which may accelerate the evolution of diabetic vascular complications (1, 2). Erythrocyte deformability

(ED), as one of rheological determinants, refers to the ability of erythrocytes to change their shapes to pass through small blood vessels and to minimize their resistance to flow at high shear rate in arteries (3, 4). In contrast, the erythrocytes with reduced deformability are incapable of passing through the vascular network under various blood flow conditions (2).

The major determinants of ED include cell geometry, internal viscosity and mechanical properties of membrane and cytoskeleton (5, 6). Metabolic alterations which are present in diabetes influence the function of erythrocytes through interaction with these determinants (7). In diabetic patients, the combined effects of both plasma glucose and serum lipids make complex changes in ED (8). Hyperglycemia contributes to glycation of hemoglobin, erythrocyte membrane and skeletal proteins (9), and dyslipidemia may adversely affect the dynamic properties of erythrocyte membrane (10). A decrease in ED is triggered by these metabolic alterations, and thus impairs circulatory flow which may increase diabetic complications (8, 11-13).

Hemoglobin A1c (HbA1c) is a form of hemoglobin which comprises about a third of the total erythrocyte volume. HbA1c has been introduced for diagnostic purpose in diabetes in the last decade (14). Impaired ED in diabetes has been repeatedly reported in numerous studies, and the correlations among HbA1c, plasma glucose, serum lipids, creatinine and ED have also been studied (11-13, 15). However, to our knowledge, no clinical studies have further investigated what an exact elevated HbA1c level initiated the impairment of ED. Accordingly, the aim of this study was to determine a cut-off point of HbA1c level leading to the impairment of ED in patients with DM.

Some reports demonstrated that impaired ED could be an early indicator of hemorheological disturbances and diabetic vascular complications (7, 11). Hence we should monitor relevant clinical indicators

*Correspondence to: Li-Zhen Yang MD, Shanghai Ninth People's Hospital affiliated Shanghai Jiaotong University School of Medicine, Division of Endocrinology and Metabolism, 639 Zhizaoju Road, Shanghai, 200011, China, E-mail: dryanginsh@yahoo.com

linked to ED to prevent or delay possible diabetic vascular complications. Previous studies have concentrated only on the employment of different rheological techniques to measure ED (11, 13, 16, 17). Blood viscosity is also determined by shear rate, red blood cell deformability and aggregation. Actually, ED can be more easily estimated by measuring the high shear reductive viscosity (HSRV) of whole blood. Therefore, we attempted to use HSRV of whole blood reflecting the behaviour of ED likewise to assess the impairment of ED indirectly in this study, the method of which was easy to handle.

PATIENTS AND METHODS

The present study was a retrospective observational study. The study protocol was approved by the Ethics Committee of our Hospital, and all the patients were informed before the study. Three hundred patients with DM (174 women and 126 men; ages ranging from 25 to 99 years, and mean $\pm$ SD as 64.79 ± 12.53 years) were enrolled from the Department of Endocrinology of our hospital from April 2013 to January 2015. Diagnosis of DM was in accordance with the criteria of the World Health Organization: FPG ≥ 7.0 mmol/L and/or 2-hr glucose ≥ 11.1 mmol/L. The patients were excluded based on the following three exclusion criteria: pregnancy, severe hepatic disease, and hematological diseases. ROC curve analysis for the utility of HbA1c as a predictor of the increasing HSRV, we categorized the HSRV values into quintiles and regarded the highest quintile as the positive group indicating the most significant decrease in ED, and then we found the optimal cut-off value of HbA1c corresponded to 9.05% (Fig. 1). Based on this cut-off value and glycemic control criteria based on the predefined levels of HbA1c as a parameter for glycemic control, all eligible patients were classified into five groups as follows: 46 patients with HbA1c $< 6.5\%$ under ideal glycemic control formed group A. Group B comprised 83 patients with HbA1c values ranged from 6.5 to 8.0 %. Group C comprised 55 poor glycemic control patients whose HbA1c ranged from 8.0 to 9.05 %. And 40 patients with HbA1c $> 9.05\%$ formed group D. Also group E consisted of 76 extremely glycemic control patients with HbA1c $> 10.0\%$.

Clinical and metabolic measurements

All participants were examined in the morning after an overnight fast for at least 8 hours. Age, duration of diabetes and past medical history were recorded. Height and weight were measured with standardized methods, as well as blood pressure, including systolic

and diastolic blood pressure. Body mass index (BMI) was calculated by dividing weight in kilograms by height in meters squared (kg/m^2).

Blood samples were collected for the routine clinical evaluation, and were drawn from the patients' antecubital vein in the morning after an overnight fast on the same day. Fasting plasma glucose (FPG), HbA1c, c-peptide and serum lipids, total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C) were measured immediately via standard procedures.

Hematological and rheological measurements

Blood samples were also simultaneously collected into potassium EDTA K3 tubes for hematological and rheological tests. Hematological parameters mainly referred to hemoglobin, erythrocyte mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were detected with an automated hematology analyzer. Rheological measurements aimed to detect the whole blood viscosity at different shear rates with a SA-6000 automatic blood rheometer (Beijing Succedder Company) by the suspension shaft cone-plate method.

Statistical analysis

All data are expressed as means $\pm$ standard deviation (SD) for continuous variables or as percentage for categorical variables. The association of HSRV with HbA1c, FPG and serum lipids was assessed by linear regression. ROC curves were plotted to estimate the optimal cut-off value of HbA1c and the areas under the curves (AUC) were calculated to assess the performance of HbA1c in predicting the increasing of HSRV and the whole blood viscosity at the shear rate 200 s^{-1} . After using Saphiro-Wilk test to check the normality of continuous variables, the differences among the five individual groups were then tested by one-way ANOVA for normally distributed variables, or Kruskal-Wallis test for non-normally distributed variables, and x²-test for categorical variables. Dunn's multiple comparisons test and Kruskal-Wallis test were also used for the comparison of the magnitude of HSRV between groups A, B, C, D and E. OR and 95% confidence intervals (CI) were calculated to determine the risk of the significantly increasing HSRV for each group of HbA1c regarding the HbA1c $< 6.5\%$ group as the reference. Multivariate stepwise regression analysis was applied to estimate the independent variables of HSRV including the following parameters: BMI, insulin medication, HbA1c, FPG,

hemoglobin, c-peptide, TG, TC and LDL-C. Statistical significance was considered as $p < 0.05$. Data processing was performed with the SPSS software package.

RESULTS

The clinical characteristics of the patients

Details of the clinical characteristics of 300 diabetic patients were presented in Table 1. Among the five groups, there was no statistical difference with regard to age, gender, BMI, blood pressure, duration of diabetes, MCV, MCH, MCHC and LDL-C. Hemoglobin was significantly higher in group E than that in groups A, B, C and D. C-peptide was significantly decreased in Group E compared to other groups (Table 1). At baseline, FPG and HbA1c of diabetic patients were 7.14 ± 2.70 mmol/L and $8.67 \pm 2.11\%$ respectively. In this study, the proportion of the patients achieving the good glycemic control target of HbA1c ($< 6.5\%$) was 15.33%.

HbA1c and HSRV

Figure 1 shows the ROC curve for HbA1c as a predictor of the increasing HSRV in diabetic groups. The optimal cut-off value of HbA1c for the increasing HSRV corresponded to 9.05% was the same as that for the increasing whole blood viscosity at the shear rate 200s^{-1} . At the HbA1c cut-off point of 9.05%, the sensitivity and specificity were 63.9% and 67.8% for HSRV and 62.1% and 66.9% for whole blood viscosity at the shear rate 200s^{-1} respectively. The Younden indexes were 0.32 and 0.29 for HSRV and whole blood viscosity at the

shear rate 200s^{-1} respectively. The AUC for the HbA1c concentration to predict the increasing HSRV and the whole blood viscosity at the shear rate 200s^{-1} were 0.65 (95% CI, 0.56-0.73) and 0.610 (95% CI, 0.524-0.695) respectively.

In type 2 diabetic patients, significantly positive correlations of HbA1c with HSRV ($P < 0.0001$, $r = 0.27$) and whole blood viscosity at the shear rate 200s^{-1} ($P < 0.0001$, $r = 0.23$) were observed in this study (Fig. 2a and c). Figure 2 (b and d) showed the comparison of magnitude of HSRV and whole blood viscosity at the shear rate of 200s^{-1} between groups A, B, C, D and E based on HbA1c levels. HSRV assessing ED increased significantly in patients of groups D and E with HbA1c levels $> 9.05\%$ in comparison to the patients in groups A, B and C ($P < 0.001$ respectively). Whole blood viscosity at the shear rate of 200s^{-1} also increased significantly in groups D and E compared with that in groups A, B and C ($P < 0.05$ respectively). But no significant difference between groups A, B and C was found. There was also no significant difference between groups D and E. This result also corresponded to that of Dunn's multiple comparisons test (Table 2). It was further demonstrated that only HbA1c $> 9.05\%$ (groups D and E) significantly increased HSRV in comparison to that in groups A, B and C. This result also corresponded to the results from Figures 2a and 2b. Moreover, higher odds ratio (OR) for significant decrease in ED was seen in the patients with HbA1c $> 9.05\%$ compared to those with HbA1c $\leq 9.05\%$ (OR: 3.78, 95% CI: 2.08-6.87, Table 3). For Groups D and E compared with group A, the ORs associating

Table 1. Clinical characteristics of 300 diabetic patients

	Group A	Group B	Group C	Group D	Group E	P-value
Patients. (n: Male/Female)	46 (22/24)	83 (32/51)	55 (22/33)	40 (17/23)	76 (33/43)	/
Age (years)	64.65 ± 14.17	65.24 ± 10.52	64.91 ± 13.21	64.53 ± 11.65	63.42 ± 14.40	0.90
Duration of diabetes (months)	98.93 ± 74.90	120.75 ± 84.18	131.67 ± 89.35	133.80 ± 92.77	130.50 ± 106.56	0.08
Body mass index (kg/m^2)	25.96 ± 5.60	26.52 ± 3.98	25.86 ± 3.23	26.56 ± 3.50	26.50 ± 4.68	0.92
Systolic blood pressure (mmHg)	134.41 ± 13.33	139.95 ± 17.85	144.83 ± 20.95	144.38 ± 24.31	140.57 ± 19.54	0.07
Diastolic blood pressure (mmHg)	76.09 ± 8.26	74.96 ± 10.73	78.00 ± 10.77	76.03 ± 10.33	76.62 ± 9.49	0.60
Usage of oral antidiabetics (%)	76.09%	79.52%	72.72%	67.50%	67.11%	0.41
Usage of insulin (%)	52.17%	50.6%	67.27%	70.0%	61.84%	0.09
Total cholesterol (mmol/L)	3.89 ± 0.97	4.12 ± 0.92	4.29 ± 0.96	4.23 ± 0.96	4.53 ± 1.14	0.011*
Triglycerides (mmol/L)	1.45 ± 0.83	1.59 ± 1.22	1.96 ± 1.13	1.37 ± 0.67	1.80 ± 1.36	0.014*
LDL cholesterol (mmol/L)	2.46 ± 0.69	2.60 ± 0.76	2.63 ± 0.81	2.72 ± 0.80	2.92 ± 0.99	0.078
Fasting plasma glucose (mmol/L)	5.30 ± 1.23	5.85 ± 1.66	7.55 ± 2.06	7.68 ± 2.56	9.10 ± 3.27	$< 0.0001^{**}$
HbA1c (%)	5.99 ± 0.32	7.16 ± 0.46	8.57 ± 0.30	9.49 ± 0.33	11.8 ± 1.43	$< 0.0001^{**}$
Hemoglobin (g/L)	121.28 ± 16.82	121.86 ± 14.61	122.74 ± 14.69	128.93 ± 15.91	129.07 ± 17.01	0.007**
MCV (fl)	90.17 ± 4.44	89.72 ± 4.60	89.86 ± 3.58	89.81 ± 3.31	89.08 ± 4.25	0.67
MCH (pg)	29.90 ± 1.59	29.79 ± 1.70	29.83 ± 1.46	29.85 ± 1.43	29.91 ± 1.70	0.99
MCHC (g/L)	331.70 ± 10.76	331.95 ± 9.32	331.79 ± 10.26	332.22 ± 9.52	335.72 ± 11.22	0.09
C-Peptide (ng/mL)	2.52 ± 1.71	2.08 ± 1.23	1.98 ± 1.20	1.89 ± 1.11	1.71 ± 1.12	0.034*

Note: LDL, low-density lipoprotein; MCV, erythrocyte mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; n, number of the patients. Kruskal-Wallis test was used to analyze the data of the clinical characteristics. * $P < 0.05$, ** $P < 0.01$.

Figure 1. ROC curve for HbA1c as a predictor of the increasing HSRV in diabetic groups (a). The optimal cut-off value (▲) for HbA1c corresponded to 9.05% with sensitivity 63.9%, specificity 67.8%. ROC curve area: 0.641 (95% CI, 0.564-0.718). ROC curve for HbA1c as a predictor of the increasing whole blood viscosity at the shear rate 200s⁻¹ in diabetic groups (b). The optimal cut-off value (▲) for HbA1c corresponded to 9.05% with sensitivity 62.1%, specificity 66.9%. ROC curve area: 0.610 (95% CI, 0.524-0.695).

Table 2. Dunn's multiple comparisons test and Kruskal-Wallis test for the comparison of magnitude of HSRV between Groups A, B, C, D and E

	Group A (n = 46)	Group B (n = 83)	Group C (n = 55)	Group D (n = 40)	Group E (n = 76)
HSRV	5.79±0.69	5.89±0.69	5.88±0.71	6.28±0.65	6.36±0.80
Dunn's multiple comparisons test:	Goups A vs. B: >0.999	Goups B vs. C: >0.999	Goups C vs. D: 0.053	Goups D vs. E: >0.999	Goups A vs. E: 0.0004**
P value	Goups A vs. C: >0.999	Goups B vs. D: 0.006**	Goups C vs. E: 0.008**		
Kruskal-Wallis test:	Goups A vs. D: 0.005**	Goups B vs. E: 0.0002**			
P value			<0.0001**		

Note: High shear reductive viscosity, HSRV; **P<0.01; number, n.

Table 3. Odds Ratios of significantly increasing HSRV for the different proposed groups of HbA1c in this study

HbA1c (%)	Odds Ratio (95% CI)	P value
Group A (<6.5)	Reference	/
Group B (6.5-8.0)	0.811(0.269-2.441)	0.709
Group C (8.0-9.05)	0.972(0.302-3.127)	0.962
Group D (9.05-10.0)	3.210(1.086-9.486)	0.03*
Group E (>10.0)	3.467(1.301-9.239)	0.01*
HbA1c>9.05 / HbA1c≤9.05	3.780(2.08-6.87)	0.000*

CI: confidence interval. *means P<0.05.

HbA1c with the significant decrease in ED were 3.21 (95% CI: 1.09-9.49) and 3.47 (95% CI: 1.30-9.24) respectively (Table 3). However, groups B and C did not experience the significant decrease in ED compared with group A (OR: 0.81, 95%CI: 0.27-2.44; OR: 0.97, 95% CI: 0.30-3.13, respectively. Table 3).

FPG concentration increased significantly with the HbA1c level elevations among the five groups (Fig. 3). HSRV was positively correlated to the FPG in the study (FPG, $r=0.41$, $P<0.0001$). FPG was significantly higher in groups C and D than these in groups A and B ($p<0.0001$, Fig. 3b). Figure 3c showed that HbA1c was

positively correlated with the whole blood viscosity at the shear rate 1.0s⁻¹ ($r=0.20$, $P=0.0006$). The comparison of whole blood viscosity at the shear rate of 1.0s⁻¹ between groups A, B, C, D and E based on HbA1c levels was shown in Figure 3d. The whole blood viscosity at the shear rate 1.0s⁻¹ increased significantly in patients with HbA1c levels >10.0% (group E) in comparison to patients with HbA1c levels ≤9.05% (groups A, B and C) ($P=0.005$, $P<0.0001$ and $P=0.034$ respectively).

Insulin therapy and HSRV

In the five study groups, 59.33% of the patients used insulin therapy. From Table 1, with respect to the medication history, no differences of the intake of oral anti-diabetes drugs and insulin usages among five groups were observed ($P > 0.05$). Figure 4 showed that there was also no significant statistical difference in the dosages of insulin in the prescriptions among groups B, C, D and E ($p > 0.05$). But, the dosage of insulin in group A was lesser than that in groups D and E ($P < 0.05$) (Fig. 4). There was no significant correlation between insulin dosages and HSRV ($r = 0.01$, $p > 0.9$).

Figure 2. Both whole blood viscosity at the shear rate $200s^{-1}$ and HSRV were linearly correlated with HbA1c, and were respectively compared with HbA1c in groups A, B, C, D and E. Significant correlations of HbA1c with the HSRV (a) and whole blood viscosity at the shear rate $200s^{-1}$ (c) were found. HSRV increased significantly in groups D and E with HbA1c > 9.05% compared with that in groups A, B and C (b). Whole blood viscosity at the shear rate of $200s^{-1}$ also increased significantly in groups D and E compared with that in groups A, B and C (d). No significant difference between groups A, B and C was found in this study (b, d). Data are means $\pm$ SD.

Table 4. A multiple stepwise regression analysis assesses the relationship between HSRV and different clinical variables in 300 diabetes patients

	Partial correlation coefficient	P value	Adjusted R^2
Body mass index (kg/m^2)	-0.098	0.056	
Insulin medication	0.072	0.173	
Fasting plasma glucose (mmol/L)	0.347	0.000**	
HbA1c (%)	0.089	0.153	
Total cholesterol (mmol/L)	-0.127	0.210	
Triglyceride (mmol/L)	0.095	0.103	0.245
LDL cholesterol (mmol/L)	0.125	0.015*	
C-Peptide (ng/mL)	0.173	0.001**	
Hemoglobin (g/L)	0.215	0.000**	

Note: * $P < 0.05$, ** $P < 0.01$.

Figure 3. HSRV was positively correlated with the FPG concentration (a). FPG concentration (b) was significantly higher in groups D and E than that in groups A and B. And whole blood viscosity at the shear rate $1.0s^{-1}$ was linearly correlated with HbA1c (c). The whole blood viscosity at the shear rate $1.0s^{-1}$ increased significantly in patients with HbA1c levels > 9.05% in comparison to patients with HbA1c levels $\leq 9.05\%$ (d). Data are means $\pm$ SD.

Serum lipids, FPG, BMI and HSRV

HSRV was also positively correlated to the serum lipids in the study (HSRV vs.: TC, $r = 0.15$, $P = 0.01$; TG, $r = 0.24$, $P < 0.0001$; LDL-C, $r = 0.17$, $P = 0.0024$) (Fig. 5a, b and c). To further estimate the independent variables of HSRV, through multiple stepwise regression analyses to adjust for BMI, insulin medication, HbA1c, FPG, C-peptide, TG, TC, and LDL-C, we found FPG had a close association with HSRV (partial correlation coefficient 0.347, adjusted $R^2 = 0.245$, $P < 0.001$, Table 3). The LDL-C, C-peptide and hemoglobin were also shown to be correlated with the HSRV (Table 4: partial regression coefficients 0.125, 0.173 and 0.215 respectively, $P < 0.05$).

DISCUSSION

Although, several studies documented that impaired ED was found in type 2 diabetic patients (8, 12, 18), we tried to further investigate the effect of glycemic control on ED in the present study. For the first time, we reported that HSRV, as a marker of ED, increased significantly in patients with HbA1c levels > 9.05%. Another important finding was that FPG concentration showed the most significant association with HSRV of whole blood.

Erythrocyte deformability and plasma glucose

In the present study, we found that HSRV was positively correlated to the FPG concentration (Fig. 3a).

Figure 4. No significant correlation between HSRV and the dosages of insulin was found in the present study (a). No significant difference in the dose usages of insulin between groups A, B, C and D was observed likewise (b). The dose usages of insulin were significantly larger in groups D and E than that in group A (b).

Figure 5. The linear correlations of HSRV with TC, TG and LDL-C in the observed subjects. HSRV was positively correlated with serum lipids including TC (a), TG (b) and LDL-C (c) in 300 diabetes patients.

Chronic hyperglycemia, as one of the fundamental abnormalities in diabetes, serves as a crucial factor involved in the development of reduction in ED5. High glucose concentration accelerates non-enzymatic glycation bringing about the glycation of hemoglobin, membrane and skeletal proteins (9). Hyperglycemia also results in the formation and accumulation of advanced glycation end-products (AGEs) which cause the direct damage to erythrocyte membrane structures via their specific receptors (19, 20). In addition, the decreased activity of Na^+/K^+ -ATPase caused by hyperglycemia can lead to the accumulation of intra-erythrocyte sodium with the subsequent increased uptake of Ca^{2+} , and thus causes the impairment of erythrocyte (9, 21). ED decreased with the increase of blood glucose concentration in diabetic patients compared to that of healthy controls (18, 22). Herein, both intensive blood glucose monitoring and management should always be priorities of treatment and not be ignored to further improvement of ED in diabetes.

Erythrocyte deformability and HbA1c

ED correlates closely with the concentration and physico-chemical properties of hemoglobin (9) which in turn tend to affect the erythrocyte internal viscosity (23). As mentioned above, hyperglycemia alters the structure and properties of hemoglobin through non-enzymatic glycosylation. The abnormally increased non-enzymatic attachment of glucose triggers the reduction of the mobility of the Lys- adjacent Cys residues, thus resulting in a significant rise in the mobility parameter of hemoglobin molecules (24). These structural changes in hemoglobin are mainly reflected by the HbA1c level, which refers to the non-enzymatic binding of specific glucose in circulation and accounts for 70% of HbA1 content (14, 25). As HbA1c connects intimately with the enhanced erythrocyte internal viscosity, high values of HbA1c induce a progressive decrease in ED ultimately (26). Several studies with nickel-mesh- filtration technique (2), system micro-fluidic ektacytometer (7), laser-assisted optical rotational cell analyzer (12), transparent microchannel capillary model and high-speed video camera system (27) have demonstrated that the erythrocyte filterability, elongation index (EI) or deformability index (DI) estimating ED were inversely correlated with HbA1c. Although it was hard to compare the results obtained by different techniques, they all provided strong evidences that high HbA1c concentration caused the impairment of ED.

As mentioned, blood viscosity is also determined by shear rates. In blood rheology, HSRV at the high shear rates of 200s^{-1} and 1s^{-1} can be used for assessing the RBC deformability and aggregation respectively. The higher value of HSRV at the high shear rate of 200s^{-1} may result in the decrease of ED causing the increase of blood viscosity; The higher value of HSRV at the high shear rate of 1s^{-1} may induce the erythrocyte aggregation causing the increase of blood viscosity.

In the present study, we found both the HSRV and the whole blood viscosity at the high shear rate of 200s^{-1} were correlated positively with HbA1c concentration. Our data likewise proved the results from previous studies (28, 29).

ED has been recognized as a critical factor to effectively perform its function of oxygen delivery and also a major determinant of blood viscosity in circulation (30, 31). Degradation of ED greatly deteriorates functioning of oxygen transportation in capillaries and perfusion in circulatory blood flow causing the impaired oxygen and nutrition supply in peripheral tissues, and eventually the diabetes-related vascular complications (31, 32). Accumulated evidences indicate that impaired ED might be involved in the evolution of diabetic

vascular complications (12, 13, 17).

HbA1c has been mainly used for diagnostic purpose in diabetes. We turned our attention to the cut-off point of HbA1c level leading to the impairment of ED, and we found HSRV increased significantly in patients with HbA1c levels $>9.05\%$ in comparison to patients with HbA1c levels $\leq 9.05\%$. In the ROC curve analysis, we obtained the same optimal cut-off point of HbA1c for the increasing HSRV and the whole blood viscosity at high shear rate of 200s^{-1} robustly affected by ED so that the accuracy of the cut-off value could be verified. And the obtained cut-off point of 9.05% presented a relatively good sensitivity and specificity. Due to no differences in the intake of oral anti-diabetes drugs among groups, we could get rid of the effect of oral therapy for diabetes on HSRV. Although here was no correlation between insulin dosages and HSRV, the dosage of insulin in group A was lesser than that in groups C, D and E. In the future, we will also investigate if the higher dosage of insulin can influence the HSRV.

In addition, we found FPG concentration increased significantly with the HbA1c level elevations among the five groups. Since HbA1c levels represent the average glucose concentration over approximately 100-120 days (14), the groups with higher HbA1c levels were certain to have higher blood glucose concentration, which could be the explanation for the above findings in this study. Overall, the alterations of HbA1c levels in different groups could reflect their effects on HSRV. Especially, based on these above findings, we concluded that poor glycemic control with HbA1c level higher than 9.05% caused a significant increase in HSRV, which could serve as a vital signal of the impaired ED. This conclusion may be a valid alternative to clinical monitoring on ED. Meanwhile, the result that whole blood viscosity at high shear rate of 200s^{-1} increased significantly in patients with HbA1c levels $>9.05\%$ in comparison to patients with HbA1c levels $\leq 9.05\%$ could also provide the basis for the conclusion. No differences in HSRV between groups A, B and C revealed that it appeared to be little variation on ED when HbA1c levels $\leq 9.05\%$. In contrast, as soon as HbA1c levels increased more than 9.05% , it appeared a significant reduction on ED ($P < 0.01$). They were in accordance with the results of the Odds Ratios calculation. Based on these findings, we could conclude that HbA1c level higher than 9.05% caused a significant increase in HSRV which could serve as a vital signal of the impaired ED. This conclusion may provide a unique approach to clinical monitoring on ED.

At present, the proportion of patients with diabetes achieving the glycemic control target of HbA1c

(<6.5%) is extremely small. In a retrospective study, only 12% of patients with DM reached $HbA1c \leq 6.5\%$ (33); and in the Diabcare-China Surveys, 23% of patients achieved the target of $HbA1c < 6.5\%$ (34). Compared with the domestic researches, in the present study, the percentage of patients achieving $HbA1c < 6.5\%$ was 15.33% which was lower than that of Diabcare-China surveys. In view of the above, we might as well regard 9.05% as a critical point of $HbA1c$ which is much easier to achieve, and is a must to achieve the goal. That is to say, the level of $HbA1c$ should be under 9.05% at least for the purpose of reducing the impairment of ED to prevent or delay possible diabetic vascular complications. However, our observations should encourage further studies to analyze whether impaired ED in the type 2 diabetic patients with $HbA1c > 9.05\%$ can be recovered by tight glycemic control.

In the present study, we also observed a significant positive correlation between $HbA1c$ level and the whole blood viscosity at low shear rate which was in agreement with the result obtained by Lc Dévéhat *et al.* (35). In the comparative analysis between groups, the whole blood viscosity at the shear rate of $1.0s^{-1}$ represented a significant increase in patients with $HbA1c$ levels $> 10.0\%$ in comparison to patients with $HbA1c$ levels $\leq 8.0\%$. Since the magnitude of whole blood viscosity at low shear rate is primarily determined by erythrocyte aggregation (EA)(5), we could infer that $HbA1c$ higher than 10.0% might increase the erythrocyte aggregation. However, there still exist several contradictory reports (36, 37) concerning the correlation between $HbA1c$ and EA in diabetic patients. Some researchers found increased EA was related with $HbA1c$ levels (37). Other authors argued that the degree of EA in diabetic patients did not directly correlate with $HbA1c$ (36). Anyway, our results might provide valuable information for improving EA by controlling $HbA1c$ levels in diabetic patients. However, the underlying mechanisms need to be further investigated.

Erythrocyte deformability and serum lipids

Furthermore, we found difference of TG among the groups, and HSRV was also positively correlated to serum lipids in the study. Because, TC, TG and LDL-C were significantly higher in group E, we could not rule out the possibility of the effect of TC, TG and LDL-C on HSRV in this grouping based on $HbA1c$ levels, and for which further research is required to illustrate this phenomenon. Diabetic patients have higher postprandial serum free fatty acids (38). The increased ratio of cholesterol to phospholipids significantly increased in diabetic erythrocyte membrane from diabetic patients which leads to the reduction in membrane fluidity and

impairment of ED ultimately (39). High serum levels of lipids cause the cholesterol enrichment of erythrocyte membranes (40). In the present study we also obtained a significant and positive relationship between serum lipids and HSRV, which is in accordance with the results of the previous study.

Moreover, plasma glucose concentration was identified as an independent variable of ED (12). In this study, we used multiple stepwise regression analyses to adjust for BMI, insulin medication, $HbA1c$, FPG, C-peptide, TG, TC and LDL-C, and confirmed that FPG showed the most significant correlation with HSRV, which was consistent with the results of the previous study. Lipoprotein-apheresis could reduce LDL-C and improve the deformability of erythrocyte (41). Based on the above information it appears that the presence of LDL-C has an apparent influence on ED. Hence, we should pay more attention on the fluctuation of LDL-C to evaluate the alterations of ED. All in all, serum lipids influence ED via altering structural properties of the erythrocyte membranes, which seem to be of a higher clinical value for improving ED by lipid-lowering therapy for the purpose of decreasing the risk of diabetic vascular complications.

In conclusion, the present study demonstrated that ED was closely related to $HbA1c$, FPG and serum lipids, and especially poor glycemic control with $HbA1c$ level higher than 9.05% caused a significant impairment of erythrocyte deformability in the patients with DM. Of course, further studies are needed to analyze whether impaired ED can be improved after the adjustment for $HbA1c$ levels.

There are two potential limitations in this study. Firstly, the results were limited in our ability to explore all connections between ED and biochemical profile in diabetic patients. Secondly, due to the small population in the study, we were incapable of classifying the subjects into more groups to achieve much higher accuracy of our findings. Anyway, our findings still provide useful clinical information and an assessment for improvement of the ED. The underlying mechanisms need to be further illustrated in experiments and in a large prospective population-based study.

Conflict of interest

The authors declare that they have no conflict of interest.

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