Research Article

Duygu Sari-Ak*, Nazli Helvaci, Alev Kural, Seher Tanrikulu, Ozgur Can, Sena Ebru Caglar, Hifa Gulru Caglar, Kursat Nuri Baydili and Yunus Karakoc

Ektacytometric examination of red blood cells' morphodynamical features in diabetic nephropathy patients

https://doi.org/10.1515/tjb-2024-0009
Received January 30, 2024; accepted August 20, 2024;
published online November 20, 2024

Abstract

Objectives: Diabetic nephropathy (DN), the primary cause of end-stage renal disease, is associated with chronic hyperglycemia and elevated blood pressure. This study investigates the correlation between DN and changes in hemorheology, which involves blood flow properties.

Methods: At the University of Health Sciences in Istanbul, Türkiye, erythrocyte deformability, aggregation, and viscosity were measured using advanced ektacytometry (Lorrca® Maxsis) on blood samples from 31 healthy individuals, 41 diabetic patients, and 45 patients with diabetic nephropathy from University of Health Sciences Haydarpasa Numune Training and Research Hospital. All hematocrit values were standardized to 40 %.

Results: Deformability of red blood cells (RBCs), indicated by the elongation index at shear stresses from 0 to 30 Pa, significantly decreased in the diabetic nephropathy group compared to diabetic patients without nephropathy and healthy controls (mean min-max SD: 0.650 (0.63–0.67) vs. 0.659 (0.64–0.68) and 0.642 (0.62–0.66), respectively; $p<0.01$). RBC aggregation significantly increased in the diabetic nephropathy group (mean min-max SD: 77.52 (57.84–90.7) vs. 69.96 (53.13–80.53) in controls; $p<0.01$), and plasma viscosity was also higher (mean min-max SD: 1.48 (1.27–2.02) mPa·s vs. 1.34 (1.22–1.72) mPa·s in controls; $p<0.001$).

Conclusions: Our findings demonstrate marked hemorheological changes in diabetic nephropathy patients. These changes suggest that evaluating RBC deformability, aggregation, and viscosity is critical for developing therapeutic strategies aimed at reducing vascular complications and preventing tissue damage in diabetics.

Keywords: red blood cells (erythrocytes); deformability; ektacytometry; diabetic nephropathy; hemorheology

***Corresponding author: Duygu Sari-Ak**, Department of Medical Biology, Hamidiye International School of Medicine, University of Health Sciences, Istanbul, 34668, Türkiye, E-mail: duygusariak@gmail.com. https://orcid.org/0000-0002-1577-7387

Nazli Helvaci, Department of Medical Biochemistry, Hamidiye School of Medicine, University of Health Sciences, Istanbul, Türkiye. https://orcid.org/0000-0001-7496-1208

Alev Kural, Department of Medical Biochemistry, Dr Sadi Konuk Research & Training Hospital, University of Health Sciences Bakırköy, Istanbul, Türkiye. https://orcid.org/0000-0003-1459-4316

Seher Tanrikulu, Division of Endocrinology and Metabolism, Department of Internal Medicine, Maslak Hospital, Acibadem University, Istanbul, Türkiye. https://orcid.org/0000-0001-9220-6118

Ozgur Can, Department of Nephrology, İstanbul Haydarpaşa Numune Training and Research Hospital, University of Health Sciences, İstanbul, Türkiye. https://orcid.org/0000-0001-8720-1400

Sena Ebru Caglar, Department of Physiology, Hamidiye School of Medicine, University of Health Sciences, Istanbul, Türkiye. https://orcid.org/0000-0002-1468-8905

Hifa Gulru Caglar, Bilecik Public Health Biochemistry Laboratory, Bilecik, Türkiye. https://orcid.org/0000-0002-8142-6817

Kursat Nuri Baydili, Department of Biostatistics and Medical Informatics, Hamidiye School of Medicine, University of Health Sciences, Istanbul, Türkiye. https://orcid.org/0000-0002-2785-0406

Yunus Karakoc, Department of Biophysics, Hamidiye School of Medicine, University of Health Sciences, Istanbul, Türkiye. https://orcid.org/0000-0001-5694-0737

Introduction

Diabetic nephropathy (DN) is a major complication of diabetes, significantly increasing morbidity and mortality rates, particularly leading to end-stage renal disease (ESRD) in developed countries. From 2000 to 2014, the number of ESRD patients in the USA due to diabetes rose from 40,000 to 50,000, while China has seen about 24.3 million DN patients with chronic kidney disease (CKD). DN poses a socioeconomic challenge, especially in developing countries, despite healthcare spending, as treatments have not decreased DN's impact on health outcomes [1–4]. Understanding the molecular mechanisms of DN is essential for developing new treatment methods and managing disease progression.

Chronic hyperglycemia and high blood pressure are key risk factors for diabetic neuropathy, causing nerve damage through oxidative stress, inflammation, and poor microcirculation. High blood pressure further worsens this condition by straining blood vessels and affecting the supply of nutrients and oxygen to nerves [5]. Microangiopathy and macroangiopathy, critical diabetic complications, are associated with hemorheological abnormalities like increased blood viscosity, indicating early-stage hemorheological disorders in diabetes onset [6–8].

Research on diabetic nephropathy (DN) often focuses on the relationship between red blood cell (RBC) deformability and aggregation, which along with factors like hematocrit and plasma proteins, influence blood viscosity. RBC deformability refers to their ability to change shape under blood flow forces, while RBC aggregation is a reversible clumping at low shear forces, forming rouleaux due to their discoid shape. Studies indicate these factors are interconnected, affecting whole blood viscosity and playing a crucial role in the vascular complications associated with diabetes [5, 9–12].

Early diagnosis and treatment can prevent or delay diabetes complications, particularly diabetic nephropathy. The urinary albumin-to-creatinine ratio (ACR) is the primary screening method for early DN, with microalbuminuria indicating a high risk for DN and cardiovascular diseases. However, despite its simplicity compared to traditional methods, ACR screening incurs costs and depends on patient compliance, prompting the search for more accessible and reliable detection tools [13–16].

Hemorheological changes, including RBC deformability due to membrane stiffening, occur early in diabetes due to the glycation of RBC membrane and hemoglobin [17]. Less deformable RBCs can damage microvascular walls, leading to narrowed or stiffened vessels and increased blood viscosity, which causes vascular complications in diabetes. Therefore, hemorheological parameters might be more reliable indicators for early DN than spot urine ACR, although studies on this are limited by small sample sizes and restricted findings [18–21].

The deformability, hydration, and membrane stability of RBCs are crucial for their role in gas transport, with osmotic gradient ektacytometry being a key diagnostic method for assessing these aspects and identifying RBC membrane disorders. The advanced Laser optical rotational red cell analyzer (Lorrca® Maxsis) represents a significant innovation in detecting RBC membrane abnormalities [22–25]. This study examined the morphodynamics of erythrocytes in diabetic nephropathy patients using the Lorrca® Maxsis to explore potential links between hemorheological changes and the disease, aiming for insightful findings.

Materials and methods

Study population

Upon receiving approval from the University of Health Sciences Hamidiye Scientific Research Ethics Committee (Date of Agreement: 10.06.2022, Decision No: 15/1, Registration No: 22/324), samples were collected from volunteers and patients for this prospective interventional study. The collection occurred over a 5-month period at the University of Health Sciences Haydarpasa Numune Training and Research Hospital, Istanbul, Turkey. The study took place at the University of Health Sciences Hamidiye School of Medicine, Medical Biochemistry Department, Istanbul, Turkey. The selection criteria for each group in this study were clearly delineated to ensure a rigorous and representative sample. Healthy volunteers were chosen based on the absence of any known diseases, with careful screening to confirm they were free from any diseases and exhibited no signs of illness. Specifically, individuals with fasting blood glucose levels of 126 mg/dL or higher, HbA_{1c} levels of 6.5 % or higher, and two-hour blood glucose levels of 200 mg/dL or higher were diagnosed with diabetes mellitus, forming the main criteria for the diabetic group patient selection. For inclusion in the diabetic nephropathy group, patients had to demonstrate the presence of albuminuria and a reduced glomerular filtration rate (GFR). Diabetic kidney disease was confirmed if albuminuria levels exceeded 300 mg/day on at least two out of three measurements and if the GFR was less than 60 mL/min/1.73 m^2 over a monitoring period of three to six months. The study group thus consisted of individuals with both diabetes mellitus and diabetic nephropathy, explicitly excluding those with other comorbidities [26, 27]. The patients diagnosed with diabetes were on oral antidiabetic medications, which they continued to take based on their renal functions and glycemic control. These medications included metformin, pioglitazone, dapagliflozin, empagliflozin, gliclazide, and insulin, all of which do not impact the rheology of the patients' blood. Additionally, these patients were either newly diagnosed or had been undergoing nephropathy treatment for no more than one year. Data were gathered from 31 healthy volunteers, 41 diabetes patients, and 45 diabetic nephropathy patients, aged 20–78 years. Demographic data and complete blood counts – including hemoglobin, hematocrit, RBC count, mean corpuscular volume, RBC distribution width (RDW), and leukocyte count – were recorded before data collection from LORRCA measurement. Demographic and complete blood count values are presented in Table 1.

Table 1: Demographic data and hemerology values of the study subjects at baseline. Values were expressed in mean ± standard deviation.

	Healthy volunteers (n=31)	Diabetic patients (n=41)	Diabetic nephropathy patients (n=45)	p-Value	Reference range
Age, years	50 (40–75)	55 (20–77)	63 (37–78)	<0.001	
HbA_{1c}, %	5.4 (5–5.7)	6.9 (5.4–10.9)	6.6 (4.6–11.3)	<0.001	%4–5.6
Creatinine, mg/dL	0.63 (0.51–0.85)	0.7 (0.44–1.05)	1.36 (0.68–7.51)	<0.001	0.7–1.2
GFR ml/min/1.73m^2	109 (77–124)	102 (63–137)	47 (8–118)	<0.001	>60[a]
Albumin excretion rate, mg/day creatinine	–	14.59 (2.04–69.67)	577.17 (5.94–5,482.95)	<0.001	<30 normal 30-300 microalbuminuria >300 clinical albuminuria
Protein excretion rate, mg/day creatinine	–	74.72 (28.5–229.36)	1,105.94 (44.06–7,045.85)	<0.001	<150
HCT, %	39.7 (32.2–49.3)	41.8 (31.7–49.6)	36.4 (29.7–51.7)	0.001	39–49
HGB, g/dL	12.8 (10–16.9)	13.7 (10.6–17.1)	11.8 (8.9–16.5)	<0.001	12.9–15.9
RBC, 10^6/mm^3	4.5 (3.94–5.52)	4.77 (3.63–5.75)	4.36 (3.26–5.95)	0.003	4.06–5.58
MCHC	33.2 (31.1–35.6)	33.3 (31.8–34.5)	32.3 (29.1–35.6)	0.004	31.8–35.4
MCV, fl	87.2 (79.3–96.3)	87.3 (74.4–95.9)	87 (64.1–94.7)	0.885	81.1–96
RDW, %	13.5 (12.3–28.3)	13.5 (12.6–17.8)	14.3 (13.1–21.5)	<0.001	11.5–14.5
PLATELET, 10^3/mm^3	245 (91–428)	255 (116–449)	270 (121–435)	0.385	155–366
WBC, 10^3/mm^3	5.76 (3.32–9.33)	7.56 (3.63–13.11)	7.94 (4.37–13.28)	<0.001	3.7–10.1

HCT, hematocrit; GFR, Glomerular Filtration Rate; RBC, red blood cell count; HGB, hemoglobin; MCV, mean corpuscular volume; RDW, red cell distribution width; MCV, mean corpuscular volume; MCHC, mean corpuscular hemoglobin concentration; WBC, leukocyte count. [a]: CDK-EPI equations used for calculations.

Hematocrit measurement and correction

Before measuring erythrocyte deformability and aggregation, the hematocrit value of the blood was determined. If the hematocrit value was below or above 40 %, it was brought to 40 % by adding to or removing from the patient's plasma at an appropriate rate. Reducing the hematocrit to 40 % is important to prevent the effect of cell number on the measurement.

Erythrocyte deformability measurement

The deformability of erythrocytes was assessed using a laser ektacytometer (LORRCA, RR Mechatronics, Hoorn, The Netherlands). Nine different "shear stress" values were entered, and the device adjusted the rotational speed in accordance with these values. Thus, shear stress was applied to the erythrocyte suspension at known levels (between 0.3 and 30 Pa). The laser diffraction pattern, shaped in accordance with the erythrocytes' form, was projected onto a screen and captured by a camera. The device then calculated the elongation index (EI) parameter based on this ellipsoid pattern. The elongation index is determined by the ratio of the difference between the long and short axes of the erythrocyte-formed shape to their sum.

In addition to the Elongation Index, another important parameter is the shear stress, which causes the shape change to be half of the maximum value of the elongation index (EImax), expressed as SS½. The SS½ parameter is calculated by applying the Lineweaver-Burk analysis. A decrease in the SS½ value and an increase in the maximum elongation index (EImax) indicate an increase in erythrocyte deformability.

According to this principle, to measure erythrocyte deformability, the hematocrit of the blood taken from the patient was first corrected to 40 %. Then, 25 µL were taken and mixed with 5 mL of PVP solution (4 % polyvinylpyrrolidone solution, Sigma PVP-360; MW 360kD, RR Mechatronics, Hoorn, The Netherlands) with a viscosity of ~30 mPa until homogeneous. Approximately 1 mL of this suspension was taken and placed in the Couette system in the device. EI values were measured under nine different shear stress values (0.3; 0.53; 0.95; 1.69; 3; 5.33; 9.49; 16.87; 30) and at 37 °C. EI, EImax, and SS½ values obtained as a result of the measurement were recorded.

Erythrocyte aggregation measurement

The erythrocyte aggregation property of the samples was evaluated using a laser ektacytometer device (LORRCA, RR Mechatronics, Hoorn, The Netherlands). Undiluted whole blood was utilized for this measurement. Since the level of

HbO_2 (oxyhemoglobin) in the blood could influence the aggregation parameters, oxygenation was routinely performed on all samples before measurement. For the oxygenation process, the whole blood sample was placed in a tube with a volume at least 10 times its own, to expose it to the air's oxygen for 10 min. During this period, the sample was gently inverted to ensure thorough oxygen contact.

After oxygenation, the whole blood sample placed in the Couette system within the device was first exposed to a high shear rate (value: 500 sˆ-1) for 5 s. Then, the speed was abruptly stopped, and the shear rate was reset to simulate a stasis state. The magnitude of aggregation-disaggregation was determined based on the backscatter of the laser beam directed at the sample. The parameters assessed during the aggregation analysis include the aggregation index (AI) (%), which is the percentage of erythrocyte aggregation in a stasis state, aggregation amplitude (AMP) (au), which represents the total change in the aggregation signal, and aggregation half-time (t½) (sec), the time it takes for the aggregation signal to reduce to half of its maximum change.

To measure erythrocyte aggregation according to the aforementioned principle, approximately 1 mL of a whole blood sample, whose hematocrit had been adjusted to 40 % with autologous plasma, was oxygenated before measurement. Then, about 900 µL of this sample was taken and placed in the device for measurement. After conducting the measurement at 37 °C, the values of the AI, AMP, and t½ parameters were recorded.

Whole blood and plasma viscosity measurement

Viscosity measurements were conducted using a rotational viscometer (Brookfield DV-III, Brookfield, Middleboro, MA, USA). For plasma viscosity measurement, an appropriate amount of whole blood was separated into plasma by centrifugation. With the temperature set at 37 °C, 525 µL of the separated plasma from the whole blood sample was placed in the device, which had a ten-digit measurement setting of 4.5; 6; 7.5; 37.5; 75; 112.5; 150; 187.5; 300; 450, respectively. Viscosity measurements were performed at "shear rates" of 300 s-1 and 450 s-1, and the results were recorded in centipoise (cP). A 40 cp disk was utilized for the measurements.

Statistical analysis

Data analysis was performed using IBM SPSS Statistics 25 software package. Frequency and percentage values were presented for qualitative variables. For elongation index values, one-Way ANOVA was employed for comparisons involving qualitative variables with more than two categories and quantitative variables. The normality of the data distribution was assessed using the Shapiro-Wilk test. For quantitative variables, median, minimum, and maximum values were reported. The chi-square test was employed for comparisons between two qualitative variables. For comparisons between qualitative variables with more than two categories and quantitative variables, the Kruskal-Wallis H test was utilized. If a significant difference was found in the Kruskal-Wallis H test, pairwise comparisons were conducted using the Bonferroni-corrected Mann-Whitney U test. The significance level was set at 0.05.

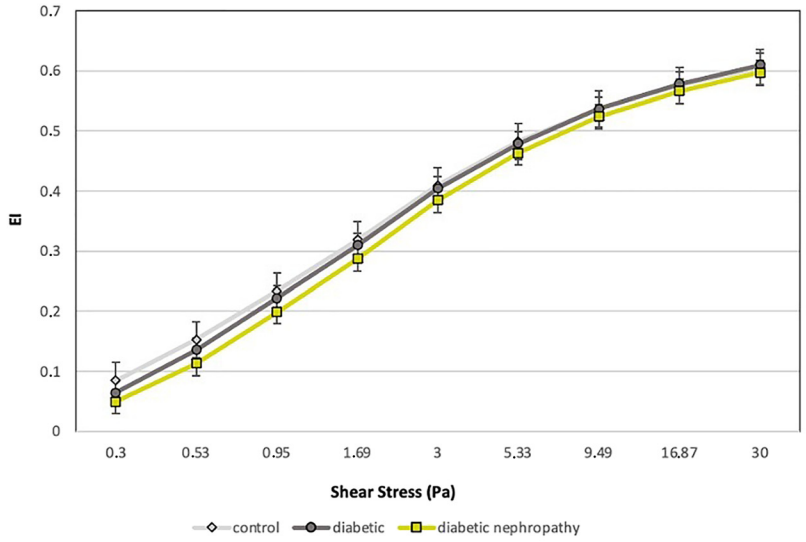
Results

RBC deformability

In our study, we examined the flexibility of red blood cells by measuring their ability to stretch under varying levels of force, or 'shear stress', from 0 to 30 Pa. This measurement, called the Elongation Index (EI), was recorded for healthy controls, diabetic patients, and diabetic patients with nephropathy. The results are displayed in Figure 1. From these data, we calculated the Maximum Elongation Index (EImax), which represents the greatest extent to which RBCs can stretch.

Additionally, we determined the amount of shear stress needed to achieve half of this maximum elongation (SS1/2), which is shown in Table 2. Lower SS1/2 values and higher EImax values generally suggest better RBC deformability, indicating that the cells can more easily change shape to navigate through the bloodstream.

Our findings revealed a significant decrease in EImax in the group of diabetic patients with nephropathy compared to those without nephropathy. This indicates a reduction in RBC deformability, which is particularly significant for patients who develop microvascular complications such as nephropathy. The decrease in deformability suggests that these patients' RBCs are less able to adjust their shape under stress, potentially contributing to the vascular complications common in diabetes. The observed increase in EImax value and concomitant decrease in SS1/2 value indicate an enhancement in erythrocyte deformability. It is anticipated that the deformability of erythrocytes will be highest in the healthy group and lowest in the diabetic nephropathy group. As the disease progresses, the deformability of cells is observed to decrease. In line with these expectations, our study found that the diabetic nephropathy group exhibited



Sheer Stress (Pa)	Healthy volunteers (n = 31)	Diabetic Patients (n=41)	Diabetic Nephropathy Patients (n = 45)	p-Value
SS 0.3	0.0849±0.03	0.0644±0.02	0.0495±0.03	<0.001
SS 0.53	0.1532±0.03	0.1359±0.03	0.113±0.02	0.012
SS 0.95	0.234±0.03	0.2218±0.02	0.1989±0.02	<0.001
SS 1.69	0.3184±0.02	0.3094±0.02	0.2871±0.02	0.036
SS 3	0.4098±0.01	0.404±0.02	0.3846±0.02	0.005
SS 5.33	0.4827±0.01	0.4796±0.01	0.4638±0.02	0.003
SS 9.49	0.5369±0.01	0.5367±0.01	0.524±0.01	<0.001
SS 16.87	0.5759±0.01	0.5785±0.01	0.5662±0.01	<0.001
SS 30	0.605±0.01	0.6097±0.01	0.5973±0.01	0.006

Figure 1: RBC deformability, as assessed using the elongation index.

Table 2: Laboratory data regarding RBC deformability for the study subjects were collected at baseline.

	Healthy volunteers (n=31)	Diabetic patients (n=41)	Diabetic nephropathy patients (n=45)	p-Value
HCT, %	40 (33–47)	43 (35–51)	35 (24–53)	<0.001
SS1/2	1.44 (0.8–1.81)	1.39 (0.83–2.25)	1.51 (1.13–2.29)	0.002
EImax	0.650 (0.63–0.67)	0.659 (0.64–0.68)	0.642 (0.62–0.66)	<0.001

The assessment of RBC deformability was conducted using the elongation index, with values expressed as mean min-max standard deviation. HCT represents hematocrit; SS ½ denotes the shear stress required for half-maximal deformation; EImax is the maximum elongation index. The Kruskal-Wallis H test and Mann-Whitney U test were used for statistical analysis.

lower EImax values and higher SS1/2 values compared to the diabetic group. The results of this study suggest that the unexpectedly high EIMAX values observed in healthy individuals may be influenced by biological variability and subclinical factors affecting erythrocyte deformability. This finding indicates that erythrocyte deformability is significantly affected not only by conditions such as diabetes and diabetic nephropathy but also by physiological and subclinical variations in healthy individuals. In light of these factors, it is crucial to consider biological variability and

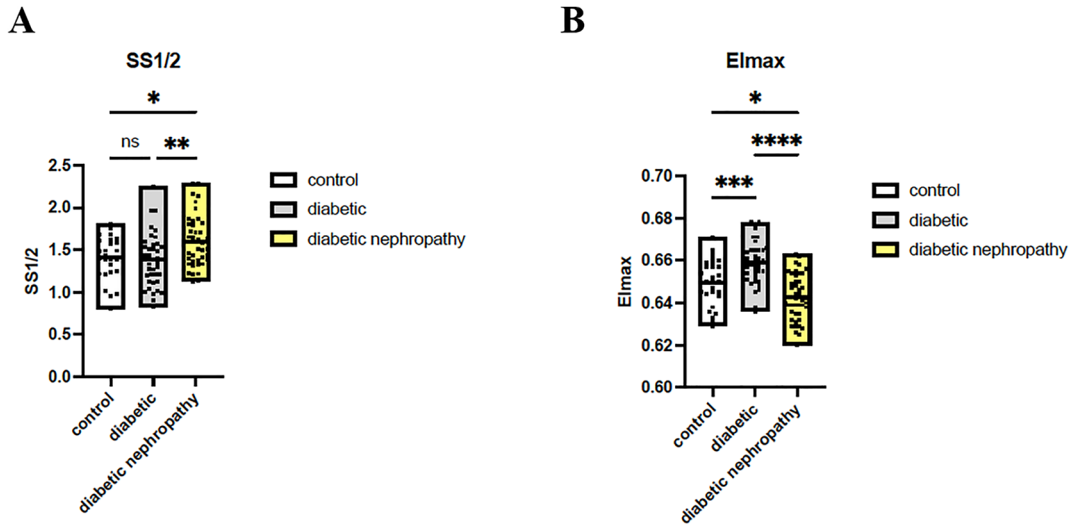


Figure 2: Lorrca indices for deformability of RBC subpopulations with (A) SS1/2 and (B) EImax datasets.

subclinical conditions in studies of erythrocyte deformability. Future research should more thoroughly investigate and control for these variables to ensure that the findings are robust and reliable [28–31] (Figure 2).

RBC aggregation

In our analysis of how red blood cells (RBCs) clump together – an indicator of potential circulation problems – we assessed several parameters related to erythrocyte aggregation: the Aggregation Measurement Parameter (AMP), Aggregation Index (AI), and the time it takes for half of the aggregation to occur (t1/2). The results for these tests, conducted across control subjects, diabetic patients, and diabetic patients with nephropathy, are summarized in Table 3.

Our findings indicate significant differences in how RBCs aggregate across the different groups. Notably, there was a clear increase in the AI as we moved from the control group to the diabetic group, and further to the diabetic nephropathy group. This suggests that RBCs in diabetic patients, especially those with nephropathy, tend to clump together more readily than in healthy controls. Conversely, the time to half-maximum aggregation (t1/2) decreased significantly across these groups, reinforcing the observation of increased RBC aggregation in diabetic conditions (Figure 3).

However, differences in the AMP across these groups did not reach statistical significance, indicating that while the propensity of RBCs to aggregate changes with disease state, the overall capacity measured by AMP remains similar (Figure 3).

Plasma viscosity

We measured plasma viscosity in three groups: healthy controls, diabetic patients, and diabetic patients with nephropathy, at two different shear rates. These measurements are detailed in Table 4.

Our analysis, illustrated in Figure 4, shows that plasma viscosity was significantly higher in the diabetic nephropathy group compared to the diabetic group without nephropathy.

Table 3: Laboratory data on RBC aggregation for the study subjects were collected at baseline.

	Healthy volunteers (n=31)	Diabetic patients (n=41)	Diabetic nephropathy patients (n=45)	p-Value
AMP, au	44.5 (36.08–51.9)	45.07 (35.7–51.4)	43.7 (29.5–52.69)	0.067
AI, %	69.96 (53.13–80.53)	74.57 (57.65–85.2)	7.52 (57.84–90.7)	<0.001
T1/2, s	1.6 (0.84–3.58)	1.28 (0.68–2.97)	1.04 (0.36–2.79)	<0.001

RBC aggregation was assessed using the Aggregation Index, with values expressed as mean min-max standard deviation. AMP stands for Aggregation Amplitude; AI for Aggregation Index; t1/2 for Aggregation Half Time. The Kruskal-Wallis H test and Mann-Whitney U test were used for statistical analysis.

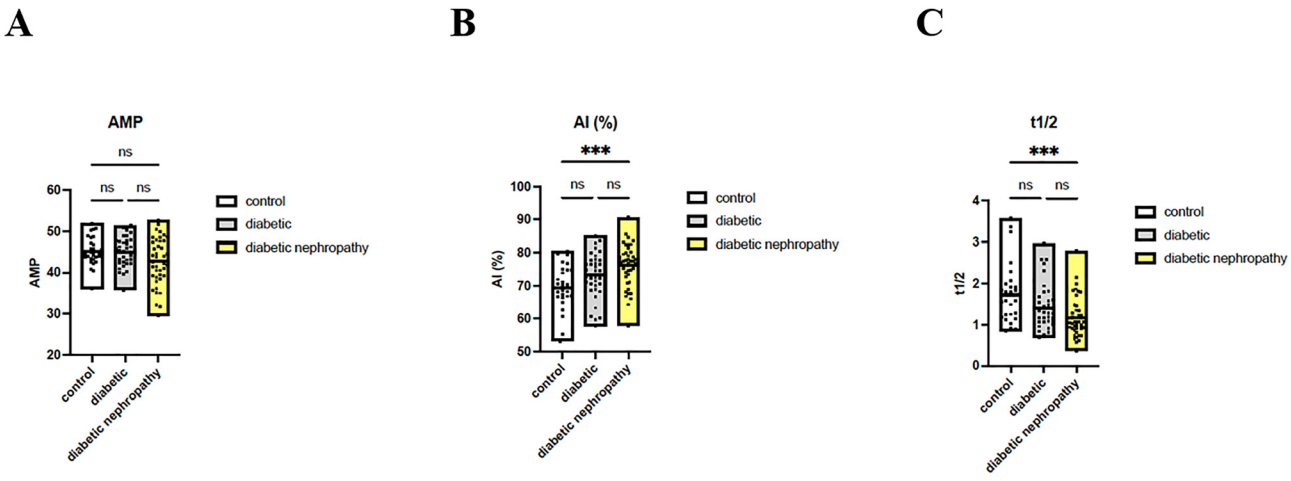


Figure 3: Lorrca indices for aggreagtion of RBC subpopulations with (A) AMP, (B) AI and (C) t1/2 datasets.

Table 4: Laboratory data on plasma viscosity for the study subjects were collected at baseline, with values expressed as mean min-max standard deviation.

	Healthy volunteers (n=31)	Diabetic patients (n=41)	Diabetic nephropathy patients (n=45)	p-Value
PV_300 s-1	1.39 (1.23–1.86)	1.44 (0.83–1.76)	1.54 (1.3–2.15)	<0.001
PV_450 s-1	1.34 (1.22–1.72)	1.41 (0.8–1.68)	1.58 (1.27–2.02)	<0.001

PV stands for plasma viscosity. Viscosity measurements were conducted at 450 s-1 shear rates, and a 40 cp disk was utilized. The Kruskal-Wallis H test and Mann-Whitney U test were used for statistical analysis.

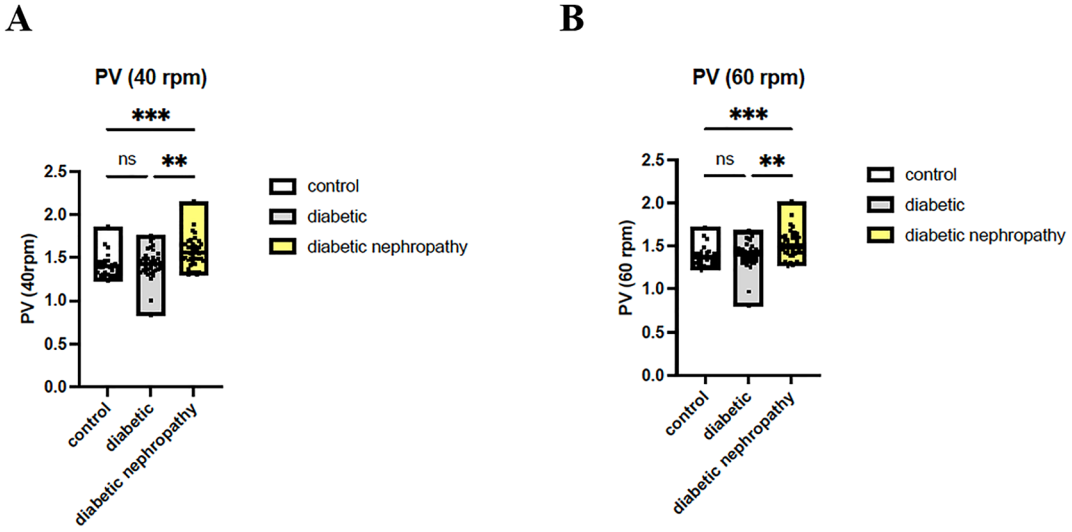


Figure 4: Lorrca indices for plasma viscosity of RBC subpopulations with (A) 300 s-1, and (B) 450 s-1 sheer rates.

This increase in plasma viscosity suggests that blood in patients with diabetic nephropathy is thicker and flows more slowly, which can contribute to complications commonly associated with this condition. Higher viscosity can impede blood flow, potentially exacerbating issues related to poor circulation and increasing the risk of vascular diseases.

Correlation analysis

The selected patients, including healthy donors, have a wide range of age distributions. To assess the impact of age variability, a correlation analysis was conducted for each parameter in the demographic data and hemorheology values based on the table provided (Table 1). The correlation

Table 5: Correlation analysis of various clinical and hemorheological parameters.

Correlation analysis		Age	HbA_{1c}	Creatinine	GFR	Albumin excretion rate	Protein excretion rate	HCT	Ss1/2	EImax	AMP	AI	T1/2
Age	Dm	1	-0.392[a]	0.048	-0.751[b]	−0.217	0.159	−0.169	−0.067	−0.292	−0.203	−0.157	0.178
	Dn	1	−0.124	−0.162	−0.137	-0.534[b]	-0.433[b]	−0.19	0	0.092	−0.111	−0.111	0.081
	Cn	1	−0.081	−0.176	−0.113	–	–	−0.19	−0.098	−0.139	−0.144	−0.049	0.092
HbA_{1c}	Dm		1	−0.272	0.493[b]	0.422[b]	−0.043	0.113	0.316[a]	0.192	0.374[a]	−0.105	0.029
	Dn		1	−0.143	−0.056	0.265	0.132	−0.006	0.041	−0.266	−0.255	0.217	−0.162
	Cn		1	−0.109	0.406[a]	–	–	0.237	0.018				
Creatinine	Dm			1	-0.568[b]	−0.256	−0.275	0.393[a]	0.178	−0.142	−0.088	−0.023	0.041
	Dn			1	-0.595[b]	0.18	0.034	-0.351[a]	0.346[a]	−0.081	0.218	0.052	−0.109
	Cn			1	-0.503[b]	–	–	0.237	0.321[a]	−0.124			
GFR	Dm				1	0.312[a]	−0.02	0.042	0.018	0.257	0.322[a]	−0.005	−0.035
	Dn				1	0.068	0.201	0.317[a]	−0.228	−0.036	−0.011	0.066	−0.014
	Cn				1	–	–	0.096	0.011	0.118			
Albumin excretion rate	Dm					1	0.23	0.03	0.145	0.071	0.175	−0.128	0.119
	Dn					1	0.779[b]	−0.087	0.231	−0.162	0.166	−0.047	0.092
	Cn					1	–	–	–	–	–	–	–
Protein excretion rate	Dm						1	−0.074	0.179	−0.176	−0.021	−0.123	0.13
	Dn						1	−0.122	0.121	−0.049	0.195	−0.012	0.051
	Cn						1	–	–	–	–	–	–
HCT	Dm							1	−0.178	0.152	0.302	−0.203	0.17
	Dn							1	−0.223	−0.146	0.034	0.093	−0.056
	Cn							1	−0.203	0.132	0.187	−0.156	0.018
SS1/2	Dm								1	-0.354[a]	0.314[a]	−0.177	0.14
	Dn								1	−0.283	0.113	0.034	0.039
	Cn								1	−321[a]	0.203	−0.256[a]	0.48
EImax	Dm									1	−0.046	0.218	−0.216
	Dn									1	0.125	−0.257	0.195
	Cn									1	−0.064	0.364	−0.462[a]
AMP	Dm										1	-0.515[b]	0.462[b]
	Dn										1	-0.627[b]	0.548[b]
	Cn										1	−348	0.376[b]
AI	Dm											1	-0.983[b]
	Dn											1	-0.977[b]
	Cn											1	−997[b]
T1/2	Dm												1
	Dn												1
	Cn												1

This Table presents the correlation analysis between various clinical and hemorheological parameters across different patient groups, including diabetic mellitus (dm), diabetic nephropathy (dn), and control (cn). Parameters analyzed include age, HbA_{1c}, creatinine, glomerular filtration rate (GFR), albumin excretion rate, protein excretion rate, Hematocrit (HCT), SS1/2, EImax, AMP, AI, and T1/2. The Table displays the correlation coefficients, with significant correlations indicated by [a] ($p<0.05$) and [b] ($p<0.01$). This comprehensive analysis reveals the interrelationships between these parameters, highlighting significant correlations that may impact the understanding of renal function and metabolic control among the different patient groups. Each cell represents the correlation coefficient for the corresponding parameter and group, elucidating how these clinical metrics interact with each other.

analysis clearly indicates that creatinine levels vary with age, primarily due to the decline in kidney function associated with aging. Consequently, GFR, albumin excretion rate, and protein excretion rate are also correlated with age since the calculation of these parameters incorporates creatinine values in the mathematical equations. As GFR calculation inherently includes age, the impact of aging on GFR values in the correlation analysis is an unavoidable outcome. Apart from GFR, albumin excretion rate, and protein excretion rate, the correlation analysis shows that age does not have a statistically significant impact on the other parameters listed, Table 5.

Discussion

Diabetic nephropathy, a serious microvascular complication of diabetes mellitus, is the primary cause of end-stage renal disease (ESRD) [32]. The histological characteristic, clinically manifested as diabetic glomerulosclerosis, is marked by a progressively escalating incidence of proteinuria, ultimately resulting in a gradual deterioration of renal function until the development of end-stage renal disease [33]. Hyperglycemia is one of the major factors for the main metabolic disruption that causes serious damage to the organs in diabetes, but the mechanism through which hyperglycemia leads to nephropathy is not fully elucidated [34, 35]. There are many glucose metabolites and reaction products that increase in volume due to hyperglycemia. The aldose reductase pathways cause the accumulation of toxic molecules in the cell, indicating how hyperglycemia can impair tissue metabolism [34, 36]. Nonenzymatic glycoxidation reactions in diabetic nephropathy lead to the accumulation of advanced glycation end-products (AGEs), which are linked to impaired red blood cell deformability. Glycoproteins on the red cell membrane and hemoglobin have been identified in diabetic patients as key factors contributing to alterations in the rheological properties of human erythrocytes in diabetes [37–39]. Such disruptions may also impair tissue oxygenation, leading to the formation of ischemic areas, then to interruptions in hemodynamics, and ultimately to organ-based disorders. In this context, the hemorheological status of patients with diabetes and diabetic nephropathy needs to be examined.

Leveraging recent advances in microfluidic technology, numerous point-of-care devices that integrate hemorheological effects have been developed. These devices enable comprehensive clinical studies through hemorheological measurements and play a crucial role in slowing the progression of various complications, thereby improving the patient's quality of life. The importance of early detection in preventing the progression of diabetic microangiopathy highlights the significance of these technological advancements. The range of hemorheological indices includes plasma viscosity, hematocrit, fibrinogen, erythrocyte deformability, and critical shear stress, with the latter defined as the minimum shear stress necessary for aggregates to disperse, serving as an indicator of red blood cell aggregation. Diabetes affects RBC function through interactions with their membrane and intracellular components, with studies showing that erythrocyte deformability is reduced in diabetes, especially when microvascular complications are present [21].

Red blood cell deformability is a passive alteration in the morphology of red blood cells due to shear forces and plays a key role in blood flow and function in the microcirculation. In our experiments, we have shown that there is a significant decrease in RBC deformability in patients who develop microvascular symptoms, in this case, nephropathy. Much research has indicated that impaired red blood cell deformability is caused by high blood glucose concentration and hyperosmolarity, hyperinsulinemia, changes in red blood cell membrane lipid distribution, increased internal viscosity, accumulation of sorbitol via the polyol pathway, and elevated erythrocyte membrane stiffness due to glycation of the erythrocyte membrane [38, 40–44]. Although the primary mechanism behind red blood cell alteration in diabetes is not well understood, checking deformability in the diabetic patient group can be crucial for treatment and to prevent potential tissue damage.

In our study, RBC aggregation in patient groups showed a statistically significant increase compared to the control group. Additionally, a strong correlation was observed between the increase in RBC aggregation in patient groups and the statistically significant increase in their plasma viscosity. These correlations suggest that the increase in plasma density, and thus plasma viscosity, causes erythrocytes to physically come closer to each other, thereby increasing their tendency to aggregate. A model, known as the bridging model, explains this phenomenon by suggesting that the increase in the density of macromolecules found in the plasma causes erythrocytes to aggregate [45]. The increase in AGEs and red cell membrane glycoproteins and hemoglobin, as previously shown, can also be one of the main factors playing a role in the deterioration of hemorheology.

Human red blood cells typically have a diameter of about 8 μm, yet they must undergo repeated deformations to traverse capillaries as small as 2 μm efficiently to deliver oxygen throughout the body. The impairment of this deformability is linked to the pathology of numerous diseases, making it a potential biomarker for assessing disease

states and treatment effectiveness. Microfluidic techniques are particularly noteworthy for their efficacy in measuring RBC deformability [46]. Given the heightened risk of cardiovascular comorbidities in individuals with diabetes and diabetic nephropathy, the inclination towards red blood cell aggregation can cause significant disturbances in microcirculation and a decline in tissue perfusion. Therefore, increased erythrocyte aggregation and viscosity in diabetic and diabetic nephropathy patients represent a risk factor that should not be overlooked during treatment. Additionally, monitoring parameters that contribute to an increased tendency for aggregation and viscosity is advantageous in patient care.

Research ethics: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study. The study supporting this work was approved by the Hamidiye Scientific Research Ethics Committee of the University of Health Sciences, Turkey, under the registration number 2022/324.

Informed consent: Not applicable.

Author contributions: D.S-A. and A.K. designed the study. N.H., S.T., O.C., S.E.C., H.G.C. and K.N.B. performed the research. D.S-A., N.H. and A.K. analyzed the data. D.S-A. wrote the paper. D.S-A., A.K. and Y.K. critically reviewed and revised the paper. All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Use of Large Language Models, AI and Machine Learning Tools: None declared.

Conflict of interest: The authors state no conflict of interest.

Research funding: None declared.

Data availability: Not applicable.

References

1. Valencia WM, Florez H. How to prevent the microvascular complications of type 2 diabetes beyond glucose control. BMJ 2017: i6505. [Internet] Available from: https://www.bmj.com/lookup/doi/10.1136/bmj.i6505.
2. Burrows NR, Hora I, Geiss LS, Gregg EW, Albright A. Incidence of end-stage renal disease attributed to diabetes among persons with diagnosed diabetes – United States and Puerto Rico, 2000–2014. MMWR Morb Mortal Wkly Rep 2017;66:1165–70. [Internet] Available from: http://www.cdc.gov/mmwr/volumes/66/wr/mm6643a2.htm.
3. Zhang L, Long J, Jiang W, Shi Y, He X, Zhou Z, et al. Trends in chronic kidney disease in China. N Engl J Med 2016;375:905–6. [Internet] Available from: http://www.nejm.org/doi/10.1056/NEJMc1602469.
4. Lopez-Vargas PA, Tong A, Howell M, Craig JC. Educational interventions for patients with CKD: a systematic review. Am J Kidney Dis 2016;68:353–70. [Internet] Available from: https://linkinghub.elsevier.com/retrieve/pii/S0272638616001499.
5. Brown CD, Ghali HS, Zhao Z, Thomas LL, Friedman EA. Association of reduced red blood cell deformability and diabetic nephropathy. Kidney Int 2005;67:295–300.
6. Lee S, Lee MY, Nam JS, Kang S, Park JS, Shin S, et al. Hemorheological approach for early detection of chronic kidney disease and diabetic nephropathy in type 2 diabetes. Diabet Technol Ther 2015;17:808–15. [Internet] Available from: http://www.liebertpub.com/doi/10.1089/dia.2014.0295.
7. Le Devehat C, Khodabandehlou T, Vimeux M. Relationship between hemorheological and microcirculatory abnormalities in diabetes mellitus. Diabete Metab 1994;20:401–4. [Internet] Available from: http://www.ncbi.nlm.nih.gov/pubmed/7843471.
8. MacRury SM, Lowe GDO. Blood rheology in diabetes mellitus. Diabet Med 1990;7:285–91. [Internet] Available from: https://onlinelibrary.wiley.com/doi/10.1111/j.1464-5491.1990.tb01391.x.
9. Zimny S, Dessel F, Ehren M, Pfohl M, Schatz H. Early detection of microcirculatory impairment in diabetic patients with foot at risk. Diabetes Care 2001;24:1810–4. [Internet] Available from: https://diabetesjournals.org/care/article/24/10/1810/20673/Early-Detection-of-Microcirculatory-Impairment-in.
10. Baskurt OK, Hardeman MR, Rampling MW, Meiselman HJ. Handbook of hemorheology and. Health Care 2007:468.
11. Singh M, Shin S. Changes in erythrocyte aggregation and deformability in diabetes mellitus: a brief review. Indian J Exp Biol 2009;47:7–15. [Internet] Available from: http://www.ncbi.nlm.nih.gov/pubmed/19317346.
12. Martínez M, Vayá A, Server R, Gilsanz A, Aznar J. Alterations in erythrocyte aggregability in diabetics: the influence of plasmatic fibrinogen and phospholipids of the red blood cell membrane. Clin Hemorheol Microcirc 1998;18:253–8. [Internet] Available from: http://www.ncbi.nlm.nih.gov/pubmed/9741665.
13. Bommer C, Heesemann E, Sagalova V, Manne-Goehler J, Atun R, Bärnighausen T, et al. The global economic burden of diabetes in adults aged 20–79 years: a cost-of-illness study. Lancet Diabetes Endocrinol 2017;5:423–30. [Internet] Available from: https://linkinghub.elsevier.com/retrieve/pii/S2213858717300979.
14. Afkarian M, Zelnick LR, Hall YN, Heagerty PJ, Tuttle K, Weiss NS, et al. Clinical manifestations of kidney disease among US adults with diabetes, 1988-2014. JAMA 2016;316:602. [Internet] Available from: http://jama.jamanetwork.com/article.aspx?doi=10.1001/jama.2016.10924.
15. Shlipak M. Diabetic nephropathy: preventing progression. BMJ Clin Evid 2010;2010. [Internet] Available from: http://www.ncbi.nlm.nih.gov/pubmed/21418671.
16. Association AD. 10. Microvascular complications and foot care: standards of medical care in diabetes–2018. Diabetes Care 2018;41:S105–18. [Internet] Available from: https://diabetesjournals.org/care/article/41/Supplement_1/S105/29707/10-Microvascular-Complications-and-Foot-Care.
17. Perkins BA, Ficociello LH, Silva KH, Finkelstein DM, Warram JH, Krolewski AS. Regression of microalbuminuria in type 1 diabetes. N

Engl J Med 2003;348:2285–93. [Internet] Available from: http://www.nejm.org/doi/abs/10.1056/NEJMoa021835.
18. Ju M, Leo HL, Kim S. Numerical investigation on red blood cell dynamics in microflow: effect of cell deformability. Clin Hemorheol Microcirc 2017;65:105–17. [Internet] Available from: https://www.medra.org/servlet/aliasResolver?alias=iospress&doi=10.3233/CH-16128.
19. Shin S, Ku Y. Hemorheology and clinical application: association of impairment of red blood cell deformability with diabetic nephropathy. Korea-Australia Rheology. Journal 2005;17:117–23.
20. Moon JS, Kim JH, Kim JH, Park IR, Lee JH, Kim HJ, et al. Impaired RBC deformability is associated with diabetic retinopathy in patients with type 2 diabetes. Diabetes Metab 2016;42:448–52. [Internet] Available from: https://linkinghub.elsevier.com/retrieve/pii/S1262363616304001.
21. Park KH, Kim U, Choi KU, Nam JH, Lee JH, Lee CH, et al. Hemorheologic alterations in patients with type 2 diabetes mellitus presented with an acute myocardial infarction. Diabetes Metab J 2018;42:155. [Internet] Available from: http://e-dmj.org/journal/view.php?doi=10.4093/dmj.2018.42.2.155.
22. Da Costa L, Suner L, Galimand J, Bonnel A, Pascreau T, Couque N, et al. Diagnostic tool for red blood cell membrane disorders: assessment of a new generation ektacytometer. Blood Cells Mol Dis 2016;56:9–22. [Internet] Available from: https://linkinghub.elsevier.com/retrieve/pii/S1079979615001679.
23. Da Costa L, Galimand J, Fenneteau O, Mohandas N. Hereditary spherocytosis, elliptocytosis, and other red cell membrane disorders. Blood Rev 2013;27:167–78. [Internet] Available from: https://linkinghub.elsevier.com/retrieve/pii/S0268960X13000192.
24. Llaudet-Planas E, Vives-Corrons JL, Rizzuto V, Gómez-Ramírez P, Sevilla Navarro J, Coll Sibina MT, et al. Osmotic gradient ektacytometry: a valuable screening test for hereditary spherocytosis and other red blood cell membrane disorders. Int J Lab Hematol 2018;40:94–102. [Internet] Available from: https://onlinelibrary.wiley.com/doi/10.1111/ijlh.12746.
25. Lazarova E, Gulbis B, Oirschot B, Van Wijk R. Next-generation osmotic gradient ektacytometry for the diagnosis of hereditary spherocytosis: interlaboratory method validation and experience. Clin Chem Lab Med 2017;55. [Internet] Available from: https://www.degruyter.com/document/doi/10.1515/cclm-2016-0290/html.
26. 2. Classification and diagnosis of diabetes: standards of medical care in diabetes—2021. Diabetes Care 2021;44:S15–33. [Internet] Available from: https://diabetesjournals.org/care/article/44/Supplement_1/S15/30859/2-Classification-and-Diagnosis-of-Diabetes.
27. Kidney Disease: Improving Global Outcomes (KDIGO) Glomerular Diseases Work Group. KDIGO 2021 clinical practice guideline for the management of glomerular diseases. Kidney Int 2021;100:S1–276. [Internet] Available from: http://www.ncbi.nlm.nih.gov/pubmed/34556256.
28. Grigorieva DV, Gorudko IV, Sokolov AV, Kosmachevskaya OV, Topunov AF, Buko IV, et al Measurement of plasma hemoglobin peroxidase activity. 2013;155:118–21., [Internet] Available from: http://link.springer.com/10.1007/s10517-013-2095-3.
29. Baskurt OK, Hardeman MR, Uyuklu M, Ulker P, Cengiz M, Nemeth N, et al. Parameterization of red blood cell elongation index – shear stress curves obtained by ektacytometry. Scand J Clin Lab Invest 2009;69:777–88. [Internet] Available from: http://www.tandfonline.com/doi/full/10.3109/00365510903266069.
30. Kuznetsova PI, Raskurazhev AA, Shabalina AA, Melikhyan AL, Subortseva IN, Tanashyan MM. Red blood cell morphodynamics in patients with polycythemia vera and stroke. Int J Mol Sci 2022;23:2247. [Internet] Available from: https://www.mdpi.com/1422-0067/23/4/2247.
31. Teległów A, Seremak J, Golec J, Marchewka J, Golec P, Marchewka U, et al. The effect of sulfur baths on hemorheological properties of blood in patients with osteoarthritis. Sci Rep 2023;13:7960. [Internet] Available from: https://www.nature.com/articles/s41598-023-35264-8.
32. Mauer SM, Chavers BM. A comparison of kidney disease in type I and type II diabetes; 1985. 299–303. https://doi.org/10.1007/978-1-4757-1850-8_16. Available from: http://link.springer.com/10.1007/978-1-4757-1850-8_16.
33. Monica T, Loredana HP, Daniela D, Ileana I. Blood viscosity in patients with diabetic nephropathy versus blood viscosity in healthy patients. ARS Medica Tomitana 2018;24:114–8. [Internet] Available from: https://www.sciendo.com/article/10.2478/arsm-2018-0021.
34. Porte D, Schwartz MW. Diabetes complications: why is glucose potentially toxic? Science 1979;272:699–. [Internet]. 1996 May 3 Available from: https://www.science.org/doi/10.1126/science.272.5262.699.
35. Friedman EA. Diabetic nephropathy is a hyperglycemic glomerulopathy. Arch Intern Med 1982;142:1269–70. [Internet] Available from: http://www.ncbi.nlm.nih.gov/pubmed/7092440.
36. Craven PA, Studer RK, Negrete H, DeRubertis FR. Protein kinase C in diabetic nephropathy. J Diabet Complicat 1995;9:241–5. [Internet] Available from: https://linkinghub.elsevier.com/retrieve/pii/1056872795800124.
37. Makita Z, Vlassara H, Rayfield E, Cartwright K, Friedman E, Rodby R, et al. Hemoglobin-AGE: a circulating marker of advanced glycosylation. Science 1979;258:651–3. 1992[Internet] Available from: https://www.science.org/doi/10.1126/science.1411574.
38. Maeda N, Kon K, Imaizumi K, Sekiya M, Shiga T. Alteration of rheological properties of human erythrocytes by crosslinking of membrane proteins. Biochim Biophys Acta Biomembr 1983;735:104–12. [Internet] Available from: https://linkinghub.elsevier.com/retrieve/pii/0005273683902651.
39. Miller JA, Gravallese E, Bunn HF. Nonenzymatic glycosylation of erythrocyte membrane proteins. Relevance to diabetes. J Clin Invest 1980;65:896–901. [Internet] Available from: http://www.jci.org/articles/view/109743.
40. Robey C, Dasmahapatra A, Cohen MP, Suarez S. Sorbinil partially prevents decreased erythrocyte deformability in experimental diabetes mellitus. Diabetes 1987;36:1010–3. [Internet] Available from: https://diabetesjournals.org/diabetes/article/36/9/1010/6574/Sorbinil-Partially-Prevents-Decreased-Erythrocyte.
41. Baba Y, Kai M, Kamada T, Setoyama S, Otsuji S. Higher levels of erythrocyte membrane microviscosity in diabetes. Diabetes 1979;28:1138–40. [Internet] Available from: https://diabetesjournals.org/diabetes/article/28/12/1138/6006/Higher-Levels-of-Erythrocyte-Membrane.
42. Leblond PF, Coulombe L. The measurement of erythrocyte deformability using micropore membranes. A sensitive technique with clinical applications. J Lab Clin Med 1979;94:133–43. [Internet] Available from: http://www.ncbi.nlm.nih.gov/pubmed/469370.
43. Schmid-Schönbein H, Volger E. Red-cell aggregation and red-cell deformability in diabetes. Diabetes 1976;25:897–902. [Internet] Available from: http://www.ncbi.nlm.nih.gov/pubmed/971793.
44. McMillan DE, Utterback NG, La PJ. Reduced erythrocyte deformability in diabetes. Diabetes 1978;27:895–901. [Internet] Available from: https://diabetesjournals.org/diabetes/article/27/9/895/5271/Reduced-Erythrocyte-Deformability-in-Diabetes.

45. Baskurt OK, Meiselman HJ. Erythrocyte aggregation: basic aspects and clinical importance. Clin Hemorheol Microcirc 2013;53:23–37. [Internet] Available from: https://www.medra.org/servlet/aliasResolver?alias=iospress&doi=10.3233/CH-2012-1573.

46. Reale R, De Ninno A, Nepi T, Bisegna P, Caselli F. Extensional-flow impedance cytometer for contactless and optics-free erythrocyte deformability analysis. IEEE Trans Biomed Eng 2023;70:565–72. [Internet] Available from: https://ieeexplore.ieee.org/document/9852302/.