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Hypoxic viscosity and diabetic retinopathy

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Abstract

Diabetic and sickle retinopathy have features in common – for example, venous dilatation, microaneurysms, and capillary closure preceding neovascularisation. Bearing in mind that haemoglobin in poorly controlled diabetes is abnormal and that extremely low oxygen tensions (known to cause sickling) exist in the healthy cat retina, we wished to explore the possibility that diabetic blood, like that of sickle cell disease, may become more viscous when deoxygenated. To do this we measured whole blood viscosity, under oxygenated and deoxygenated conditions, of 23 normal persons, 23 diabetic patients without retinopathy, and 34 diabetic patients with retinopathy. The shear rate used was 230 s^{-1}, which is similar to that thought to prevail in the major retinal veins. The viscosity of blood from normal persons, corrected for packed cell volume, did not change significantly on deoxygenation: mean 4·54 (SD 0·38) cps, versus, 4·57 (0·39) paired *t* test, p=0·66. Similarly the blood from diabetics without retinopathy showed no change: 4·42 (0·45) versus 4·42 (0·30), p=0·98; whereas the blood from patients with retinopathy changed from 4·82 (0·48) to 4·95 (0·63), p=0·027. The hypoxic viscosity ratio (deoxygenated divided by oxygenated viscosity) correlated with total serum cholesterol (r=0·44, p=0·018) but not with HbA1, serum glucose, triglycerides, or age. A disproportionate increase in venous viscosity relative to arterial viscosity would lead to increased intraluminal and transmural pressure and therefore exacerbate leakage across capillary walls.

Diabetic retinopathy is the commonest cause of blindness in the working age group in England[1] and the United States.[2] Since its pathophysiology is still not understood, all possible factors should be explored. We considered the well known fact that diabetic retinopathy has features in common with sickle cell retinopathy, namely, venous dilatation, microaneurysms, and capillary closure[3] preceding neovascularisation,[4] even though the new vessels are usually in different areas.

Both diseases are also characterised by abnormal levels of different haemoglobin components, which in sickle cell disease, under conditions of hypoxia, cause a marked rise in viscosity. Even in health the functional reserve of oxygen of the human retina lasts only seconds,[5] and the oxygen tension (in cats) just proximal to the outer nuclear layer has been shown to be 12 mmHg[6] and even zero,[7] and the PO_2 of blood in human retinal veins has recently been estimated to be as low as 25 mmHg.[8] If low oxygen tensions interfere with blood flow anywhere, it is likely to happen in the retina. Venous dilatation, one of the first clinical signs of diabetic retinopathy, could be the result of the blood becoming more viscous as it enters the venous side of the circulation. The aim of this study was to test the hypothesis that blood from diabetic patients becomes more viscous under conditions of extreme hypoxia such as are thought to exist even in the healthy retina.

Patients and methods

PATIENTS

Consecutive patients were entered into the study from the Diabetic Retinopathy and General Diabetic Clinics of the Hammersmith Hospital. The 57 patients in the study were later grouped, from clinical records and reference to fundus photographs, according to the retinopathy status of the worse eye: 23 had no retinopathy, 11 had background retinopathy (at least one microaneurysm but no macular oedema nor preproliferative changes), nine had more severe background retinopathy with exudative maculopathy (one also having cotton-wool spots), and 14 had previously undergone argon laser panretinal photocoagulation (at least 2000 burns 500 μm in size and strong enough to cause moderate blanching) for proliferative retinopathy. No patients in the study had active proliferative retinopathy. Twenty-three normal subjects were recruited from staff and relatives of patients. The details of patients and controls are shown in Table I.

Methods

After informed consent was obtained, blood samples were collected from the antecubital vein in lithium heparin containers (15 IU/ml). The samples were collected from five subjects at a time. From each sample 1·5 ml was transferred to each of two 10 ml syringes, which were immediately capped. There were five syringes containing blood to be equilibrated with an oxygenating gas mixture and five syringes containing blood to be equilibrated with a deoxygenating gas mixture. A frame was constructed which allowed five syringes of blood/gas to be equilibrated simultaneously.

TABLE I *Details of patients and normal persons*

	Number	*Age (yr)**	*Duration of diabetes (yr)*
Normal	23	38·5 (15·5)	–
No retinopathy	23	55·7 (15·0)	7·7 (6·0)
Background	11	53·7 (15·2)	9·7 (4·2)
Maculopathy	10	55·1 (8·2)	12·1 (5·4)
Postphotocoagulation	14	50·1 (11·5)	16·1 (10·9)

* Means (with standard deviations).

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The oxygenating gas mixture was 95% air/5% CO_2 by volume and the deoxygenating mixture 1·4% O_2/7·0% CO_2/balance N_2. . Both gas mixtures were passed through a humidifyer (Sintaglass Ltd, London) before being exposed to the blood samples. The first five syringes containing blood samples were deoxygenated by filling the remaining 8·5 ml of the syringes with the appropriate gas mixture and then rotating the syringes at approximately 30 RPM for 20 minutes. This duration was chosen because a preliminary test had shown that another 10 minutes only lowered the PO_2 by a further 1 or 2 mmHg. The syringes were submerged in water at 37°C. At 5-minute intervals the gas volumes were expelled and replaced with fresh gas mixture. The blood samples in the second set of five syringes were then oxygenated in the same manner as above with the oxygenating gas mixture.

After agitation a small volume from each syringe was injected into the Corning 178 pH/blood gas analyser (Ciba Corning Diagnostics Ltd, Halstead, UK) and drawn into capillary tubes for packed cell volume PCV) measurement by the microhaematocrit procedure (Hawksley Ltd, England). The remaining volume in each syringe was reduced to 1 ml for viscometry. Apparent whole blood viscosities were measured at 37°C with a Wells-Brookfield Cone-Plate rotational viscometer (model LVT, Stanford, CA) which was calibrated immediately prior to the study. Our viscosity assays were 'apparent' because they were only at one shear rate, whereas viscosity of a non-Newtonian fluid is theoretically the slope of the tangent of the curved plot of shear stress against shear rate. The viscometer was purged with the appropriate gas mixture to minimise gas exchange during the period of the assay. This was achieved by passing the gas through a nozzle attached to the barrel of the viscometer which did not interfere with any moving parts. It took 3½ hours to complete the above procedure for five subjects from venepuncture to the last viscosity measurement. The investigator who did all the studies was masked for the patients' retinopathy status.

Besides measuring whole blood viscosity we also report on the viscosity corrected for PCV variation by the methods described by Dormandy.[9] At venepuncture samples were also taken for HbA1 levels. All diabetic patients underwent regular biochemical screening. Plasma glucose (Beckman Glucose analyser), and HbA1 were measured at every visit. Total serum cholesterol and triglyceride levels, however, were assayed less frequently but, because of an association with retinopathy,[10 11] were recorded for those patients who had these tests on blood taken at the same time as that for viscometry.

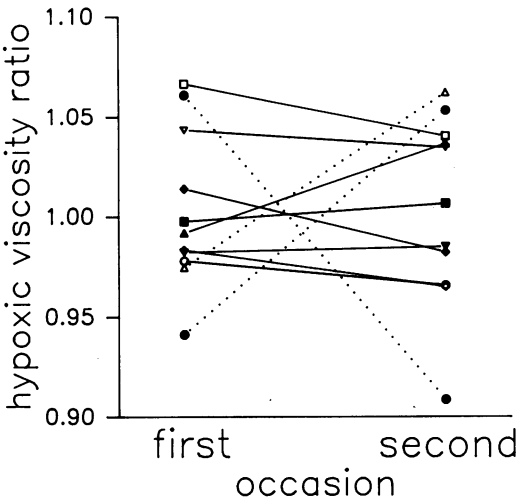

Figure 1: Reproducibility of the hypoxic viscosity ratio (deoxygenated divided by oxygenated viscosity) of 11 normal subjects, the two measurements being several weeks apart and both at the shear rate of 230 s^{-1}. The solid lines represent reproducibility scores greater than 95%, whereas the dotted lines represent those between 84 and 95%.

To study the reproducibility of the relationship between the oxygenated and deoxygenated viscosities the viscosity measurements were repeated several weeks later on 11 normal persons. The results on each occasion were expressed as a ratio of the deoxygenated divided by the oxygenated viscosity, which was called the 'hypoxic viscosity ratio'. Reproducibility scores were calculated for each individual by expressing the difference between the two ratios (the signs being ignored) as a percentage of the mean and then subtracting this from 100. Correlation tests (least squares method) were carried out between viscosity values and ratios described above and independent variables – that is, HbA1, blood glucose, serum total cholesterol, serum triglycerides, and age. Student's *t* tests were used to compare groups of data, and p values less than 0·05 were considered significant.

Results

For the 11 normal persons the reproducibility

TABLE II *Results of viscometry of normal persons and diabetics. The shear rate was 230 s^{-1}, which is similar to that thought to prevail in the major retinal veins*

	Normal		*Diabetics without retinopathy*		*Diabetics with retinopathy*
Number	*23*	*p value**	*23*	*p value§*	*34*
Measured viscosities (cps)†					
Oxygenated viscosity	4·31 (0·53)	0·73	4·26 (0·40)	0·001	4·72 (0·56)
Deoxygenated viscosity	4·32 (0·47)	0·53	4·22 (0·55)	<0·001	4·84 (0·71)
Paired *t* test	0·89		0·53		0·041
Corrected viscosities (cps)‡					
Oxygenated viscosity	4·57 (0·39)	0·14	4·42 (0·30)	<0·001	4·82 (0·48)
Deoxygenated viscosity	4·54 (0·38)	0·31	4·42 (0·45)	0·001	4·95 (0·63)
Paired *t* test, p value	0·66		0·98		0·027
Hypoxic viscosity ratio	0·996 (0·065)	0·65	0·990 (0·017)	0·014	1·025 (0·064)

* Unpaired *t* tests between normal persons and diabetics without retinopathy. † Expressed as means (with standard deviations). ‡ Corrected to standard PCV of 45%. § Unpaired *t* tests between diabetics with and without retinopathy.

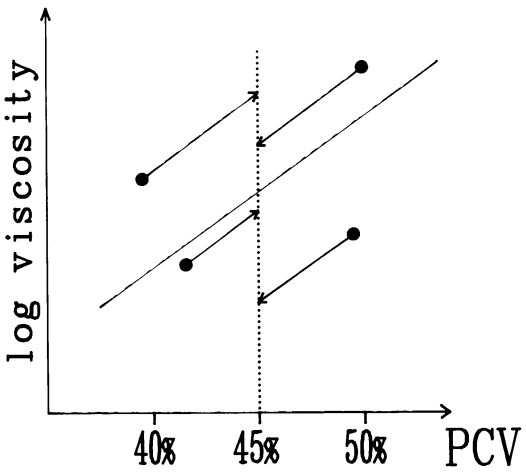

Figure 2: Correction of viscosity for variations of packed cell volume (PCV) for all subjects and patients. Data points (four sample ones are shown) were moved by computer to the 45% PCV line in a direction parallel to the linear regression line relating the log of the measured viscosity to the PCV for the group concerned.

scores ranged from 84·6 to 99·7 (mean 95·4). These scores are illustrated in Figure 1.

The viscosities of oxygenated and deoxygenated blood of normal persons and diabetics without and with retinopathy are presented in Table II. The PCV corrected viscosity is shown in Figure 2, where the slope of the linear regression line was calculated separately for each group concerned, at the same shear rate of 230 s^{-1}. The log of viscosity is used because of its greater correlation with PCV. The whole blood viscosity of normal persons and diabetics without retinopathy did not change significantly when deoxygenated (paired *t* tests, p=0·66 and 0·98 respectively for corrected values). In contrast the blood from patients with retinopathy became more viscous when deoxygenated (p=0·027). This pattern was seen with both the measured and corrected values.

Blood gas analyses on 56 samples (20 normals and 36 diabetics) showed that after oxygenation the mean values for PO_2 were 124·7 (SD 17·2) mmHg and for PCO_2 40·4 (1·6) mmHg. After deoxygenation the values for PO_2 were 18·3 (SD 2·8) mmHg and for PCO_2 were 54·1 (2·3) mmHg. The desired changes in blood gases were achieved with good reproducibility, indicated by the relatively small standard deviations.

The results of corrected viscosities of separate retinopathy groups together with the results of the other blood tests performed are presented in Table III. The biggest differences of viscosities are between the no-retinopathy group and the maculopathy and postphotocoagulation groups. In particular there were no significant differences between the HbA1 levels of the retinopathy groups. A scatterplot of the corrected deoxygenated viscosities is shown in Figure 3 to illustrate the spread of data.

Correlation studies showed a significant relationship between total serum cholesterol and the hypoxic viscosity ratio for the 29 patients who had these assays at the same time as the viscometry:

$$HVR=0{\cdot}02\times C+0{\cdot}89,\ r=0{\cdot}44,\ p=0{\cdot}018.$$

where HVR is the hypoxic viscosity ratio and C is the total serum cholesterol level (mmol/l). The relationship was the same for corrected and measured viscosities and is illustrated in Figure 4. Cholesterol also correlated with deoxygenated viscosity ($0{\cdot}24\times C+3{\cdot}3$, r=0·45, p=0·013) but not with oxygenated values. There were no other statistically significant correlations between any of the viscosity values and any of the other independent variables tested, namely, glucose, HbA1, triglycerides, and age. There was no correlation between cholesterol and either HbA1 or blood glucose.

The first 18 samples (6 normal and 12 diabetic) had PCVs measured of both oxygenated and deoxygenated portions. Of these, seven (three normal) showed a slight increase of PCV on deoxygenation (mean 2·6%) seven (three normal) showed a decrease (mean 2·5%), and four (one normal) showed no change. Only seven of the 18 pairs of PCVs reflected the change in viscosity. Owing to this lack of pattern all the following samples only had one estimate of PCV, performed on the original venous sample.

Discussion

The study was undertaken to test the hypothesis that blood from diabetic patients becomes more viscous when deoxygenated. The hypothesis has been supported in part: blood from diabetics with retinopathy was significantly more viscous when deoxygenated in contrast to that of normals and diabetics without retinopathy. However, this tendency (expressed as a 'hypoxic viscosity ratio') did not correlate with HbA1 but did with total serum cholesterol to a modest degree (r=0·44).

The correlation with cholesterol indicates that, of the factors influencing the hypoxic ratio, only 19% (r^2) can be attributed to the serum cholesterol level. Although cholesterol has been reported to affect membrane viscosity[12] and rigidity[13] and to correlate with the severity of

TABLE III *Results of viscometry and blood tests for 57 diabetic patients divided into retinopathy groups. The viscosities have been corrected to a PCV of 45%. The values are expressed as means (with standard deviations). The shear rate was 230 s^{-1}. The figures in parentheses, unless otherwise obvious, are p values (<0·05) for unpaired t tests with the no-retinopathy group*

	No retinopathy	*Background*	*Maculopathy*	*Postphotocoagulation*
Number	23	11	9	14
Oxygenated viscosity cps	4·42 (0·30)	4·47 (0·47)	4·86 (0·51) (0·005)	4·86 (0·50) (0·002)
Deoxygenated viscosity cps	4·42 (0·45)	4·77 (0·59)	5·00 (0·58) (0·005)	5·05 (0·70) (0·002)
Hypoxic viscosity ratio	0·990 (0·017)	1·007 (0·069)	1·028 (0·030) (<0·046)	1·037 (0·075)
PCV%*	42·7 (3·8)	43·4 (4·5)	42·7 (4·9)	43·9 (4·3)
HbA1%	7·7 (2·1)	7·7 (1·5)	7·6 (2·1)	8·0 (2·0)
Blood glucose mmol/l	9·2 (3·6)	14·2 (6·7)	10·2 (2·8)	9·6 (6·2)
Total serum cholesterol mmol/l	5·3 (1·4) (n=5)	5·6 (1·4) (n=8)	7·3 (1·8) (n=5)	6·5 (1·2) (n=11)
Serum triglycerides mmol/l	1·2 (0·6) (n=5)	2·3 (2·2) (n=8)	4·0 (4·8) (n=5)	1·7 (1·2) (n=11)

*Normal persons in this study: PCV 42·2 (3·7)%, HbA1 4·3 (0·6)%. Normal ranges for the hospital: blood glucose 3·5–5·0 mmol/l, total serum cholesterol 4·0–6·5 mmol/l, and triglycerides 0–2·0 mmol/l.

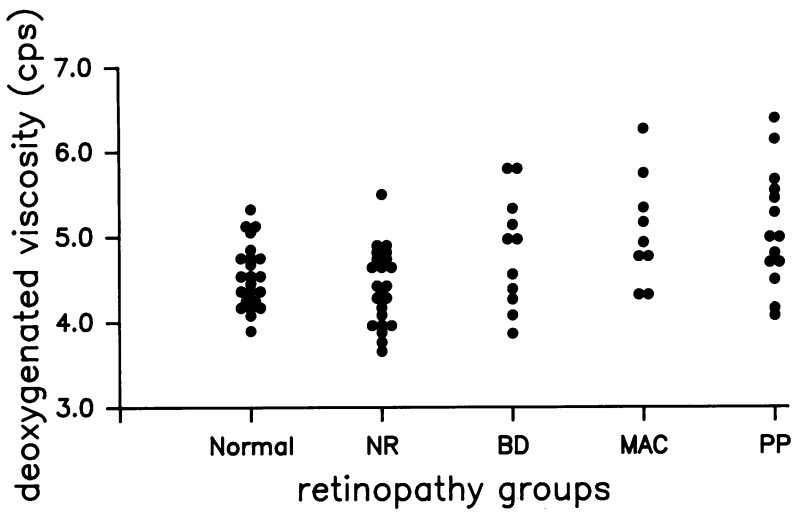

Figure 3: Deoxygenated viscosity of 23 normal persons and 57 diabetic patients measured at 230 s^{-1} and corrected to a PCV of 45%. NR=no retinopathy. BD=background retinopathy only. MAC=maculopathy. PP=postphotocoagulation. The deoxygenated viscosity of the patients in the maculopathy and postphotocoagulation groups were 13% (p=0·013) and 14% (p=0·007) higher than that of patients without retinopathy respectively.

diabetic retinopathy,[10] it is just as likely that the diabetic metabolic state itself is interfering directly with blood viscosity rather than through abnormal cholesterol levels. It was recently shown that red cell deformability was the same in diabetics with and without retinopathy (n=30, presumably under conditions of full oxygenation).[14] In the same study red cell membrane cholesterol content was lower in patients with retinopathy than those without ($p<0·025$) in contrast to the plasma levels, which were the other way around ($p<0·05$). In our study there was no relationship between cholesterol and HbA1 (or blood glucose), which might have been expected, since bad control is associated with increased levels of cholesterol.

The deoxygenating gas mixture was designed to bring the PO_2 down from the normal mixed venous level of about 40 mmHg to just below that of retinal venous blood, which is approximately 25 mmHg.[8] It is probable that the oxygen tension of blood in the deepest retinal capillaries will be lower still than that of mixed retinal venous blood. The PO_2 of the oxygenated samples is about 25 mmHg higher than true arterial blood, but the difference in saturation of the erythrocytes is of no consequence. The phenomenon of hypoxic viscosity may be a result of the combination of highly glycated haemoglobin and high cholesterol or other components of the blood. Haemoglobin is glycated at the terminal valine of the β chain – the same site of attachment of 2,3 diphosphoglycerate (DPG).[15] This substance, apart from facilitating the release of oxygen from haemoglobin, favourably affects the solubility of normal deoxygenated haemoglobin,[16] which may therefore be reduced if DPG is displaced by glycation. Fibrinogen is one blood component, for example, which has been reported to influence blood viscosity in diabetes[17] but was not measured in this study.

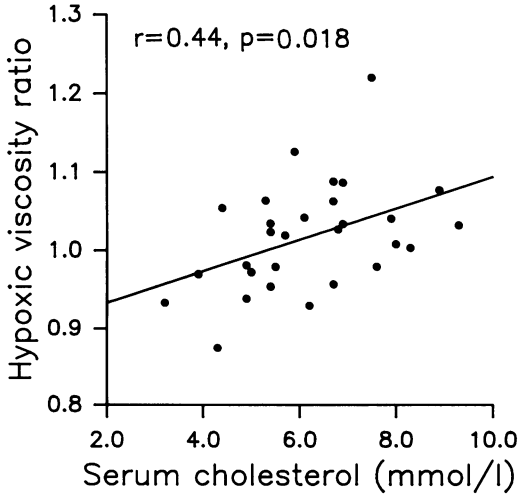

Figure 4: The hypoxic viscosity ratio (HVR) plotted against total serum cholesterol (C) for 29 diabetic patients. The HVR is the ratio of the deoxygenated divided by oxygenated viscosities, measured at the shear rate of 230 s^{-1} (similar to that in the major retinal veins). The regression line is represented by HVR=0·02×C+0·89.

Some increase in viscosity on deoxygenation may be expected in normal persons because the chloride shift is supposed to cause a 3% rise in PCV in venous blood.[18] This rise was not seen consistently in this study perhaps because the microhaematocrit method was not sensitive enough. The viscosity measurements in this study were carried out at the shear rate of 230 s^{-1} because this most closely resembles the shear rate thought to prevail in the retinal veins. The data of Riva *et al*[19] and Feke *et al*[20] show that the centre-line velocity of blood in a retinal vein of 180 μm diameter is 1·9 and 3·2 cm/s respectively, indicating average shear rates of 281 and 474 s^{-1}.* Red blood cells are subjected to changing shear rates during their passage through the vascular system. The plug flow in capillaries requires them to change shape rapidly in order to maximise contact between their cell membranes and the capillary endothelium. The shear rate in this situation is not well defined but is probably high.

Blood from the patients with retinopathy was 12% more viscous when deoxygenated than deoxygenated blood from diabetic patients without retinopathy (p=0·001, Figure 3, Table III). This could mean reductions in blood flow, which may not be adequately corrected by autoregulation because the PO_2, PCO_2 and pressure of blood in the retinal arteries is normal. Previous studies on retinal vascular autoregulation have demonstrated vascular responses to abnormal changes in *arterial* blood including PO_2, PCO_2[21], systemic blood pressure,[22] blood glucose,[23,24] and pharmacological agents.[25,26] Initial demonstrations of an increase in blood flow in response to dark adaptation[27,28] were later questioned[29] and explained as an artifact of the laser doppler system.[30] There is no evidence that retinal blood flow can increase *in the absence* of changes in arterial blood parameters, but the vessels may be able to respond directly to tissue hypoxia.

To maintain volume blood flow in the face of increased viscosity there must be an increase in pressure gradient and/or dilatation of vessels (and hence a reduction in velocity). Venous dilatation and lower velocities are seen in practice in diabetic retinopathy.[31] Any increase in pressure gradient would cause a rise in capillary intraluminal and transmural pressure and predispose to oedema. In the event of volume blood flow not being fully corrected the consequence

*Average shear rate=(−4/3)×(max velocity/radius). This is derived from an integration of the radially dependent shear rate over the cross sectional area, assuming Poiseuille flow since the Reynolds number is only 0·7.

could be chronic mild hypoperfusion of the retinal tissue, which could lead to hypoxia and a rise in concentration of waste products of metabolism, such as toxic oxidative radicles, which are considered by some to be important in the development of diabetic complications.[32] In advanced retinopathy increased hypoxic viscosity could set up a vicious circle by leading to reduced blood flow and lower oxygen tensions, which would cause further increase in viscosity. However, the fact that it was only the patients with the most severe retinopathy who showed an increase in hypoxic viscosity suggests that this is the result of the same diabetic microenvironment which caused the retinopathy rather than the cause of the retinopathy itself; nevertheless the retinopathy could be worsened by it.

The phenomenon of hypoxic viscosity could be important because it might present a new target for pharmacological attack. A future study will examine whether the deformability of red blood cells from diabetic patients is affected by oxygen tension.

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