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Increased erythrocyte aggregation in patients with primary open angle glaucoma

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Background: The rationale of this study is to determine alterations in blood rheology (erythrocyte aggregation and deformability) and relationship between structural measurements obtained from optical coherence tomography (OCT) in different stages of primary open angle glaucoma (POAG).
Methods: This prospective controlled study comprised 23 POAG patients (glaucoma group) and 23 age- and sex-matched healthy subjects (control group). Elongation index (EI), which is the indicator of erythrocyte deformability and erythrocyte aggregation was measured using an ektacytometer. Optic nerve head (ONH) morphology and peripapillary retinal nerve fibre layer (RNFL) thickness were evaluated using a spectral domain (SD) OCT.
Results: There were no significant differences between the groups regarding the elongation index values ($p > 0.05$). On the other hand, erythrocyte aggregation amplitude (AMP) and mean corpuscular haemoglobin concentration (MCHC) were significantly higher in the glaucoma group than in the control group ($p = 0.015$, $p = 0.003$ respectively). A significant correlation was also found between the elongation index and retinal nerve fibre layer (average and superior) thickness ($p < 0.05$) in patients with late glaucoma.
Conclusions: In patients with POAG, erythrocyte aggregation appears to be higher. It can be speculated that higher erythrocyte aggregation and deformability may be involved in the pathogenesis of glaucoma by affecting microperfusion of the optic nerve head and retina. Modification of rheological parameters in patients with glaucoma may be considered as an adjuvant future therapy in glaucoma management, whereas further studies in larger groups are needed.

Key words: erythrocyte aggregation, optic nerve head, optical coherence tomography, primary open angle glaucoma, red blood cell deformability

Glaucoma is one of the leading causes of blindness worldwide, and it is characterised by progressive optic nerve damage. Elevated intraocular pressure (IOP) is the major and only modifiable risk factor in glaucoma development.[1,2] The Ocular Hypertension Treatment Study (OHTS) demonstrated that IOP lowering therapy prevents or delays the onset of glaucoma;[1,2] however, in some cases, glaucomatous progression cannot be prevented despite controlling IOP. This contradiction suggests that secondary mechanisms may also contribute to the mechanical theory of development and progression of glaucoma. Based on the mechanical theory, high IOP leads to laminar stretching and secondary damage to retinal ganglion cell axons.[3,4]

In recent years, studies focused on ocular blood flow in glaucoma and a vascular theory was put forward.[3,4] Blood hyperviscosity, coagulopathy, uncontrolled systemic hypertension or hypotension and vascular inflammatory diseases were found to be related to glaucomatous visual field (VF) defects.[5] Furthermore, most of the previous Doppler-based studies suggested that patients with glaucoma had reduced ocular and papillary (optic nerve head) blood flow.[6–8]

Blood rheology has a significant effect on distal microcirculation and impaired rheological parameters are involved in many diseases.[5] Haemorrheology is the scientific field that deals with blood flow properties and the relationship between vessel and flowing blood. Erythrocyte (red blood cell [RBC]) deformability and aggregation, haematocrit, whole blood viscosity (WBV) and plasma viscosity (PV) are the main components of haemorrheology.[9,10]

Erythrocyte deformability is ability of the entire cell to change shape under a given level of applied stress without haemolysing, and an increased elongation index (EI) at a given shear stress indicates greater cell deformation.[9] The RBC deformability is of crucial importance for performing its function of oxygen delivery. The physiological importance of erythrocyte aggregation, which is the reversible adhesion of adjacent erythrocytes in circulation, is its tendency to increase blood viscosity at low shear flow and disturb the passage in capillary circulation.[5,9,10] In small vessels, where cells have to deform to pass through, erythrocyte deformability and aggregation are the major determinants of resistance to flow and play an important role in pathogenesis of local ischaemia.[11–14] Hence, impaired RBC deformability and aggregation may be involved in glaucoma pathogenesis.[11–17] A general reduction in RBC deformability may diminish retinal oxygenation and so ganglionic cell functions. Similarly, increased erythrocyte aggregation may lead

to a decrease in papillary blood flow, which has been suggested to be associated with axonal degeneration in glaucoma.[15–17] Therefore, alterations in erythrocyte mechanical properties might be related to ischaemia of the optic nerve head, ganglion cells and retinal nerve fibre layer, which is the common endpoint in the pathogenesis of glaucoma.[5,15–17]

Optical coherence tomography (OCT), as a non-invasive laser technology, allows quantitative assessment of optic nerve head morphology and peripapillary retinal nerve fibre layer thickness, which has a critical importance in glaucoma diagnosis and follow-up.[18]

Previous literature comprises conflicting results from a number of studies investigating haemorrheological characteristics of patients with glaucoma based on different patient selection criteria (sample size, exclusion criteria, guidelines for the diagnosis of glaucoma, different stages of glaucoma and presence of hypertension) and methods used for determination of RBC deformability and aggregation.[2,5,15–17,19–21] Furthermore, the relationship between haemorrheological parameters and optic nerve head morphology in patients with primary open angle glaucoma (POAG) is unclear. Hence, the present study aims to determine alterations in erythrocyte mechanical properties (erythrocyte aggregation and deformability) and the association between rheological alterations and OCT measurements (ONH and RNFL parameters) in patients with POAG. Additionally, alterations in these parameters in different stages of POAG were also assessed. Results of this study may contribute to the current knowledge about the role of haematovascular theory in the pathogenesis of glaucoma. We hypothesised that decreased erythrocyte deformability and/or increased erythrocyte aggregation might lead to deterioration in microperfusion of retina and optic nerve head in patients with POAG.

MATERIALS AND METHODS

The tenets of the declaration of Helsinki were followed and local ethics committee approved the study protocol. The sample size was calculated at 95 per cent power and 0.05 significance level (95 per cent confidence interval) using statistical software (PASS version 11.0.1, NSCC, LLC, Utah, USA) and found to be 21 subjects per group. Written informed consent was obtained from all participants. Twenty-three patients with a confirmed diagnosis of POAG (glaucoma group) and 23 age- and sex-matched healthy subjects (control group) were included into the study. All participants underwent the following ophthalmological examinations: visual acuity measurement (Snellen charts), slitlamp biomicroscopic examination, IOP measurement (using Goldmann applanation tonometry), gonioscopy, dilated fundoscopic examination with non-contact + 90 D lens, OCT imaging (Zeiss Cirrus HD-OCT 400, Carl Zeiss Meditec, Dublin, California, USA) and automated perimetry (Humphrey Visual Field Analyser, Carl Zeiss Meditec, Inc, Dublin, California, USA).

Study groups

GLAUCOMA GROUP

Inclusion criteria were aged between 40 and 70 years, clinical diagnosis of POAG, initial IOP greater than 21 mmHg, glaucomatous optic disc appearance (such as cupping, focal or diffuse neuroretinal rim thinning, notching and nerve fibre layer defect), glaucomatous optic nerve damage confirmed with OCT imaging and visual field tests, normal anterior segment and gonioscopic examination. All patients were receiving topical anti-glaucoma treatment. None of these topical treatment modalities is known to be associated with changes in haemorrheological parameters. None of the participants received IOP lowering surgery including laser.

The European Glaucoma Society (EGS) guidelines were followed for glaucoma (POAG) diagnosis.[22] The participants had at least two reliable and consecutive automated perimetric records (24-2 test pattern and Swedish interactive thresholding algorithm standard, Humphrey Visual Field Analyser. Tests with artefacts, fixation losses greater than 20 per cent and false positive or negative responses more than 15 per cent were excluded. A glaucomatous visual field defect was defined as follows; two or more non-edge contiguous points with a sensitivity loss at $p < 0.01$ level or three or more non-edge contiguous points with a sensitivity loss of at $p < 0.05$ level in the superior or inferior arcuate areas or 10-dB difference across the nasal horizontal midline at two or more adjacent locations and coexisting abnormal result in the glaucoma hemifield test (GHT).[22]

The patients with glaucoma were also graded according to the Hodapp-Parrish-Anderson (HPA) classification system as early and late (included moderate and advanced defect) glaucoma for severity based statistical analyses.[23]

CONTROL GROUP

Subjects were enrolled into the study according to the following eligibility criteria; age between 40–70 years, IOP less than 21 mmHg, cup/disc ratio less than 0.5, cup/-disc asymmetry between the two eyes less than 0.2, absence of disc haemorrhage, normal appearance of optic disc and normal neuroretinal rim (absence of cupping, focal or diffuse neuroretinal rim thinning, notching and nerve fibre layer defect). None of the control subjects was taking medications, which had a known influence on haemorrheological parameters studied herein.

Exclusion criteria

Subjects with any of the following conditions were excluded from the study; presence of systemic inflammation or systemic disease (uncontrolled hypertension, renal or hepatic dysfunction and haematological diseases), current anti-inflammatory, antioxidant or anti-aggregant therapies, a visual acuity less than 6/12 (Snellen equivalent), refractive error greater than 5.00 D of sphere or 3.00 D of cylinder, unclear media, history of intraocular surgery (other than uncomplicated cataract surgery), diabetic retinopathy, macular degeneration and optic neuropathies. The glaucoma group included only patients with POAG and patients with all secondary conditions, such as pseudoexfoliation (PXF), narrow angle, pigment dispersion syndrome and ocular inflammation were excluded.

Optical coherence tomographic imaging

The same experienced technician conducted OCT. Optical coherence tomographic images were acquired using optic disc cube 200 by 200 scan protocol with a signal strength over 7/10. The device automatically centres and places the peripapillary retinal nerve fibre layer calculation circle (3.4 mm diameter) around the optic nerve head for precise and reproducible measurements. The OCT parameters included average, superior, inferior, nasal and temporal RNFL thicknesses, RNFL symmetry, rim area, disc area, average cup/disc (C/D) ratio, vertical C/D ratio and cup volume.

Blood samples and measurements

Venous blood samples were drawn by venepuncture after eight hours of fasting. Blood for haematological parameters was collected into standard tubes containing ethylenediaminetetraacetic acid (EDTA) and blood count was determined by using an electronic haematological analyser (Siemens ADVIA 2120i System, Siemens Healthcare Diagnostics, Japan). Haemorrheological measurements were performed in accordance with 'new guidelines for haemorrheological laboratory techniques' within three hours after blood collection.[24]

ERYTHROCYTE DEFORMABILITY MEASUREMENTS

RBC deformability was determined at various fluid shear stresses by laser diffraction analysis using an ektacytometer (Laser-Assisted Optical Rotational Cell Analyzer (LORCA); RR Mechatronics, Hoorn, The Netherlands). Briefly, a low haematoctit suspension of RBC in an isotonic viscous medium (4% polyvinylpyrrolidone 360 solution; MW 360 kD; Sigma P 5288; St. Louis, Missouri, USA) was sheared in a Couette system composed of a glass cup and a precisely fitting bob, with a gap of 0.3 mm between the cylinders. A laser beam was directed through the sheared sample and the diffraction pattern produced by the deformed cells was analysed by a microcomputer. On the basis of the geometry of the elliptical diffraction pattern, an elongation index was calculated as EI = $(L - W)/(L + W)$, where L and W are the length and width of the diffraction pattern, respectively. Elongation index values were determined for nine shear stresses between 0.3 and 30.0 Pascal (Pa) and similar patterns of RBC deformability alterations were obtained between groups at all stress levels. All measurements were carried out at 37 °C.[25]

DETERMINATION OF ERYTHROCYTE AGGREGATION

Erythrocyte aggregation was also measured by LORCA as described elsewhere. [25] The measurement is based on the detection of laser back-scattering from the sheared (disaggregated), then unsheared (aggregating) blood, performed in a computer-assisted system at 37 °C. Back-scattering data are evaluated by the computer and aggregation index (AI), amplitude of the aggregation (AMP), which is the total extent of aggregation, aggregation half time ($t_{1/2}$) are calculated on the basis that there is less light back-scattered from aggregating red cells. Aggregation measurements were determined using RBCs in autologous plasma adjusted to 40 per cent haematocrit (Hct). Blood was fully oxygenated before measurements.

Statistical analysis

Statistical analysis was performed with the version 18.0 (Statistical package for social sciences (SPSS) Inc, Chicago, Illinois, USA). Values were expressed as the mean and standard error (SE). Qualitative variables were analysed using the Chi squared test. The Kolmogorov–Smirnov test was used to determine whether statistical data were normally distributed. Comparisons of age, OCT parameters and haemorrheological measurements between the control and glaucoma groups were performed using the independent samples t-test. Pearson correlation coefficients were used to determine relations between OCT measurements and haemorrheological parameters (in the entire glaucoma group and severity-based glaucoma groups). At 95 % confidence interval, p values less than 0.05 were accepted as statistically significant.

RESULTS

The study enrolled 23 patients (11 female) with POAG (mean age of 65.86 ± 1.23, range 53 to 70 years) and 23 (13 female) age- and sex-matched healthy controls (mean age of 66.66 ± 2.12, range 54 to 70 years) ($p = 0.743$, $p = 0.559$, respectively). Statistical tests showed that all data used in the study had normal distributions.

Table 1 shows comparison of age, gender distribution and haematological measurements between the glaucoma and control groups. The mean corpuscular haemoglobin concentration (MCHC) in the glaucoma group was higher compared to those of the control group ($p = 0.003$). There were no other statistically significant differences between the glaucoma and control groups in terms of haematological parameters ($p > 0.05$) (Table 1).

Erythrocyte deformability was determined as the elongation index and it was measured at nine shear stresses between 0.3 and 30.0 Pa. The differences between the glaucoma and control groups regarding elongation index values were not statistically significant ($p > 0.05$) (Table 2).

Erythrocyte aggregation parameters (amplitude of the aggregation, aggregation index and aggregation half time) were shown in Table 3. There was no statistically significant difference in AI and $t_{1/2}$ measurements between the groups ($p > 0.05$). On the other hand, AMP value was significantly higher in the glaucoma group than in the healthy controls ($p = 0.015$).

When the glaucoma patients were grouped regarding disease severity, haemorrheological measurements did not differ between the early (n = 9) and late (n = 14, [moderate = 8, advanced = 6, HPA classification]) glaucoma groups ($p > 0.05$).

Average (63.15 ± 2.71 versus 95.39 ± 1.11 μm), superior (73.84 ± 3.90 versus 117.47 ± 2.59 μm), inferior (69.10 ± 3.55 versus 124.56 ± 1.82 μm), nasal (54.57 ± 1.45 versus 73.30 ± 1.97 μm) and temporal (48.42 ± 3.06 versus 66.47 ± 1.58 μm) retinal nerve fibre layer thicknesses, nerve fibre layer symmetry (47.31 ± 5.69 versus 85.43 ± 2.02), rim area (0.80 ± 0.04 versus 1.41 ± 0.0) values were significantly lower in the glaucoma group, when compared to those of the control group ($p = 0.0001$). On the other hand, average C/D ratio (0.73 ± 0.02 versus 0.47 ± 0.03), vertical C/D ratio (0.74 ± 0.02 versus 0.42 ± 0.02) and cup volume (0.48 ± 0.01 versus 0.13 ± 0.02) values were significantly higher in the glaucoma patients than in the healthy controls ($p = 0.0001$), as expected.

The correlation analysis showed no significant associations between the haemorrheological parameters (EI values, AMP, AI and $t_{1/2}$) and OCT measurements ($p > 0.05$) in the entire glaucoma group. When a subgroup analysis was performed, in the late glaucoma group, there was a significant correlation between average retinal nerve fibre layer thickness and elongation index (at 9.49 Pa, $r = 0.659$, $p = 0.020$; at 16.87 Pa $r = 0.671$, $p = 0.016$; at 30.0 Pa $r = 0.667$, $p = 0.019$) and superior nerve fibre thickness thickness was significantly related to elongation index values at 16.87 and 30.0 Pa shear rates ($r = 0.629$, $p = 0.010$, for both) in the late glaucoma group. On the other hand, there was no significant correlation between haemorrheological parameters and OCT measurements in the early glaucoma group ($p > 0.05$).

DISCUSSION

Glaucoma is a progressive neuro-degenerative ocular disease and the main treatment strategy is based on lowering IOP, which is the main risk factor.[1,2] Previous studies showed significant reduction in ocular blood

Parameter	Control group (n = 23)	Glaucoma group (n = 23)	p
Age (years)	66.66 ± 2.12	65.86 ± 1.23	0.743 *
Gender (F/M)	13/10	11/12	0.559 **
Hb (g/dl)	13.27 ± 0.43	14.16 ± 0.32	0.109 *
Hct (%)	41.30 ± 0.68	41.57 ± 0.75	0.790 *
RBC (10^6/µl)	4.82 ± 0.088	4.71 ± 0.110	0.448 *
WBC (10^6/µl)	7.63 ± 0.41	8.43 ± 0.47	0.215 *
MCV (fL)	86.58 ± 1.92	87.97 ± 1.29	0.553 *
MCH (pg)	27.63 ± 0.96	30.11 ± 0.47	0.051 *
MCHC (g/dl)	31.81 ± 0.66	34.25 ± 0.32	0.003 *
RDW (%)	14.60 ± 0.26	15.75 ± 1.14	0.334 *
Plt (K/uL)	251.47 ± 12.26	242.47 ± 14.79	0.643 *

*= Independent samples t-test
**= Chi square test
Values are expressed as mean and standard error. Hb: haemoglobin, Hct: haematocrit, RBC: red blood cell count, WBC: white blood cell count, MCV: mean corpuscular volume, MCH: mean corpuscular haemoglobin, MCHC: mean corpuscular haemoglobin concentration, RDW: red blood cell distribution width, Plt: platelet count. $p < 0.05$ indicates statistical significance

Table 1. Comparison of age and haematological measurements between the control and glaucoma groups

Shear stress (Pa)	Control group (n = 23)	Glaucoma group (n = 23)	p*
0.30	0.035 ± 0.004	0.043 ± 0.003	0.175
0.53	0.065 ± 0.005	0.084 ± 0.007	0.052
0.95	0.142 ± 0.009	0.152 ± 0.140	0.581
1.69	0.247 ± 0.110	0.239 ± 0.018	0.716
3.00	0.356 ± 0.010	0.331 ± 0.020	0.293
5.33	0.447 ± 0.010	0.417 ± 0.020	0.184
9.49	0.517 ± 0.007	0.490 ± 0.018	0.189
16.87	0.572 ± 0.005	0.546 ± 0.017	0.160
30.00	0.609 ± 0.005	0.588 ± 0.015	0.229

*Independent samples t-test, $p < 0.05$ indicates statistical significance
Values are expressed as mean and standard error; EI: elongation index, Pa: pascal.

Table 2. The erythrocyte elongation index (EI) values in the study groups at different shear stresses

	Control group (n = 23)	Glaucoma group (n = 23)	p*
AMP (au)	23.40 ± 0.97	26.49 ± 0.71	**0.015**
AI (%)	69.25 ± 1.93	71.30 ± 1.53	0.409
$t_{½}$ (sn)	1.76 ± 0.18	1.52 ± 0.13	0.279

*Independent samples t-test

Table 3. Comparison of erythrocyte aggregation parameters between the study groups

flow in patients with glaucoma using laser Doppler flowmetry. Impaired ocular hemodynamics might contribute to the ganglion cell and retinal nerve fibre layer damage in the pathogenesis of glaucoma.[26,27] In addition to normal ocular blood flow, distal oxygen and nutrients delivery is also critical and erythrocyte mechanical properties (erythrocyte aggregation and deformability) were suggested to have an important role in microperfusion of ocular endorgans. For instance, the retina is very sensitive to alterations in blood supply because of its high metabolic rate and retinal circulation has a limited capacity to adapt rheological problems.[5,28] Moreover, ischaemia has been suggested to be involved in the pathogenesis of glaucoma.[29,30] Therefore, rheological parameters may be taken into account, while investigating the aetiopathogenesis of glaucoma.

Although several studies investigated the role of many factors, such as decreased blood flow and release of vasoactive substances from capillary endothelium, the exact mechanisms leading to glaucomatous damage have not been clarified.[19] Only a few studies evaluated erythrocyte aggregation and deformability in glaucoma patients and these studies used different laboratory techniques and patient selection criteria.[2,5,15–17,19–21] Cheng et al[15] and Vetrugno et al[19] using ektacytometry, demonstrated a lower index of erythrocyte deformability in patients with normal tension glaucoma (NTG). Ates et al[5] found no difference in RBC deformability in normal tension glaucoma and high tension glaucoma using the Cell Transit Analyser. These studies differed from ours in the type of glaucoma. On the other hand, a number of studies also assessed RBC deformability and aggregation in patients with POAG using different non-computerised techniques. For instance, decreased RBC deformability and unaltered erythrocyte aggregation were reported in POAG patients using Hanss hemorheometer,[2] while an increase in RBC aggregation was demonstrated using erythroaggregameter.[21]

Recent advances in instrument design have resulted in the production of laser diffraction-based devices. The main advantage of this up-to-date laser technique is its repeatability and accuracy. Using laser assisted optical rotational cell analyser (LORCA), Vetrugno et al[17] reported no difference in RBC deformability at a shear stress of 30 Pa between patients with POAG

and healthy controls. In contrast, Michalska-Małecka and Słowi□ska-Ło□y□ska[16] demonstrated decreased erythrocyte deformability using the same device at shear stresses of 18.49, 33.20 and 60.03 Pa. Based on a laser Doppler velocimetric system, the average wall shear stress values in the first-order retinal arterioles and venules were reported as 54 dyne/cm^2 and 24 dyne/cm^2 in healthy humans, corresponding to 5.4 Pa and 2.4 Pa, respectively.[31,32] This means that RBC deformability was measured at high shear stresses, which actually do not match with the wall shear stress values of retinal vessels in these studies. On the other hand, Sekeroglu et al[20] measured RBC deformability of POAG patients at shear stresses of 3 and 30 Pa and found no difference. In the present study, RBC deformability was measured using LORCA but in a wider range of shear stresses between 0.30 and 30 Pa covering the average wall shear stress values of retinal vessels. Although slight alterations in different shear stresses were observed, we did not determine any statistically significant alteration in RBC deformability in POAG patients, when compared to that of the controls.

It is known that erythrocyte deformability is affected by membrane skeleton elasticity, cytoplasmic viscosity and cell geometry (surface-volume ratio).[9] Decreased deformability not only diminishes tissue oxygenation but also shortens erythrocyte life span.[33,34] Young erythrocytes have less mean corpuscular haemoglobin concentration and higher mean corpuscular volume (MCV) and deformability. [35] In our study, mean corpuscular haemoglobin concentration values in the POAG patients were significantly higher when compared to those of the controls. The mean corpuscular haemoglobin concentration is directly proportional to osmolality and increased MCHC is known to impair RBC deformability via increasing internal viscosity.[36] It can be suggested that increment of mean corpuscular haemoglobin concentration may have contributed to the slight decrement of RBC deformability (between 1.69 and 30.00 Pa shear stresses) of the POAG patients in our study.

Another haemorrheological parameter, RBC aggregation, which is defined as reversible adhesion of adjacent RBCs was also evaluated in this study.[37] The fibrous proteins especially plasma fibrinogen, erythrocyte membrane properties and erythrocyte morphology are the most important determinants of erythrocyte aggregation.[9] It is known that, RBC aggregation affects the fluidity of blood in larger blood vessels, where the shear rate is low enough to allow RBC to aggregate.[10,38] The reports on RBC aggregation parameters in patients with glaucoma are very scarce. In patients with normal tension glaucoma, higher aggregation index values were demonstrated, when compared to those of the healthy controls.[15,26]

A number of studies also investigated the role of RBC aggregation in POAG pathogenesis. Hamard et al[21] and Hamard, Hamard and Dufaux[26] reported reduced optic nerve blood flow and velocity and higher blood viscosity as well as erythrocyte aggregation in POAG patients. Erythrocyte aggregation was measured by an erythroaggregameter in their study. They suggested that erythrocyte hyperaggregability was secondary to erythrocyte membrane modifications.[21] In line with these results, higher fibrinogen content was also demonstrated in POAG.[39] In contrast, Mary et al[2] reported no difference in erythrocyte aggregability using Hanss hemorheometer in POAG patients. There are two previous studies in the current literature determining RBC aggregation using ektacytometer (LORCA) in POAG patients, as we did. These studies reported inconsistent results. No significant difference in aggregation parameters between glaucomatous (POAG and exfoliative glaucoma) and healthy patients were reported by Sekeroglu et al.[20] In contrast, increased erythrocyte aggregation index, decreased amplitude of the aggregation and $t_{1/2}$ in patients with POAG were also shown by another study.[16] They proposed that these results indicate an increase in power of erythrocyte chains in aggregates, reflected by the aggregation index and amplitude of the aggregation values, which requires higher shear stress to break them down as well as acceleration of the aggregation phenomenon-reflected by the values of $t_{1/2}$.[16] In our study, although aggregation index and amplitude of the aggregation were higher and $t_{1/2}$ was lower in the POAG patients compared to those of the controls, only the alteration in amplitude of the aggregation was statistically significant. It is worthy to note that increments in amplitude of the aggregation and aggregation index and decrement of $t_{1/2}$ are concordant with each other and indicate increment of RBC aggregation. The discussion whether alterations in haemorrheological parameters are the cause or the result of some systemic diseases, such as hypertension and coronary artery disease still goes on in the literature.[40,41] Our experiments do not enlighten this issue directly for POAG because glaucoma is a local disease and far from affecting the determinants of RBC aggregation systemically. Instead, it may sound more acceptable that RBC properties and haemorrheological parameters might have taken place in the pathogenesis of POAG. Increased RBC aggregation in POAG patients might affect dynamics of optic nerve head microcirculation and oxygen delivery, which contribute to the ischaemic glaucomatous optic nerve damage.

In a clinical perspective, structural optic nerve head and RNFL damage in the pathogenesis of glaucoma can be quantitatively measured using OCT as a gold standard diagnostic method. It is well known that patients with glaucoma have decreased peripapillary RNFL thickness, rim area and increased optic disc cupping compared to those of the healthy subjects. As expected, the patients with POAG in our study had significantly reduced retinal nerve fibre layer thicknesses, rim area, rim symmetry and increased C/D ratio and cup volume compared to the healthy controls. Moreover, the relationship between the mechanical properties of erythrocytes and glaucomatous damage (such as RNFL thinning and optic disk cupping) was first investigated in patients with POAG based on OCT parameters in the current study. Although we found increased erythrocyte aggregation in POAG patients, which may be speculated to affect microcirculation of the optic nerve head and retinal nerve fibre layer, correlation analysis did not reveal a significant association between the haemorrheological parameters and structural glaucomatous damage detected with OCT (such as RNFL thicknesses, rim area, C/D ratio and cup volume) in the entire glaucoma group. It is worthy to note that, when patients with glaucoma were divided into two subgroups as early and late glaucoma (derived from HPA classification), average and superior RNFL thicknesses were found to be significantly correlated with erythrocyte deformability (EI values at 9.49, 16.87 and 30.0 Pa) in patients with only late glaucoma; however, it should be stated that the modest sample size in the present study is a limitation.

In conclusion, secondary mechanisms in pathogenesis of glaucoma are still being investigated and ocular haemodynamics is

the main research area. According to the results of the present study, it can be speculated that, increased erythrocyte aggregation may lead to predisposition to stasis in retinal and papillary microcirculation and subsequent ischaemia, which was suggested to be associated with glaucomatous optic nerve damage. Hence, treatment strategies for improving diminished erythrocyte aggregation may be involved in management of POAG. On the other hand, average and superior retinal nerve fibre layer thicknesses appear to be correlated with erythrocyte deformability in patients with late glaucoma. It can be hypothesised that impaired erythrocyte deformability might lead to reduction in blood flow in small vessels nourishing retina and ONH, which might be related with axonal damage. Further studies in larger groups are needed to enlighten the role of haemorrheological parameters on glaucomatous damage and future management of glaucoma.

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