Retinal Hemodynamics in Proliferative Diabetic Retinopathy

A Laser Doppler Velocimetry Study

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Purpose. This study investigated retinal hemodynamic changes associated with different pathologic features observed on fundus color and fluorescein angiography in patients with proliferative diabetic retinopathy.

Methods. Retinal circulatory characteristics were investigated in 25 eyes of 23 diabetic patients with proliferative retinopathy using a combination of bidirectional laser Doppler velocimetry and monochromatic fundus photography.

Results. Eyes with severe capillary nonperfusion had 32% less average volumetric blood flow rate (Q) than eyes with less severe nonperfusion ($P = 0.0005$). In addition, eyes with severe vessel staining with fluorescein had 20% less average Q than eyes without staining ($P = 0.0508$). Eyes with severe fluorescein leakage in the macula had a 17% larger total venous cross-section than eyes with milder leakage ($P = 0.027$). Eyes with clinically significant macular edema had 11% larger average venous diameter than eyes without this feature ($P = 0.0085$).

Conclusions. Severe capillary nonperfusion and vessel staining with fluorescein are associated with decreased retinal blood flow rates. Vasodilatation may be an important factor for increased vascular permeability and macular edema in proliferative diabetic retinopathy. Invest Ophthalmol Vis Sci. 1993;34:66–71.

Several laser Doppler velocimetry studies have described retinal hemodynamic changes in patients with diabetic retinopathy.[1-8] In these studies, diabetic retinopathy was graded into large subgroups, such as no retinopathy, mild background, severe background, and proliferative retinopathy.

In the present investigation, we studied eyes with proliferative diabetic retinopathy (PDR) in an attempt to characterize the hemodynamic changes associated with different pathologic features observed on fundus color and fluorescein angiographic photographs. Assessment of retinal blood flow was performed with a combination of laser Doppler velocimetry and monochromatic fundus photography.

MATERIALS AND METHODS

Bidirectional laser Doppler velocimetry and monochromatic fundus photographs were performed in 25 eyes of 23 diabetic patients with proliferative retinopathy and high-risk characteristics.[9] Nineteen patients had type I and four had type II diabetes mellitus. Patients ranged in age from 19–63 yr (35 ± 11 yr; mean ± 1 standard deviation). Duration of known diabetes ranged from 9–30 yr (mean, 20 ± 7 yr). Twenty-one

patients received insulin and two patients took oral hypoglycemics. None of the studied eyes had received photocoagulation therapy before the study.

No patients had history or evidence of ocular disease other than diabetic retinopathy. Eyes studied had a best-corrected visual acuity of 6/15 or better, refractive errors between +4.00 and −4.00 diopters, and pupillary dilatation of 6 mm or more. The protocol used in this study was approved by our institutional human experimentation committee. Tenets of the Declaration of Helsinki were followed. Informed consent was obtained from all subjects after the nature of the procedure had been explained.

Ophthalmologic examination included best-corrected visual acuity, slit-lamp biomicroscopy, Goldmann applanation tonometry, and dilated direct and indirect funduscopy. Seven-field color fundus photography and fluorescein angiography were obtained in all eyes. Information on the approximate arteriovenous transit time was not available on all eyes; therefore, we could not investigate the association between arteriovenous transit times and laser Doppler velocimetry measurements.

After pupillary dilatation with Mydriacyl 1% (Alcon, Puerto Rico) and Neo-Synephrine 10% (Winthrop Pharmaceuticals, New York, NY), a Polaroid (Cambridge, MA) color fundus photograph of the posterior fundus was obtained to document the laser Doppler velocimetry measurement site. Bidirectional laser Doppler measurements of erythrocyte velocity in a main superior or inferior temporal retinal vein were performed in each patient. The erythrocyte velocity was measured on straight portions of the veins, at a distance less than 2 disc diameters from the center of the optic nerve head. Sites close to venous junctions or arteriovenous crossings were avoided, as were sites where two vessels were close to each other. We performed flow measurements in veins, because the minimal flow pulsatility in these vessels permits a more accurate determination of the average erythrocyte velocity.[3]

The maximum or centerline velocity of the erythrocytes (V_{max}) was determined according to the relation

$$V_{max} = k\Delta f \qquad (1)$$

where k is a constant of proportionality that depends on the scattering geometry and the wavelength of the laser light, and Δf is the average of the Δf measured from 10 pairs of spectra.

$$\Delta f = f_{1max} - f_{2max} \qquad (2)$$

where f_{1max} and f_{2max} are the cutoff frequencies of the Doppler shift power spectra recorded simultaneously in two different directions of the scattered light.[10,11]

Fundus photographs were taken in monochromatic light at 570 nm just before the bidirectional laser Doppler velocimetry recordings. The diameter (D) of each vessel at the site of the bidirectional laser Doppler velocimetry recordings was determined from projected photographic negatives using a caliper. For each vessel, D was obtained from an average of the values measured from three photographs.

Retinal venous volumetric blood flow rate (Q) was estimated, as described previously,[3] from the relation:

$$Q = V_{mean} \cdot \pi D^2 / 4 \qquad (3)$$

with the mean blood velocity (V_{mean}) calculated as

$$V_{mean} = C \cdot V_{max}. \qquad (4)$$

A value of C equal to 1/1.6 was used to estimate V_{mean}.

All measurements of D were performed by one trained examiner, and all V_{max} determinations were done by another. Each individual was masked with regard to the results of the other and the result of color photograph and fluorescein angiogram assessment.

Blood glucose levels from finger capillary blood samples were determined at the time of bidirectional laser Doppler velocimetry measurements using an Accu-check Blood Glucose Monitor (Boehringer Mannheim, Indianapolis, IN). Blood glucose values ranged between 54 and 400 mg/dl (mean, 220 ± 99 mg/dl). Brachial artery blood pressure was measured by sphygmomanometry. Systolic pressure ranged between 98 and 136 mmHg (mean, 114 ± 11 mmHg) and diastolic pressure ranged between 60 and 86 mmHg (mean, 73 ± 8 mmHg). Five patients were receiving antihypertensive medication.

Fundus fluorescein angiograms and seven 30° field color photographs were assessed in a masked fashion. The studied eyes were divided into two categories according to each of the following parameters: (1) capillary nonperfusion (mild/severe); (2) intraretinal microvascular abnormalities (present/absent); (3) cotton wool spots (present/absent); (4) vessel staining with fluorescein (present/absent); (5) fluorescein leakage in the macula (mild/severe); (6) clinically significant macular edema as defined by the EDTRS study[12] (present/absent); (7) neovascularization of the disc (NVD; (present/absent); (8) neovascularization of the retina (NVE; present/absent); and (9) both NVD and NVE (present/absent).

We are aware of that this is a coarse evaluation of the severity of each lesion. The relatively small number of patients studied did not allow for a more detailed grading of each of the mentioned lesions.

Eyes also were divided into three categories according to the number of microaneurysms within 2 disc diameters of the center of the fovea (group A: 1–10 microaneurysms, group B: 11–50 microaneurysms, and group C: 51 or more microaneurysms).

In one patient, the quality of the sweep frames did not permit accurate evaluation of the severity of capillary nonperfusion; therefore, the eye was excluded from this analysis.

Measurements of Q were obtained in one major temporal vein in each eye. Because Q depends on the diameter of the vein measured and the size of the retinal area drained by this vessel, we also estimated a total venous volumetric blood flow rate (Q_T). Q_T was calculated using the formula

$$Q_T = Q \cdot S_T / D^2 \qquad (5)$$

where Q is the measurement obtained in a major retinal vein, S_T is the total cross section of all visible veins around the optic nerve head calculated from diameter measurements, and D is the diameter of the vein in which Q was measured[13].

Nonpaired t-test and correlation analysis were used to statistically evaluate the results. The Wilk-Shapiro test was used to assess the normal distribution of the results. All results were normally distributed. Probability values smaller than 0.05 were considered statistically significant.

RESULTS

In the studied eyes, average D was 166 ± 17 μm, V_{max} was 1.25 ± 0.3 cm/sec, Q was 10.3 ± 3.0 μl/min, S_T was 96.2 ± 17.0 cm$^2 \times 10^{-5}$, and Q_T was 35.6 ± 11.0 μl/min.

The study eyes were divided into two groups according to the severity and extent of the areas of capillary nonperfusion (CNP; (Table 1). Twelve eyes with severe CNP had an average Q of 8.2 ± 1.6 μl/min, which was significantly smaller (32%) than the average Q of 12.1 ± 2.9 μl/min measured in 12 eyes that had mild CNP (nonpaired, two-tailed Student's t-test; P = 0.0005). Significantly smaller average D (10.3%, P = 0.0098), S_T (21.2%, P = 0.0002), and Q_T (33.9%, P = 0.0004) also were observed in eyes with severe CNP. Although V_{max} was 14% smaller in eyes with severe CNP, the difference was not statistically significant. No significant differences in age or duration of diabetes were observed between eyes with mild and severe CNP.

Eyes also were divided into two groups according to presence or absence of vessel staining with fluorescein (Table 1). Average Q in 13 eyes with staining was smaller than Q in 12 eyes without staining (20.2%). This difference was borderline significant (P = 0.0508). Average Q_T was smaller by 23.7% in eyes with staining (P = 0.0263). No significant differences in D, V_{max}, S_T, and age were observed between the two groups of eyes. Average duration of the disease was

TABLE 1. Retinal Hemodynamics According to Different Diabetic Retinopathy Characteritics

	No. of Eyes	D (μm)	V_{max} (cm/sec)	Q (μl/min)	S_T (cm$^2 \cdot 10^{-5}$)	Q_T (μl/min)
CNP						
Mild	12	174 ± 13	1.35 ± 0.30	12.1 ± 2.9	107.6 ± 15.1	43.1 ± 10.2
Severe	12	157 ± 16*	1.15 ± 0.29	8.2 ± 1.6†	84.8 ± 9.3†	28.5 ± 6.6†
Staining						
Mild	12	169 ± 16	1.35 ± 0.30	11.5 ± 3.1	101.4 ± 20.0	40.6 ± 11.7
Severe	13	163 ± 18	1.17 ± 0.28	9.1 ± 2.5	90.2 ± 11.9	31 ± 8.4‡
FL						
Mild	11	162 ± 18	1.24 ± 0.31	9.5 ± 2.7	87.0 ± 11.5	31.9 ± 8.7
Severe	14	169 ± 15	1.27 ± 0.30	10.8 ± 3.2	102.2 ± 17.8‡	38.5 ± 12.1
CSME						
No	17	160 ± 16	1.26 ± 0.28	9.6 ± 2.8	91.2 ± 14.6	34.2 ± 10.9
Yes	8	178 ± 11*	1.23 ± 0.34	11.7 ± 3.2	104.9 ± 18.7	38.5 ± 11.5
IRMA						
No	10	163 ± 21	1.15 ± 0.27	9.0 ± 2.3	90.3 ± 19.5	30.5 ± 7.8
Yes	15	168 ± 13	1.32 ± 0.3	11.1 ± 3.2	99.0 ± 14.6	39.0 ± 11.8
CWS						
No	16	164 ± 16	1.27 ± 0.26	10.1 ± 2.4	93.5 ± 16.4	35.6 ± 11.0
Yes	9	169 ± 18	1.23 ± 0.38	10.5 ± 4.0	99.2 ± 18.2	35.6 ± 11.9

Significantly different from the other group by two-tailed nonpaired t-test: *P < 0.01; †P < 0.001; ‡P < 0.05.

Results shown as mean ± standard deviation. D, venous diameter at the site of laser Doppler velocimetry determination. V_{max}, maximum or centerline erythrocyte velocity. Q, volumetric blood flow rate. S_T, total venous cross-section. Q_T, total retinal volumetric blood flow rate. CNP, capillary nonperfusion. FL, fluorescein leakage in the macula. CSME, clinically significant macular edema. IRMA, intraretinal microabnormalities. CWS, cotton wool spot.

significantly longer in eyes with staining (24 ± 4 yr) than in eyes with no staining ($16 \pm$ yr, $P = 0.0023$).

A larger average S_T in eyes with severe fluorescein leakage in the macula (17.5%, $P = 0.027$) was the only significant difference observed between 11 eyes with mild leakage of fluorescein in the macular area and 14 eyes with severe leakage (Table 1). Although the other parameters also were larger in eyes with severe leakage, the differences did not reach statistical significance.

Average diameter was significantly larger (11.3%, $P = 0.0085$) in eight eyes with clinically significant macular edema (CSME) than in 17 eyes without CSME (Table 1). Average S_T also was larger in eyes with CSME, but the difference was of borderline statistical significance ($P = 0.056$). Although most of the other parameters also were larger in eyes with CSME, the differences were not statistically significant.

Fifteen eyes with intraretinal microvascular abnormalities (IRMA) had larger (27.9%) average Q_T than 10 eyes without IRMA. This difference, however, was borderline significant ($P = 0.0579$; Table 1). No significant differences were observed in any other parameters between eyes with and without IRMA.

No significant differences were detected between eyes with cotton wool spots (CWS) and eyes without CWS or between eyes with NVD, NVE, or both. Also, no significant differences were detected between the three groups of eyes divided according to number of microaneurysms.

No significant correlations were observed between Q and any of the following: age, duration of disease, blood glucose, mean blood pressure, intraocular pressure, or perfusion pressure.

DISCUSSION

The purpose of this study was to investigate whether retinal hemodynamic characteristics are associated with specific clinical and angiographic features observed in patients with PDR.

As a first approach, we classified the study eyes into two to three groups for each of the investigated pathologic features. The relatively small number of eyes studied did not allow us to perform a meaningful statistical analysis using a more sensitive grading system with more categories. Our analysis, which divides the eyes into two groups with or without a specific pathologic finding, does provide an estimate on whether the retinal circulatory parameters are different between these two groups.

Our results show that eyes with proliferative retinopathy and extensive areas of CNP have significantly smaller D, Q, S_T, and Q_T than eyes with less severe CNP, a finding that is not surprising, because a more

atrophic retina probably has smaller metabolic requirements. Although eyes with extensive CNP also showed a lower average V_{max} than eyes with less severe CNP, the difference was not statistically significant.

Vascular staining usually is associated with retinal vascular occlusion and ischemia and is thought to be the result of endothelial cell dysfunction and breakdown of the blood-retinal barrier.[14] In our study, eyes with vascular fluorescein staining had significantly smaller Q_T than eyes without staining, a finding that fits with the fact that vascular staining is associated with decreased vascular perfusion.

Eyes with more severe macular leakage of fluorescein had significantly larger S_T than eyes with milder leakage. Average D also was larger, but the difference was not statistically significant. In addition, eyes with CSME had a significantly larger D than those without CSME and had a larger S_T of borderline significance.

Vasodilatation is known to occur in eyes with diabetic retinopathy.[3,15] It is interesting that within our group of eyes with proliferative retinopathy, those eyes with CSME or those with more severe macular leakage of fluorescein had veins that were more dilated. Dilated vessels probably are more permeable to the passage of substances from blood to the retinal tissue; therefore, they may be an important factor in the formation of CSME. Stefansson et al[16] suggested a model for the development of diabetic retinopathy in which "dilatation and increased endothelial wall tension in the retinal capillaries and vesicles will cause them to leak and proliferate."

Eyes with more severe staining belonged to patients with a longer duration of diabetes. Our small sample size, however, did not allow us to assess separately the relationships between vessel staining, vasodilatation, and length of the disease.

No significant differences in D, V_{max}, Q, S_T, or Q_T were observed in eyes with or without CWS or IRMA. One explanation for such a lack of significant differences may be that measurements obtained in the major retinal veins may not represent sensitively abnormal flow occurring in specific small areas of the fundus. CWS are areas of nerve fiber layer infarction that usually appear on fluorescein angiograms as small focal areas devoid of perfusion. IRMAs also are seen in association with areas of capillary nonperfusion. If these areas are very small compared to the total area drained by the major veins measured, the total flow passing through these veins may not be significantly affected.

No significant differences were observed between eyes with NVD, NVE, or both, indicating that similar blood flow parameters are associated with these different types of retinal neovascularization. Also, no significant differences between eyes with smaller and larger numbers of microaneurysms were detected in

this study. This indicates that in eyes with PDR, the number of microaneurysms is not associated with changes in retinal blood flow parameters.

For the group of 25 eyes studied, average D, V_{max}, and Q were close to those of a previous report in which we found an average D of 170 ± 21 μm, V_{max} of 1.2 ± 0.1 cm/sec, and Q of 10.4 ± 3.4 μl/min in a group of 11 eyes with PDR.[3] Comparing the results of our current study with those reported by us in a group of 26 normal subjects matched for age and systolic and diastolic pressure[3] shows that eyes with PDR have a significantly larger than normal average venous diameter (9%, $P < 0.01$) and a significantly slower than normal V_{max} (26%, $P < 0.001$). Although average Q is 9% smaller than normal, the difference is not statistically significant ($P > 0.05$). Within this group of PDR eyes that has a larger average venous diameter than normal, those with severe CNP have smaller diameters than those with mild CNP. Therefore, it seems that the retinal vasodilation produced by the diabetic process may be somewhat counteracted by the development of severe capillary nonperfusion that leads to a decrease in retinal blood flow and venous diameter.

Although there has been some controversy in the literature regarding the hemodynamic changes that occur in patients with no diabetic retinopathy or background diabetic retinopathy,[2,3,5,7,8,13,17,18,19] several previous studies of the retinal circulation in eyes with PDR have suggested results that are not very different from those obtained in our current study.

Kohner et al[17] studied the retinal hemodynamics in 12 patients with severe proliferative retinopathy using the dye dilution technique. They found no significant difference from normal in mean dye transit time or segmental blood flow (SBF). Koerner et al[20] used the same technique and reported retinal circulation times that were significantly longer than normal in seven patients with PDR. Estimation of vessel diameters was not provided, however. Therefore, conclusions on vascular volume and blood flow changes in PDR could not be obtained from this study. Blair et al[18] studied 10 patients with proliferative retinopathy and found increased mean circulation times (MCT). In five of these patients, MCT could not be quantified. Therefore, quantitative comparison with normal eyes could not be obtained. Their results, however, suggested decreased retinal SBF in these patients. Yoshida et al[19] performed measurements in 12 patients with early PDR and found an average 16% significant increase above normal in MCT and an average 9% decrease in SBF that was not statistically significant (a result very similar to the average 9% nonstatistically significant Q change from normal observed in our study).

Feke et al[1,8] used laser the Doppler velocimetry technique to study the retinal circulation in patients

with PDR. The unidirectional laser Doppler velocimetry technique used by Feke et al[1,8] is different from the bidirectional laser Doppler velocimetry technique employed in the present study. Unlike the bidirectional technique that provides absolute determinations of red blood cell velocity in retinal vessels,[21] the unidirectional technique provides only relative velocity measurements. As a result, only the red blood cell velocity pulsatility (ie, the ratio between the systolic and diastolic velocities) could be assessed. No significant difference from normal in velocity pulsatility was found in 12 eyes with PDR.

In summary, our results show that PDR eyes with more severe CNP or staining of fluorescein have smaller volumetric blood flow rates than eyes with less severe CNP or staining. Eyes with CSME or more severe leakage of fluorescein have larger venous cross-section than those without SCME or less severe leakage of fluorescein. Further studies are needed to clarify the relationship between these hemodynamic measurements and other parameters, such as the retinal arteriovenous circulation times observed on fluorescein angiography.

Key Words

fluorescein angiography, laser Doppler velocimetry, proliferative diabetic retinopathy, retinal hemodynamics, venous diameter.

Acknowledgments

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