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ORIGINAL ARTICLE

Erythrocyte deformability correlates with microvascular ischemia and disease severity in diabetic retinopathy

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ABSTRACT

Aims: Impaired red blood cell (RBC) deformability is a potential contributor to microvascular dysfunction in diabetes. This study aimed to determine the association between erythrocyte deformability and the severity of diabetic retinopathy (DR), focusing on quantitative measures of macular and peripheral ischemia.

Methods: We retrospectively analyzed 79 patients with treatment-naïve DR. RBC deformability was measured using a microfluidic ektacytometer, and the elongation index at 3 pascals (EI@3P) was calculated. Optical coherence tomography angiography (OCTA) and ultra-widefield fluorescein angiography (UWFFA) were performed to quantify macular vessel density, foveal avascular zone (FAZ) area, and peripheral ischemic index. Associations between EI@3P and imaging parameters were assessed across DR severity groups using correlation and regression analyses.

Results: EI@3P significantly decreased with advancing DR stages (P = 0.010). Eyes with lower EI@3P exhibited reduced vessel density and enlarged FAZ in the deep capillary plexus on OCTA (P = 0.042 and P = 0.041, respectively). In severe non-proliferative and proliferative DR, reduced EI@3P was associated with a higher peripheral ischemic index on UWFFA (r = −0.206, P = 0.035). No significant association was observed between EI@3P and glycated hemoglobin or diabetes duration.

Conclusions: Decreased erythrocyte deformability correlates with microvascular ischemia and disease severity in DR, suggesting a hemorheological mechanism linking systemic blood rheology to retinal microcirculatory impairment. EI@3P may serve as a novel biomarker for assessing microvascular complications in diabetes.

INTRODUCTION

Diabetic retinopathy (DR) is the most prevalent microvascular complication in diabetic patients and is the leading cause of blindness among the working-age population[1]. Hyperglycemia-mediated oxidative stress and inflammation damage the endothelial cells in retinal capillaries[2]. The adhesion and infiltration of leukocytes, coupled with endothelial damage, cause the physical occlusion of capillaries through thrombosis and the release of inflammatory mediators[3]. Recently, it has been suggested that the biophysical properties of erythrocytes may affect platelet aggregability and adherence to the endothelium, resulting in thrombosis formation and microvascular complications in diabetes.[4,5]. We previously reported that impaired erythrocyte deformability was associated with the risk of DR[6]. However, to date, no clinical research has demonstrated how the biophysical properties of erythrocytes contribute to the pathogenesis and progression of DR.

Erythrocytes are highly specialized cells with a biconcave shape, and their unique structure enables them to deform readily under mechanical force[7]. Erythrocyte deformability, defined as the ability of erythrocytes to deform and elongate under

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externally applied shear stress[6]. is essential for passing through the capillary bed, where retinal capillaries are smaller than the diameter of erythrocytes. Impaired erythrocyte deformability can result in perfusion insufficiencies in various diseases, including sickle cell disease, hereditary erythrocyte diseases, and malaria[8–10]. Although several intrinsic and extrinsic factors determine the rheological properties of erythrocytes, metabolic changes in diabetes are considered one of the critical factors causing erythrocytes to stiffen through the modification of cell membranes[11].

Diabetic macular ischemia is a significant cause of visual impairment in patients with DR[12]. Clinically, it is defined by the enlargement of the foveal avascular zone (FAZ) and areas of capillary nonperfusion in the paramacular region[13]. The advent of optical coherence tomography (OCT) angiography has enlarged the visualization of macular capillary loss in DR[14]. Ultra-widefield (UWF) angiography enables the visualization of peripheral non-perfusion areas[15] that contribute to neovascularization, the hallmark of advanced DR, and poor visual outcomes.[16] However, the quantitative correlation between macular and peripheral ischemic indices is controversial and usually does not exhibit a linear relationship[17]. Therefore, a common pathogenic mechanism derived from blood cells, rather than blood vessels, is needed to address the fundamental limitations of DR treatments.

Here, we investigated whether the biophysical changes in erythrocytes correlate with the severity of DR. We also evaluated the association between the deformability of erythrocytes and macular ischemia or peripheral retinal non-perfusion areas in DR using multimodal imaging techniques.

MATERIALS AND METHODS

Study design and patients

This retrospective cohort study enrolled outpatients with a confirmed diagnosis of type 2 diabetes mellitus (DM) with any stage of DR who visited Yeungnam University Hospital. The protocol for this research project was approved by the Ethics Committee of Yeungnam University Hospital, Daegu, Republic of Korea (Approval No. 2020-01-013), and it conforms to the provisions of the Declaration of Helsinki. The requirement for informed consent was waived due to the retrospective nature of the study. Patient anonymity was fully preserved throughout the study. Clinical trial registration was not required due to the retrospective observational design of the study.

We obtained patients' medical histories, performed ophthalmic examinations, and measured hemoglobin A1c (HbA1c) and red blood cell (RBC) deformability through venous sampling in all participants. Inclusion criteria were age 18 or older and clinical signs of DR according to the international classification. The diagnosis of DR was made based on a fundus examination and documented using color fundus photographs. Only treatment-naïve DR patients without a history of laser photocoagulation or intravitreal injections were enrolled. We included patients who underwent venous sampling within one month of their initial visit to our ophthalmology department, during which the severity of DR was evaluated. The exclusion criteria included the presence of type 1 diabetes, gestational diabetes, previous history of ocular trauma, intravitreal injections, intraocular surgery and/or laser treatment (cataract surgery within 6 months), concomitant retinal disease (e.g., dystrophy, vascular occlusion, or age-related macular degeneration), refractive error greater than ±6 diopters (as the spherical equivalent), glaucoma, significant media opacity that precluded high-quality fundus imaging and examination, low signal strength index (SSI < 50), presence of one or more blink artifacts, and poor fixation leading to motion artifacts.

The eyes of patients were divided into three groups based on their DR grades as determined by fundus photography and examination, guided by the ETDRS (Early Treatment Diabetic Retinopathy Study) severity scale: the mild-to-moderate non-proliferative diabetic retinopathy (NPDR) group, the severe NPDR group, and the proliferative diabetic retinopathy (PDR) group.

Erythrocyte deformability measurements

Erythrocyte deformability was analyzed in all patients according to a protocol previously reported by our group[6]. Venous samples were acquired from the antecubital veins of patients and placed into the EDTA tubes. Within six hours of collection, the venous samples were processed into an erythrocyte suspension by adding 0.5 mL of a phosphate-buffered saline solution containing highly viscous polyvinyl pyrrolidone (PVP; Sigma-Aldrich, St. Louis, MO, USA) to 5 μL of whole blood. Subsequently, the solution was placed in a microfluidic ektacytometer (Figure 1a, RheoScan-D, Sewon Meditech Inc., Seoul, Korea). Within the ektacytometer, erythrocytes were driven through a slit by a pressure difference of 3 Pa generated from a vacuum generator. A laser beam was directed through the slit, casting shadows of the erythrocytes as they passed through; these images are captured on a projection screen connected to a CCD (charge-coupled device) video camera. The projection images were used to obtain numerical data for the major and minor axes of the erythrocytes. The elongation index (EI) was computed as a percentage (EI@3P, %) by dividing the difference between the major (length, L) and minor (width, W) axes of the erythrocytes by their sum at 3 Pa: $EI = (L - W)/(L + W)$. A lower elongation index indicates impaired deformability of erythrocytes (Figure 1b). The patient information was blinded during the analysis process.

Baseline ophthalmic evaluation

A comprehensive ophthalmic examination was conducted for all whole participants, which included history taking, best-corrected visual acuity (BCVA) assessment (values were converted into LogMAR VA), slit-lamp biomicroscopy, intraocular pressure measurement, fundus examination, color fundus photographs, and fluorescein angiography photographs (Optos California; Optos plc, Dunfermline, UK). Additionally, OCT

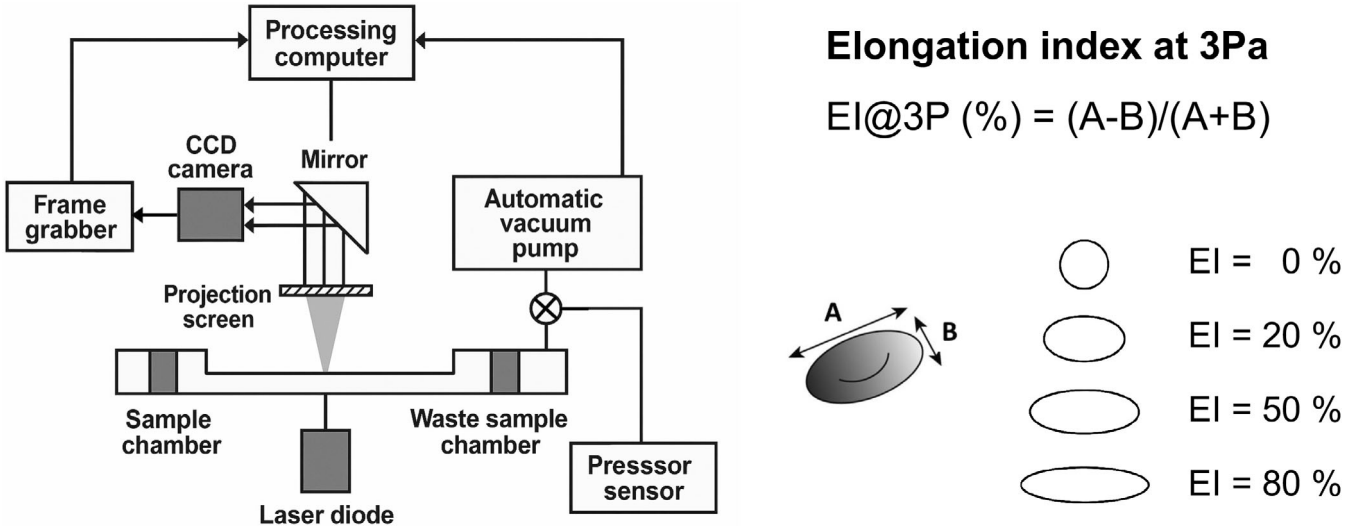

Figure 1 | Schematic illustration of the ektacytometer and determination of erythrocyte elongation index. The ektacytometer measures red blood cell deformability under controlled shear stress using a laser diffraction system with CCD detection. The elongation index (EI) is calculated as (A − B)/(A + B), where A and B denote the major and minor axes of the elliptical diffraction pattern.

angiography (Optovue RTVue XR AVANTI; Optovue, Inc., Fremont, CA, USA) and spectral-domain optical coherence tomography (Spectralis OCT; Heidelberg Engineering, Heidelberg, Germany) were performed (Figure S2). All imaging scans were acquired by an experienced examiner while the participants' pupils were dilated. All imaging evaluations, including OCT, OCTA, and UWFFA performed within 2 months—closest to the time of venous sampling for erythrocyte deformability—were included in the analysis.

Measurements of the ischemic area in UWFFA

The ischemic index of peripheral retinal nonperfusion was calculated using ultra-widefield fluorescein angiography (UWFFA). To correct peripheral distortion of UWF imaging, we used stereographic projection software. The ImageJ software (version 1.48; National Institutes of Health, Bethesda, MD, USA) was used to manually mark the entire picture area and the region of capillary nonperfusion seen during the arteriovenous phase. The ischemic index was calculated by dividing the pixels of the nonperfusion area by the pixels of the total image area (Figure S3). Two independent examiners (IK and JL) examined all manual measurements, and the averaged values were used in the final analysis. Generally, the interperformer agreements were good (ICC value = 0.952).

Assessment of diabetic macular edema

OCT images were analyzed to detect the presence and extent of diabetic macular edema (DME). SD-OCT scans were obtained in a 6 mm cross-hair fashion pattern centered on the fovea, both horizontally and vertically. Additionally, a volume scan comprising 49 single scans was used to measure the central macular thickness (CMT, μm) within a 1-mm-diameter circle centered on the fovea. CMT was automatically measured using the mapping software of the coherence tomography system (Heidelberg Eye, version 1.7.0.0). DME was defined as CMT of more than 300 μm.

Quantifications of vascular parameters in OCTA

We also captured 3-mm × 3-mm images of the macula centered on the fovea to obtain OCT angiography volume scans (Figure S2). OCT angiography data was extracted from split-spectrum amplitude-decorrelation angiography. For the superficial plexus (from 2.6 μm below the internal limiting membrane to 15.6 μm below the junction between the inner plexiform and inner nuclear layers [IPL/INL]) and the deep plexus (from 15.6 μm below the IPL/INL to 70.2 μm below the IPL/INL), segmentation was performed automatically by the OCT device using an integrated algorithm. The area of the foveal avascular zone (FAZ, mm^2) and vessel density (VD, %) at the levels of the superficial (SCP) and deep retinal capillaries (DCP) were calculated automatically by the program (RTVue-XR version: 2017.1.0.151) for a percentage of the total area.

Statistical analysis

We presented the patients' baseline characteristics as frequencies with percentages for categorical variables and as mean ± standard deviation (SD) for continuous variables. One-way analysis of variance (ANOVA) was used to analyze the clinical characteristic trends across the DR group. Student's *t*-test was used to compare RBC deformability with DME, OCTA parameters, and ischemic index between patient groups. The

associations between RBC deformability and retinal vascular parameters were determined using Spearman's correlation coefficient. All statistical analyses were performed with IBM SPSS Statistics, version 22.0 (IBM Inc., Armonk, NY, USA), and *P*-values <0.05 were considered statistically significant.

RESULTS

Baseline characteristics of participants

A total of 79 patients were included in the study, with 14 [18%] in the mild-to-moderate NPDR group, 28 [35%] in the severe NPDR group, and 37 [47%] in the PDR group. The mean age of the participants was 58.7 ± 8.9 years, the mean duration of diabetes was 12.1 ± 8.9 years, and the mean glycated hemoglobin (HbA1c) was 8.81% ± 2.28%. The characteristics of the patients according to the DR grade are summarized in Table 1. There were no significant differences among the groups in terms of age, sex, spherical equivalent, and visual acuity. However, the mild-to-moderate NPDR group had a shorter duration of diabetes ($P < 0.001$) and lower HbA1c ($P = 0.013$) compared with the other groups. Figure 1 illustrates the schematic design of the ektacytometer and the determination of the erythrocyte elongation index. The elongation index (EI) was computed as a percentage (EI@3P, %) by dividing the difference between the major (length, L) and minor (width, W) axes of the erythrocytes by their sum at 3 Pa. EI@3P did not show significant associations with HbA1c ($P = 0.319$) or the duration of diabetes ($P = 0.167$) (Figure S1).

Differential erythrocyte deformability by DR severity, not by DME presence

The mean EI@3P (%) value of all patients was 28.26 ± 2.41. The EI@3P level differed significantly among the three DR groups ($P = 0.010$, Table 1). The mild-to-moderate NPDR group had a significantly higher EI@3P than the other groups, while the PDR group had a significantly lower EI@3P than the severe NPDR group. Next, we evaluated whether erythrocyte deformability was associated with DME in any stage of DR. A total of 147 eyes that were suitable for OCT assessment were analyzed. When we compared the EI@3P values between the eyes with or without DME, there was no statistically significant difference between the groups in all DR stages (Table 2).

Table 2 | Comparison of erythrocyte deformability according to the presence of DME

	Eyes with or without DME (*n*, Y/N)	EI@3P in Eyes with DME†	EI@3P in Eyes without DME	*P*-value*
Mild-to-moderate NPDR	0/27	NA	31.10 ± 1.76	NA
Severe NPDR	17/38	28.78 ± 1.69	28.74 ± 1.88	0.93
PDR	31/34	26.61 ± 1.70	26.77 ± 2.07	0.74
Total eyes	48/99	27.38 ± 1.98	28.70 ± 2.54	0.27

DR, diabetic retinopathy; DME, diabetic macular edema; EI@3P, elongation index calculated at 3 pascal; NA, not available; NPDR, non-proliferative diabetic retinopathy; PDR, proliferative diabetic retinopathy. *Student's test. †DME: CMT ≥300 μm.

Erythrocyte deformability and macular ischemia

Based on our hypothesis that impaired erythrocytes may cause retinal capillary closure, we evaluated the association between EI@3P and macular capillary status in eyes with DR. Table 3 shows the results of macular capillary parameters in OCTA from the eyes without DME. We excluded the eyes with DME because intraretinal fluid or exudate can cause artifacts, segmentation errors, and capillary displacement in OCTA images. Based on the median EI@3P value, we divided the eyes into high RBC elongation (EI@3P ≥ 29.20, $n = 32$) and low RBC elongation (EI@3P < 29.20, $n = 17$) groups. Compared to the eyes with high erythrocyte elongation, eyes with low RBC elongation had a lower vessel density (VD) and a larger FAZ area in the deep capillary plexus (DCP) ($P = 0.042$ and $P = 0.041$, respectively, Table 3). However, VD and FAZ areas in the superficial capillary plexus (SCP) did not show a significant difference between the two groups. DCP VD was significantly positively correlated with EI@3P values (r = 0.3893, p = 0.007,

Table 1 | Baseline demographics and clinical characteristics of patients

	Total patients	Mild-to-moderate NPDR	Severe NPDR	PDR	*P*-value*
No of patients (*n*, %)	79 (100)	14 (17.7)	28 (35.5)	37 (46.8)	
Age (years)	58.7 ± 8.9	56.7 ± 11.4	62.1 ± 7.2	56.9 ± 8.7	0.09
Male:Female (% of M)†	51:28 (64.6)	9:5 (64.3)	18:10 (64.3)	24:13 (64.8)	0.35
Duration of DM (years)	12.1 ± 8.9	6.3 ± 1.8	12.7 ± 7.7	13.6 ± 9.8	<0.001
Visual acuity (logMAR)	0.3912 ± 0.631	0.0968 ± 0.131	0.1534 ± 0.219	0.6163 ± 0.784	0.14
SE (diopter)	−0.04 ± 1.60	−0.03 ± 1.46	0.03 ± 0.18	0.13 ± 3.16	0.11
HbA1c (%)	8.81 ± 2.28	8.68 ± 2.86	8.74 ± 2.17	8.98 ± 2.22	0.013
EI@3P	28.26 ± 2.41	31.13 ± 1.78	28.79 ± 1.82	26.77 ± 0.18	0.010

DM, diabetes mellitus; EI@3P, elongation index calculated at 3 pascal; NPDR, non-proliferative diabetic retinopathy; PDR, proliferative diabetic retinopathy; SE, spherical equivalent. *One-way analysis of variance. †Analyzed using the proportion with percentages of males.

Table 3 | Macular capillary parameters in non-DME eyes according to the degree of erythrocyte deformability

	Total eyes	High EI@3P group†	Low EI@3P group‡	Linear regression	
				B	*P*-value*
SCP VD (%)	39.10 ± 4.84	40.14 ± 4.92	37.75 ± 4.75	43.161	0.94
DCP VD (%)	42.71 ± 4.75	46.45 ± 4.02	39.50 ± 4.89	29.466	0.042
SCP FAZ (mm^2)	0.44 ± 0.21	0.43 ± 0.20	0.44 ± 0.22	0.370	0.92
DCP FAZ (mm^2)	0.57 ± 0.32	0.51 ± 0.21	0.62 ± 0.27	−4.548	0.041

DCP, deep capillary plexus; DME, diabetic macular edema; FAZ, foveal avascular zone; SCP, superficial capillary plexus; VD, vessel density. *Linear regression analysis. †EI@3P ≥ 29.20. ‡EI@3P < 29.20.

Figure 2). These data suggest that impaired erythrocyte deformability is associated with macular capillary loss, especially in the DCP.

Erythrocyte deformability and peripheral non-perfusion

The aforementioned results encouraged us to evaluate the association of erythrocyte deformability with the development or severity of the peripheral non-perfusion area, the major ischemic component that drives the progression of DR. The ischemic index values measured using UWFFA in each group divided by DR severity are shown in Table 4. As expected, patients with mild-to-moderate NPDR did not have a non-perfusion area. In addition, the PDR group had a higher ischemic index than did the severe NPDR group (0.315 ± 0.21 vs. 0.147 ± 0.16; $P < 0.001$). When we divided the eyes with severe NPDR or PDR according to the presence of a non-perfusion area in UWFFA, the mean EI@3P value was significantly lower in eyes with a non-perfusion area (27.64 ± 0.88, $n = 65$) compared to those without (31.13 ± 1.78, $n = 14$).

When the eyes were categorized according to the degree of RBC elongation (i.e., high vs. low), those with impaired RBC deformability showed a significantly higher ischemic index (Table 4, $P = 0.044$). The ischemic index was negatively

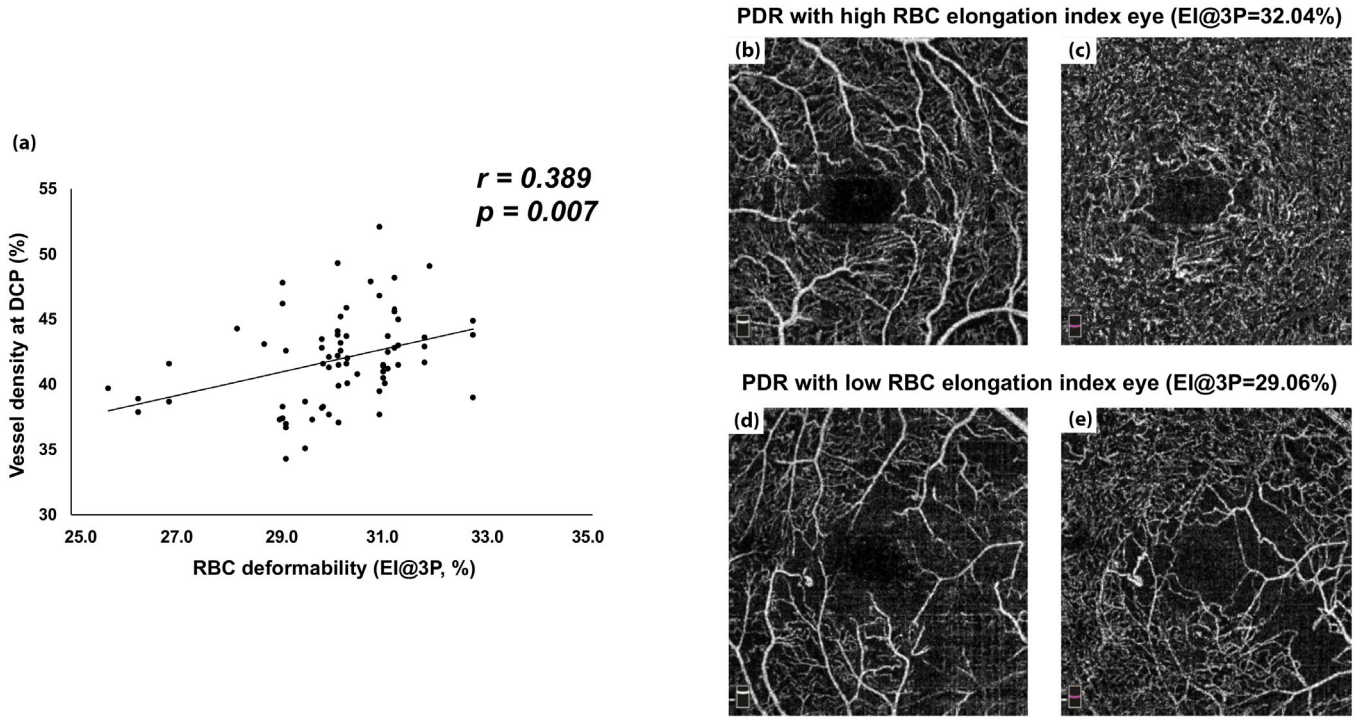

Figure 2 | Correlation between VD at DCP and RBC elongation index (a). Representative OCTA images of two patients with PDR. The (b) SCP and (c) DCP images are from a 53-year-old man with a 10-year history of T2DM and a high RBC elongation index eye (EI@3P = 32.04%). The OCTA images of (d) SCP and (e) DCP are from a 52-year-old man with an 8-year history of T2DM and a low RBC elongation index eye (EI@3P = 29.06%). Note the larger FAZ area and lower VD in the patient with a low RBC elongation index.

Table 4 | Peripheral non-perfusion area and erythrocyte deformability

	Without non-perfusion area	With non-perfusion area	*P*-value*
EI@3P (%)	31.13 ± 1.78	27.64 ± 0.88	0.004
	High EI@3P group†	Low EI@3P group‡	*P*-value*
Ischemic index	0.144 ± 0.213	0.242 ± 0.203	0.044

EI@3P, elongation index calculated at 3 pascal. *Student's test.
†EI@3P ≥ 29.20. ‡EI@3P < 29.20.

correlated with the EI@3P value in the severe NPDR and PDR groups with a non-perfusion area (r = –0.206, P = 0.035, Figure 3). These data suggest that impaired erythrocyte deformability is associated with the severity of peripheral non-perfusion areas in DR.

DISCUSSION

This study demonstrated that impaired erythrocyte deformability is associated with the severity of DR, macular ischemia, and peripheral ischemia. The elongation index of erythrocytes was strongly correlated with the degree of deep capillary loss in OCTA and the ischemic index from UWFFA. This is the first clinical study to demonstrate that the biophysical properties of erythrocytes can contribute to the pathogenic mechanisms of DR.

Previous studies[6,18] reported that erythrocyte deformability was lower in patients with DM. Several hypotheses have been proposed to explain the impaired RBC deformability in DM, including elevated blood glucose concentration and hyperosmolarity, hypoinsulinemia, alterations in RBC membrane lipid composition, increased internal viscosity, accumulation of sorbitol via enhanced activity of the polyol pathway, and increased erythrocyte membrane rigidity caused by glycation of erythrocyte membranes. Martinez *et al.*[19] reported changes in RBC membranes in diabetic patients compared to healthy controls and noted that the increase in the saturated nature of the phospholipids of the erythrocyte membrane in diabetic patients is significantly related to the increased RBC aggregability observed in DM patients. Furthermore, elevated blood glucose can cause hemoglobin, membrane, and skeletal proteins to become glycosylated, which can change cell shape and cause echinocytes or enlarged RBCs. In addition, oxidation of spectrin in the RBC cell membrane or decreased enzyme activity as a result of a malfunctioning Na-K-ATPase pump can cause oxidative damage to the RBC membrane. All of these DM-related factors can contribute to alterations in the RBC, making them more rigid and less deformable.

In this study, DR severity was significantly correlated with RBC deformability. In particular, the size of the ischemic area in DCP and FAZ was significantly associated with RBC deformability. Numerous studies have reported that blood

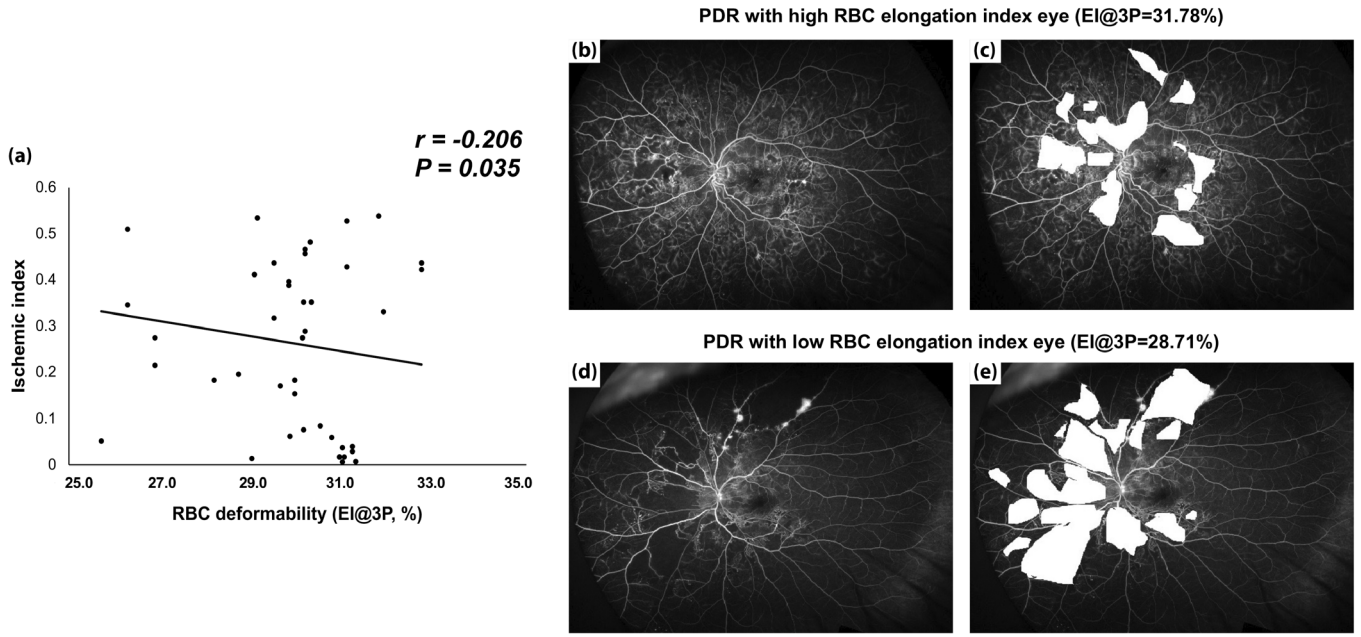

Figure 3 | Correlation between the ischemic index and RBC deformability (a). Representative UWFFA images of two patients with PDR. The stereographic projection images (B,D) and images of manually displayed ischemic lesions (C,E) are shown. (b, c) UWFFA images from a 44-year-old man with a 10-year history of T2DM and a high RBC elongation index eye (EI@3P = 31.78%). (d, e) UWFFA images from a 41-year-old man with a 1-year history of T2DM and a low RBC elongation index eye (EI@3P = 28.71%). Note the larger peripheral ischemic area in the patient with low RBC elongation.

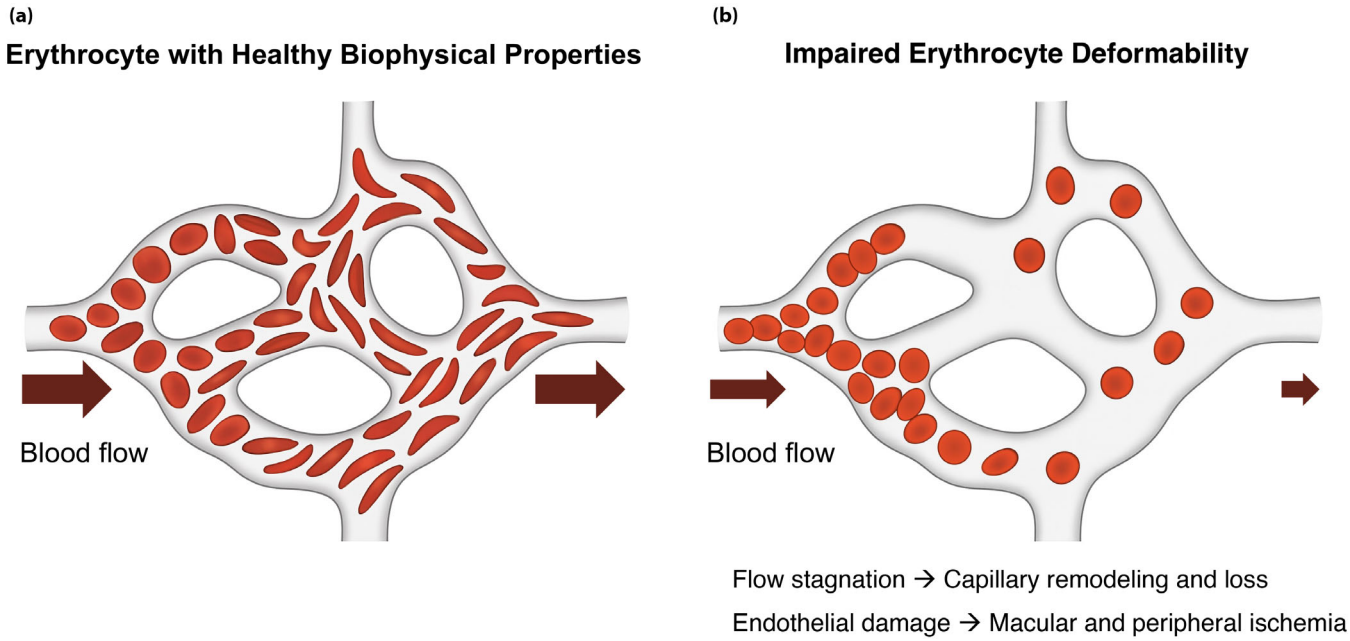

Figure 4 | Working hypothesis: Impaired erythrocyte deformability leads to capillary flow stasis. (a) Under physiological conditions, deformable erythrocytes pass smoothly through the capillary network, maintaining retinal capillary perfusion. (b) In pathological conditions, reduced erythrocyte deformability causes irregular passage and stagnation within capillaries, resulting in localized blood flow stasis and microvascular congestion, leading to macular and peripheral retinal ischemia.

viscosity is significantly increased in DM patients, and there have been many studies on the correlations between deformability of erythrocytes and diabetic micro- and macroangiopathies. Rigid RBCs can cause extensive damage to the retinal microvasculature, including vascular lumen narrowing and extensive damage to endothelial cells, pericytes, and extracellular matrix. Additionally, alterations in RBC function and structure can increase their adherence to endothelial cells and aggregability, which consequently induce platelet adherence and result in thrombus formation (Figure 4).

DCP is more vulnerable to capillary occlusion in cases with impaired RBC deformability. Rigid RBCs can damage the endothelium, leading to endothelial dysfunction and vascular occlusions within the less-flexible DCP and right-angled vertical branches. DR is characterized by its unique vascular structure, distinguishing it from other complications. The retinal vascular system consists of three interconnected layers of laminar capillaries at various retinal depths. These trilaminar networks are vulnerable to shear stress and turbulent flow because they are connected by many vertical branches, with branch points producing right angles. According to recent OCTA-based studies, vascular alterations in DR primarily take place in the deepest capillary plexus of the three capillary networks. Compared to the other two layers, the DCP is smaller and has a less pronounced vasodilatory response to external stimuli.

In this investigation, we did not observe significant differences in the RBC deformability between eyes with and without DME. DME is primarily induced when the blood-retinal barrier (BRB) is disrupted, which increases fluid collection in the macula's intraretinal layers. DR has always been thought to result from microvascular damage to the retinal capillaries. Yet, there is mounting evidence showing that vascular alterations may be preceded by retinal neural dysfunction. Macular edema's pathophysiology seems to be complex, encompassing elements like inflammation, hypoxia, retinal ischemia, and altered blood flow. These factors may partially explain the lack of a significant association between RBC deformability and the presence of DME.

Previous studies[6,18] reported that erythrocyte deformability was lower in patients with a longer duration of DM and higher HbA1c. However, there was no significant correlation between erythrocyte deformability and HbA1c or the duration of DM in this study. This is likely due to the fact that we only included treatment-naïve DR patients who may have neglected DR screening and blood sugar control, which led to less variation of HbA1c according to DR severity. Although erythrocyte deformability was primarily affected in the severe NPDR group and the PDR group, the duration of DM did not show significant differences between the two groups. Nevertheless, erythrocyte deformability was associated with macular and peripheral ischemia, suggesting that erythrocyte deformability is a more critical factor than HbA1c and the duration of DM.

This study has several limitations. First, it is a relatively small study with 79 participants and few patients with NPDR. This

study is limited by an unequal distribution of patients among DR severity groups and a relatively small sample size, which may affect the strength and generalizability of the conclusions. Second, although we were able to observe a relatively wide range of hemorheological values in DR patients, we could not provide a cutoff value for the criteria for assessment due to the small sample size. Third, hemorheological parameters are known to be affected by age, smoking, metabolic status, and coexistent conditions, which could not be controlled in this retrospective study. Therefore, we cannot exclude the possibility that some confounders affected our results. Consequently, large-scale controlled trials are needed. Fourth, we could not establish a causal relationship between hemorheological alterations and DR; a longitudinal study is required to monitor how these parameters contribute to the progression of DR. Fifth, although DR is a bilateral disease, it sometimes shows asymmetric progression between the two eyes, and hemorheological features cannot explain such differences. In this study, we selected one or both eyes from the same patient depending on the image analysis. Lastly, the ischemic index of the peripheral retinal nonperfusion area was measured manually; thus, the determination of the outer border may be subjective, and there is concern about reproducibility.

In conclusion, we found that impaired RBC deformability was associated with the severity of DR, and macular ischemia was correlated with increased RBC stiffness in DR. Also, the area of peripheral ischemic lesions showed a linear correlation with RBC stiffness in eyes with advanced retinopathy with non-perfusion areas. Our results suggest that the rheological properties of RBC contribute to retinal capillary closure in DM, leading to the progression of DR, ischemic maculopathy, and peripheral retinal ischemia. Therefore, erythrocyte deformability may serve as a useful predictive marker for microvascular complications, and the potential diagnostic value of EI@3P in the screening process for DR should be investigated in future studies.

AUTHOR CONTRIBUTIONS

Conception and design: Ye Eun Han, Joon Seo Lim, Inhye Kim, Junyeop Lee. Analysis and interpretation: Ye Eun Han, Inhye Kim, Jun Sung Moon, Junyeop Lee. Data collection: Ye Eun Han, Yulim Kim, Inhye Kim. Writing—original draft: Ye Eun Han, Joon Seo Lim, Inhye Kim. Writing—review & editing: Jun Sung Moon, Junyeop Lee. Overall responsibility: Ye Eun Han, Joon Seo Lim, Junyeop Lee.

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DISCLOSURE

The authors declare no conflict of interest.
Approval of the research protocol: This study was approved by Ethics Committee of Yeungnam University Hospital (approved no. 2020-01-013) and was conducted in accordance with the Declaration of Helsinki.
Informed consent: The requirement of informed consent was waived by Ethics Committee due to the retrospective nature of the study.
Registry and the registration no. of the study/trial: N/A.
Animal studies: N/A.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Correlation between RBC elongation index and HbA1c or duration of diabetes.

Figure S2. Schematic representation of vessel density and foveal avascular zone (FAZ) area analysis using OCTA.

Figure S3. Schematic representation of UWF fundus images and ischemic index analysis using UWFFA.