Optic nerve head blood flow using a laser Doppler velocimeter and haemorheology in primary open angle glaucoma and normal pressure glaucoma

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Abstract

Optic disc blood flow velocity was measured in healthy patients, those with primary open angle glaucoma (POAG), and patients with normal pressure glaucoma (NPG). The velocity of the red blood cells (RBCs) in the capillaries of the optic nerve head (ONH) has been measured with a laser Doppler velocimeter (LDV), and blood viscosity has been evaluated notably by determining the aggregability of the RBCs with an erythroaggregameter. Our results in POAG patients and NPG patients showed that their optic nerve blood flow velocity was reduced and that the aggregability of the RBCs was increased. The hyperaggregability of the erythrocytes is responsible for the increase of the local viscosity in the papillary capillary network. These haemodynamic modifications observed in patients with glaucoma support the hypothesis of a vasogenic mechanism that could impair the optic nerve in glaucoma patients.

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The pathogenesis of optic nerve injury and visual defects in glaucoma is not well known. Two different theories have been proposed to explain the optic nerve damage. The mechanical theory emphasises that excavation of the optic nerve head is due to elevated intraocular pressure (IOP), with interruption of axoplasmic flow and late optic nerve fibres degeneration.[1 2] However, optic nerve excavation and glaucoma visual field defects have been described without elevated IOP, leading to the 'low tension' concept of glaucoma. The vasogenic theory supports the hypothesis that hypoperfusion of the papilla is responsible for optic nerve head impairment.[3] This approach is backed up by the fact that glaucomatous field losses are also encountered in cases of untreated systemic hypertension or hypotension, positive histories of major vascular crisis,[4] coagulopathy, hyperviscosity, and vascular inflammatory diseases. Histological and fluorescein angiographic studies have also supported this theory.[3 5]

In order to assess optic nerve head circulation, the blood flow velocity can be measured in a given area of the papilla with a non-invasive technique using the laser Doppler velocimeter (LDV).[6] It determines the maximum velocity of the moving cells in the papillary capillaries. However, to achieve the circulation in a capillary network, blood viscosity must be taken into account since it is known that the blood viscosity is proportional to the rate of flow of the blood, which is a non-Newtonian fluid; thus, the blood flow rate is influenced by the blood viscosity. Blood viscosity itself depends on numerous factors. However, in the microcirculation, it is essentially the capacity of the red cells to aggregate and to change form which controls the viscosity of the blood.[7 8] Abnormality in the aggregability of the red blood cells has been described in numerous diseases such as arterial hypertension,[9] diabetes mellitus,[10] coronary insufficiency,[11] and ocular venous thrombosis.[12]

In an earlier study,[13] we showed that patients with primary open angle glaucoma (POAG), whose IOP was controlled with topical hypotonic therapy, had a decrease in their papillary capillary blood flow velocity measured with LDV, and that they had an increase in their erythrocyte aggregability. These two findings suggested that their optic nerve head perfusion was impaired.

In this present study, we wished to investigate whether patients with normal pressure glaucoma (NPG) had the same haemodynamic changes in their optic disc as was shown in POAG patients, independently of IOP and of any glaucoma therapy. We measured the velocity of the red blood cells on the papillary area with an LDV and the aggregability of the red blood cells in NPG patients. The results were compared with control and POAG patients.

Material and methods

We selected three groups of patients: (1) a control group of 14 healthy subjects (eight men and six women); (2) a second group of 17 POAG patients (11 men and six women); (3) a third group of 18 NPG patients (three men and 15 women).

The patients in the study were a subset of a larger population followed in the National Ophthalmology Hospital Centre in Paris, for a chronic open angle glaucoma or for a normal pressure glaucoma. Healthy volunteers with the appropriate inclusion criteria and without the predefined exclusion criteria were chosen from the hospital staff.

Patients were excluded if they had any disease known to modify the aggregability of the red blood cells or the blood flow velocity such as: arterial hypertension, hyperlipidaemia, diabetes mellitus, cardiovascular disease (in particular carotid artery stenosis), smoking; and any patients with other ocular disease excluding glaucoma. Patients were excluded if they had pigmentary dispersion or exfoliation. Also patients with secondary glaucoma were excluded. The only concurrent treatments allowed were local hypotonic agents in POAG patients (β adrenergic blockers for all patients except one taking anticholinergic agents). Also patients

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were excluded who had undergone laser trabeculoplasty or filtering surgery. All women were post-menopausal and none was receiving substitution therapy.

Furthermore, as it is difficult to focus the laser beam correctly on the papillary area in patients with ametropia (more than 3 dioptres myopia or hyperopia) or in patients with lens opacities, these patients have also been excluded.

Patients were included if they fulfilled the following criteria:

(1) Control group: bilateral IOP less than 19 mm Hg without treatment, normal visual field (automated perimeter, Octopus G1 program), and normal papilla (cup/disc ratio vertical and horizontal (C/D<0·3).

(2) POAG patients: a diagnosis of bilateral primary chronic open angle glaucoma – that is,

- open angle on gonioscopy,
- bilateral IOP over 21 mm Hg before treatment,
- glaucoma visual field defects and a glaucoma papillary excavation (C/D vertical >0·5).

During the study, all POAG patients had IOP less than 20 mm Hg under local hypotonic agents.

(3) NPG patients: a diagnosis of bilateral normal pressure glaucoma – that is, bilateral IOP less than 20 mm Hg without therapy (the IOP was measured every 2 hours for 24 hours), bilateral glaucoma visual field defects, and glaucoma papillary excavation.

The number of patients in the glaucoma groups was insufficient to allow determination of subgroups according to the increasing severity of visual field loss.

A velocimeter assessment was carried out in each patient at the right and the left optic nerve head (ONH), and baseline measurements of fibrinogen, plasma protein, a packed cell volume, blood viscosity, and aggregability of the erythrocytes were carried out. All data were analysed in a blinded manner as to whether the source was from patients or volunteers. Informed consent was obtained from all patients.

THE LASER DOPPLER VELOCIMETER

We use a prototype laser Doppler[14] based on the model described by Riva *et al*[6] and built in a laboratory of the University of Paris VII (LBHP).

A weak laser beam (power of 0·025 mW on the cornea, from an HeNe laser, wavelength 632 nm) is focused for 2 to 3 seconds on a papillary area of 50 μm, at a depth of between 100 and 200 μm as far as possible from visible vessels. The light is partly scattered by the tissue and the moving red blood cells, having undergone a shift in its frequency (Δf), owing to the Doppler effect. This frequency shift is proportional to the velocity of the red cells in the volume exposed to the laser. The collected signal is then analysed. A Fourier transform reconstitutes the frequencies which compose the spectrum and allows determination of the maximum frequency shift of the light (Δf max). The maximum velocity of the red blood cells (Vmax) in the volume exposed to the laser can be calculated using the formula:

$$V(max) = 1/2.\lambda.\ \Delta f\,(max)$$

with V(max)=maximum velocity of the red blood cells; λ=wavelength of the laser beam (632 nm); Δf (max)=maximum frequency shift.

THE HAEMORHEOLOGICAL PARAMETERS

Venous blood samples were drawn from the antecubital vein and collected in various plastic tubes according to the different assays – that is, anticoagulated with EDTA salt for packed cell volume, with trisodium citrate for plasma fibrinogen, and heparinised for the erythrocyte aggregability study. Fibrinogen was assayed using a chronometrical method, following the addition of thrombin.[15] The capacity of the red blood cells to aggregate and disaggregate was determined using the following apparatus:

A Couette viscometer[16]

This viscometer enables measurement of the viscosity coefficient of a given blood sample in different flow conditions, at constant temperature (37°C). The Couette system consists of two concentric cylinders, with an annular gap of 0·5 mm. The shear rate is generated across the gap by rotating the outer cylinder, the inner cylinder remains stationary. The shear rate can vary from 0·017 s^{-1} to 128 s^{-1} in 30 discrete steps. For each shear rate, the viscometer calculates the corresponding in the value of blood viscosity.

An erythroaggregameter (Sefam)[17]

This apparatus uses a modified Couette viscometer. The blood sample (1·5 ml) is placed between the two cylinders. The external cylinder is transparent and rotates at variable speeds controlled by a microcomputer which allows the blood suspension to be exposed to varying shear rates between 7 and 400 s^{-1}. An infrared laser beam illuminates the blood sample through the transparent external cylinder. The backscattered light is collected on a photodiode. The reflectivity signal is directly related to the mean bridging area of the red blood cells per unit volume of the suspension at a given shear rate. The blood is first subjected for 10 seconds to a high shearing (400 s^{-1}) which leads to a break up of any aggregate and disorientates the red blood cells. The shearing is suddenly stopped, allowing aggregate formation. This corresponds to a decrease in the backscattered light. The blood is then subjected to 40 decreasing shear rates, starting at 400 s^{-1} and ending at 7 s^{-1}. Each shear rate is applied for 5 seconds and suddenly stopped for 5 seconds. When the shear rate is insufficient to dissociate the packed red blood cells, the backscattered light amplitude diminishes. This given point corresponds to the lowest shear rate beyond which the red cells will no longer disaggregate and is called the disaggregation shear rate. This parameter along with the corresponding viscosity is used to calculate the disaggregation shear stress (τ dyne/cm^2) which reflects the forces binding the erythrocytes together as packed red cells.

The Mann Whitney test and Student's two tailed *t* test were used. Significance was considered if $p<0·05$.

Table 1 Patient characteristics

	Controls (n=14)	POAG (n=17)	NPG (n=18)
Mean age (years) (SD)	44·8 (12·7)	51·6 (11·6)	57·4 (5·4)†
Mean packed cell volume (SD)	42·7 (2·9)	42·2 (4·2)	39·2 (3·5)
Mean plasma protein (g/l) (SD)	68·3 (30)	70·6 (4·4)	70·2 (3·2)
Mean fibrinogen (g/l) (SD)	2·7 (0·3)	3·1 (0·8)	3·1 (0·6)

*Not significant. †p<0·05.
POAG=primary open angle glaucoma patients. NPG=normal pressure glaucoma patients.
There is no significant difference in any parameter between the two groups apart from age: NPG patients are older than healthy controls.

Table 2 An age-paired comparison using Student's t test on seven pairs of data to confirm the absence of influence of age on the study outcome

Variable	N	Mean	SE	t Test	p>t
Age	7	−0·143	0·340	−0·420	0·6891
Shear stress	7	1·694	0·413	4·102	0·0063
RE (t)	7	−421·429	87·870	−4·796	0·0030
LE (t)	7	−407·143	52·812	−7·709	0·0002

RE=right eye; LE=left eye.

Results

As shown in Table 1, we observed that the three groups were matched for packed cell volume, plasma protein level, and fibrinogen level. However, there was a significant difference in age between the NPG group and the healthy patients. The mean age in the NPG group was higher than that in healthy patients. We wished to verify whether the difference observed in the disaggregation shear stresses and the papillary blood velocity were in any way linked to the fact that patients in the NPG group were slightly older. An age-paired comparison using Student's *t* test was carried out on seven pairs of data. It was found that the significance observed in the overall sample persisted and therefore was not age-related (Table 2).

The mean IOP was significantly higher in the POAG group compared with the healthy group (p<0·05), but there was no difference in the mean IOP between the healthy and the NPG groups (Table 3).

As shown in Table 3 we observed that: (1) The disaggregation shear stress was significantly increased. This implies that the aggregability of the red blood cells was increased in patients with glaucoma compared with the control subjects. (2) The maximum frequency shifts were significantly decreased suggesting that the ONH blood flow velocity was decreased in glaucoma patients compared with the control subjects.

There was no difference in the maximum frequency shifts between the right eye and the left eye in the healthy group. There was no difference in the maximum frequency shifts and the disaggregation shear stresses between the NPG group and the POAG group. There was a negative correlation ($r=-0·41$, $p=0·003$) between the increase in aggregation of the red blood cells and the extent of the decrease in circulation in the papillary capillaries in the whole population (controls, POAG, and NPG patients). However, this correlation was not found within each group; perhaps the number of patients in each group was not high enough.

Table 3 Comparison of intraocular pressure (IOP), laser Doppler velocimetry, and haemorheological parameters in primary open angle glaucoma (POAG) patients, normal pressure glaucoma (NPG) patients and healthy subjects (controls)

		Controls (n=14)	POAG (n=17)	NPG (n=18)
Mean IOP (mm Hg) (SD)	R	14·9 (2·6)	17·0 (3·0)*	14·7 (2·2)
	L	14·6 (2·5)	17·0 (2·8)*	15·2 (2·3)
Mean shear stress (dynes/cm²) (SD)		4·1 (0·6)	5·4 (0·9)**	5·3 (1·0)**
Mean Δf (max) (Hz) (SD)	R	853 (118)	494 (117)***	373 (141)***
	L	850 (78)	476 (165)***	386 (139)***

*p<0·05; **p<0·001; ***p<0·0001.
Mann Whitney test.

Discussion

It is difficult to perform optic nerve head perfusion measurements on humans. Laser Doppler velocimetry is a non-invasive technique that allows an evaluation of the blood flow velocity in the capillaries of the optic disc and thus gives an indication of tissue perfusion. Some limits of this technique are that the information obtained from the optic nerve head capillaries is global: we cannot determine independently the velocity of the red blood cells in the capillaries coming from the short ciliary arteries and that from the central retinal artery, since the laser light is scattered by red blood cells moving in capillaries embedded in a volume of tissue of the ONH and not only on the surface of the ONH. We know that the blood flow rate depends on several factors: the diameter of the blood vessel, the average blood pressure, the IOP, the local autoregulation, and the blood viscosity. Changes in one of those factors may compromise the microcirculatory efficiency. We were specially interested in the determination of the red blood cell capacities to aggregate and disaggregate, since it is known that in the microcirculatory network, the capacities of the erythrocytes to aggregate and to deform are mainly responsible for the value of the viscosity.[7] Red blood cell aggregability and deformability are physiological phenomena to facilitate the blood flow. The disaggregation of the red blood cells is possible if the local shear rates are sufficient. When the blood flow is reduced, the shear stresses decrease and the erythrocytes tend to aggregate. The packed red cells themselves induce a decrease of the blood flow velocity. This haemorheological phenomenon is a vicious circle.

In our study, we showed that POAG and NPG patients had an increase in their erythrocyte aggregability. A possible consequence of this anomaly was that the blood flow rate would decrease first in the venule network where the local shear stresses are weak in the basal state, and then upstream in the capillary network (notably in the papillary capillaries). We showed, in fact, a decrease in the papillary capillary blood flow velocity in patients with glaucoma; however, it did not show a correlation with the increase in the aggregability of the red blood cells.

In previous studies, an abnormality in blood viscosity in POAG patients has been suspected and confirmed by many authors.[18-20] However, Carter[21] showed no difference in blood viscosity between patients with glaucoma and control subjects. But the method he used for viscosity measurements differs from the one we used, and therefore the results cannot be compared. Carter used a law for the determination of blood

viscosity with four parameters of blood viscosity obtained at four shear rates chosen between 0·03 s^{-1} and 400 s^{-1}. The use of such a model requires matching the curve to four points, and we have to extrapolate the values of the viscosity at shear rates inside the reference values. Thus, this model only provides approximate values and not the real viscosity at a given rotation point. Furthermore, the curve starts at a very low value of rotation (0·03 s^{-1}), a point at which false low values for viscosity can be interpreted: this is one of the limitations of the use of the viscometer.

Klaver,[18] Trope,[19] and Foulds[20] showed an increase in blood viscosity in patients suffering from glaucoma, without giving any definite explanation for the exact cause. There is no published evidence that the increase in blood viscosity could be explained by an anomaly in the aggregability of the erythrocytes. However, Fouds's findings[20] suggested that the aggregability of the red blood cells could be increased since he showed that blood viscosity measured with a Contraves viscometer tended towards high values at low shear rates. It is well established that the aggregability of the red cells is responsible for the values of the blood viscosity at low shear rates,[7] but the aggregability of the red cells was not measured directly as this can be done with an erythroaggregameter.[17]

It is known that blood viscosity depends on several parameters: packed cell volume, plasma viscosity,[22] especially the level of fibrinogen and proteins,[23] and capacities of the red cells to aggregate and to deform. In our study, we found no difference beween the level of packed cell volume in the control group or in patients with glaucoma. Elsewhere, we found no increase in plasma viscosity in patients with glaucoma, since plasma protein levels, notably the level of fibrinogen, were similar to those in control subjects. This agrees with previous studies[19] but it is in contradiction with the results of Garcia[24] who showed an increase in the level of proteins in POAG patients. The increase in blood viscosity at low and high shear rates could be explained, therefore, by modifications directly linked with the erythrocytes – that is, modification in the capacity of the red cells to disaggregate and to deform. In a recent study, Mary[25] showed that the deformability of the erythrocytes was impaired in POAG patients. However, this study has failed to detect a modification in the aggregability of the erythrocytes in POAG patients. This discrepancy with our results has been discussed elsewhere.[13]

Erythrocyte hyperaggregability and decrease in the blood flow velocity in the optic disc seem to be common factors in both types of glaucoma studied. But we could not prove the connection between the hyperaggregability of the red cells and the decrease in the papillary blood flow velocity. This means that factors other than blood viscosity are reponsible for the blood flow velocity in the ONH capillaries. One possibility is that the decrease of the ONH blood flow velocity depends on the level of the IOP. We found that the mean IOP in the POAG group was slightly higher compared with the mean IOP in the healthy group. However, we found no statistical difference between the mean IOP in the NPG group and the healthy group although the decrease in the maximum frequency shifts was the same in the POAG and the NPG group. The effect of IOP on the decrease in the blood velocity in the papillary capillaries cannot be excluded but the maximum threshold IOP beyond which the ONH circulation could be affected should be determined. A second possible explanation for the decrease of the ONH blood flow velocity could be the pathological structure of the optic nerve head caused by cupping in optic atrophy. If the nutrient capillaries that are located within the laminar sheets are compressed and their direction modified because of the misalignment of the pores of the lamina cribrosa, the blood velocity can decrease. The consequence is that the local shear rates will decrease and will become insufficient to dissociate the red cell from each other especially if there is an impairment, even minor, in the aggregability of the red blood cells. The packed red cells will in turn reduce the blood velocity. This is the haemorheological vicious circle.

There are other factors which could modify the blood flow velocity in response to an haemorheological anomaly, in particular the level of papillary autoregulation, which might be different depending on the severity of the glaucoma.[26-28] Thus, the blood flow velocity response to the same haemorheological anomaly could be different in two patients. Finally, the lack of correlation between the decrease in the ONH blood flow velocity and the increase in the aggregability of the red cells in these glaucoma patients could be explained by the presence of another haemorheological anomaly – namely, impaired deformability of the red cells. Indeed, in our study, the hyperaggregability of the red cells was not linked with modification of the plasma environment, but might be caused by modifications of the coating of the red blood cells – that is, modification in the capacity of the red cells to deform as shown by Mary *et al.*[25]

Even if the hyperaggregability of the erythrocytes that we observed in our glaucoma patients cannot alone explain the decrease in the papillary blood flow velocity, this does show that there must be a vascular pathology which adds to or helps the degeneration of the nervous fibres in glaucoma.

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