Detection of Early Retinal Changes in Diabetes by Vitreous Fluorophotometry

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SUMMARY

A series of 77 patients with overt diabetes and with apparently normal fundi on ophthalmoscopy and fluorescein angiography was examined by vitreous fluorophotometry. Breakdown of the blood-retinal barrier, which appears to be the earliest clinically detectable change in the retina in diabetes, was a constant finding. Quantitative measurement by vitreous fluorophotometry of the breakdown of the blood-retinal barrier could be correlated with degree of metabolic control and previous duration of diabetic disease. Significantly higher vitreous fluorophotometry values, indicating a more marked breakdown of the blood-retinal barrier, were recorded in patients under poor metabolic control than in patients whose diabetes was under relatively better control. Similarly, patients who had had diabetes for longer periods of time showed higher vitreous fluorophotometry values than those recorded in patients with diabetes of shorter duration.

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The blood-retinal barrier appears to play an important role in the mechanisms of retinal pathophysiology, particularly in diabetic retinopathy.[1,2]

In human retinal disease, our knowledge of the importance of the blood-retinal barrier has been derived from the widespread clinical use of fluorescein angiography, a direct correlation between fluorescein "leakage" and the disruption of the blood-retinal barrier being made after the demonstration of an active transport of fluorescein at the blood-retinal barrier level.[3] Fluorescein angiography, however, is difficult to quantitate and has relatively low sensitivity in regard to fluorescein permeability. It was only recently that vitreous fluorophotometry, a clinical quantitative method of studying the blood-retinal barrier, was developed.[2]

The possibilities offered by this technique prompted us to examine a series of diabetic patients, particularly those who had no evidence of retinal lesions or of breakdown of the blood-retinal barrier by fluorescein angiography. The disturbance of the blood-retinal barrier was a constant finding in every diabetic patient examined, well before any lesion was visible in the fundus,[2] a finding recently confirmed by Waltman et al.[4]

From this study it was apparent that a breakdown of the blood-retinal barrier (possibly by a functional mechanism) is one of the earliest changes known to occur in the retina in diabetes.

To examine the relationships between the breakdown of the blood-retinal barrier in diabetes and the systemic disease, a series of diabetic patients who had no clinically visible retinal lesions was examined using vitreous fluorophotometry. The degree of breakdown of the blood-retinal barrier was correlated with the degree of metabolic control and the duration of diabetes.

MATERIALS AND METHODS

Assessment of the breakdown of the blood-retinal barrier by vitreous fluorophotometry. The apparatus for vitreous fluorophotometry consists essentially of a slit lamp that has been modified by adaptation with a new source of illumination, appropriate filters, a photometric detection system, and a device for electrical registration of the movement of the instrument. The source of illumination is a fiberoptic system connected to a 150-W unit with ventilation. The filters used are either a Haag-Streit cobalt blue filter (T = 460 nm) or a Balzer FITC-3 with a red suppression filter as an exciter and an Ilford 110 as a barrier filter. The photometric detection system is comprised of a modified eyepiece containing a fiberoptic probe, which is designed to be superimposed on any area of the image of the optical cross section, connected to a photomultiplier tube, an auto-

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ranging photometer, a recorder, and an oscilloscope with storage. The aperture of the fiberoptic probe, a 75 × 2,500 μm slit, is designed so that it may be superimposed on any area of the image of the optical cross section. The sensor tip of the probe is focused with the optical section, thus allowing the fluorescein concentration to be measured in all parts of the eye that are visible in the eyepiece of the slit lamp as it scans through the vitreous from the retina to the lens.

As the instrument scans, its movement is registered electrically, using a linear carbon potentiometer connected to the X plates of the recorder. All measurements are made after a Worst low-vacuum contact lens is applied to the eye under examination.

In this study, the macular region was selected for the initiation of the recordings. The recordings were then transferred to fluorescein concentration curves by dividing the recordings into three equal parts, which were ascribed to posterior vitreous, middle vitreous, and anterior vitreous. Only posterior vitreous values were taken into consideration for this study.

Protocol. Every patient was tested for visual acuity and examined by direct ophthalmoscopy and fluorescein angiography before being submitted to vitreous fluorophotometry. Fluorescein angiography was performed using either a Zeiss camera with Baird atomic filters or a Topcon TRC-F_3 with original filters. After a series of pictures was taken at 1-s intervals, additional photographs were taken at 5, 10, 15, and 30 min using Kodak Tri-X-Pan film.

Each patient was given an intravenous injection of 1 g of sodium fluorescein, either as a 10 or 20% solution, which was followed immediately by fluorescein angiography. Vitreous fluorophotometry was performed exactly 1 h after the injection. The fluorescein concentrations in the vitreous were corrected taking into account the amount of fluorescein injected per kilogram of body weight, a dose of 14 mg/kg being used as the standard. Blood samples were taken from six patients for measurement of fluorescein concentration in the plasma. An average value of $9 \pm 0.9 \times 10^{-6}$ g/ml of fluorescein was found 1 h after the intravenous injection.

Subjects. Of the large number of diabetic patients who were examined by vitreous fluorophotometry, those included in this study fulfilled the following criteria: apparent retinal normality on fluorescein angiography, and available information about the state of control of their diabetes in the 1-yr period immediately preceding the vitreous fluorophotometry examination. The apparent retinal normality was determined by a 10/10 visual acuity and normal ophthalmoscopic and fluorescein angiographic examinations. Any retinal macular lesions seen at the time of the examination automatically excluded the patient from this study.

The patients were considered to have their diabetes under relatively good control if, during the 1-yr period before examination, they satisfied the following conditions: (1) absence of major hypoglycemic symptoms; (2) absence of ketoacidosis; (3) satisfactory plasma glucose concentration (50% of fasting glucose determinations between 100 mg/dl and 200 mg/dl and total test levels consistently less than 250 mg/dl); (4) 50% of all tests for glycosuria less than +; and (5) absence of ketonuria. The patients were asked to test their own urine daily using Ketostix and Clinistix. All blood and urine tests were performed at a local laboratory at 1-mo intervals and at the University Hospital at each clinic attendance, which was made at 3-mo intervals.

Vitreous fluorophotometry examinations were performed in 77 eyes satisfying the study criteria. There were 41 female patients and 36 male patients. The eyes were grouped according to the type of diabetes the patient had: whether the patient was insulin dependent or noninsulin dependent, and whether the duration of diabetes was less than 5 yr or 5 yr or more. There were 27 eyes of patients with insulin-dependent diabetes and 50 eyes of patients with noninsulin-dependent diabetes who were being treated by diet and oral sulfonylurea agents. Most of these patients presented with maturity-onset diabetes. In only 11 of the 27 insulin-dependent diabetic patients was the age of onset less than 30 yr.

Statistical analysis among study groups was determined using the Student's *t* test adjusted for degrees of freedom.

Reproducibility studies and results from vitreous fluorophotometry examinations on normal volunteers have been previously reported.[2] Further observations in normal volunteers older than 50 yr have shown that vitreous fluorophotometry values increase with age. However, no values higher than 1.5×10^{-8} g/ml have been seen in the posterior vitreous of normal human eyes. A total of 45 normal human eyes has now been examined by vitreous fluorophotometry.

RESULTS

All the eyes included in this study showed normal fundi. In spite of the normality of the fluorescein angiograms, from which no lesions could be detected, the vitreous fluorophotometry readings were consistently abnormal, showing values greater than 2×10^{-8} g/ml in the posterior vitreous. The fluorescein concentration indicated an abnormal penetra-

FIGURE 1. Vitreous fluorophotometry measurements in an eye of a diabetic patient that revealed no abnormality with fluorescein angiography. Plotted curve of fluorescein concentration from the retina to the lens.

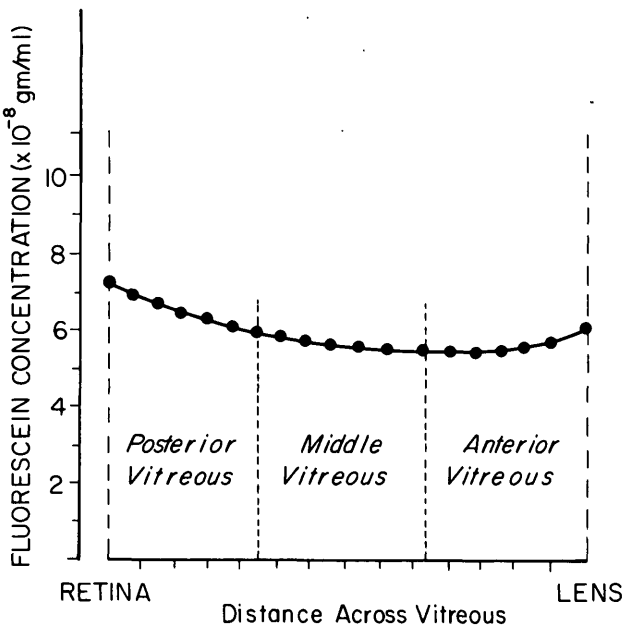

TABLE 1
Vitreous fluorophotometry values in insulin-dependent diabetics

Metabolic control	Vitreous fluorophotometry—posterior vitreous ($\times 10^{-8}$ g/ml; Mean ± SD)				
	No. of eyes	<5 yr*	No. of eyes	≥5 yr*	P-values
Relatively good	10	3.6 ± 1.2	3	5.9 ± 0.8	<0.005
Poor	11	5.5 ± 2.4	3	7.7 ± 1.5	NS
P-values		<0.025		NS	

* Duration of diabetes.

tion of fluorescein into the vitreous of all 77 eyes examined. The fluorescein concentration curves followed a typical pattern (Figure 1); the fluorescein concentration profile demonstrated the penetration of fluorescein across the blood-retinal barrier.

To examine the possible existence of correlation between the alteration of the blood-retinal barrier and the quality of metabolic control of diabetes, the vitreous fluorophotometry measurements in insulin-dependent and noninsulin-dependent diabetes in patients under relatively good metabolic control were compared with those of patients under poor diabetic control (Table 1). In the group of insulin-dependent patients with known diabetes for less than 5 yr, there were 10 eyes from patients under good control and 11 eyes from patients under poor control. Statistical analysis comparing these two groups revealed a significant difference ($t = 2.4$; Df = 19; $P < 0.025$). Insulin-dependent diabetics who had had diabetes for less than 5 yr and were in relatively good metabolic control had vitreous fluorophotometry values significantly lower than those registered in a similar group of diabetic patients who had poor control of their diabetes.

In the group of patients who had had diabetes for 5 yr or more, there were only three eyes from patients under better control and three eyes from patients under poor control. Although higher vitreous fluorophotometry values were recorded for patients under poor metabolic control, the difference was not statistically significant, partly due to the small number of eyes included in these groups.

The vitreous fluorophotometry values observed in noninsulin-dependent diabetics who were divided according to the duration of their diabetes are shown in Table 2. In the group of diabetic patients who had known diabetes for less than 5 yr, there were 12 eyes from patients under better diabetic control and eight eyes from patients under poor diabetic control. Statistical analysis of the comparison between these two groups showed a very significant difference ($t = 4.5$; Df = 18; $P < 0.0005$).

TABLE 2
Vitreous fluorophotometry values in noninsulin-dependent diabetics

Metabolic control	Vitreous fluorophotometry—posterior vitreous ($\times 10^{-8}$ g/ml; Mean ± SD)				
	No. of eyes	<5 yr*	No. of eyes	≥5 yr*	P-values
Relatively good	12	4.6 ± 1.4	26	6.3 ± 1.8	<0.01
Poor	8	8.3 ± 2.4	4	8.7 ± 3.4	NS
P-values		<0.0005		<0.0125	

* Duration of diabetes.

Noninsulin-dependent diabetic patients of less than 5 yr duration under relatively good metabolic control have vitreous fluorophotometry values significantly lower than those observed in a similar group of diabetic patients under poor metabolic control. The same conclusion was observed in the group of noninsulin-dependent diabetic patients who had had diabetes for 5 yr or more. Statistical analysis of the comparison between groups with better metabolic control and poor metabolic control showed a significant difference ($t = 2.4$; Df = 28; $P < 0.0125$). Again, significantly higher vitreous fluorophotometry readings were seen in diabetic patients under poor metabolic control.

Results regarding duration of diabetes and early breakdown of the blood-retinal barrier are included in Tables 1 and 2. A clear difference between vitreous fluorophotometry values was visible in patients with diabetes for less than 5 yr and those with known diabetes for 5 yr or more. This difference was especially significant in patients under relatively good metabolic control.

In the group of insulin-dependent diabetic patients under better metabolic control (Table 1), there were 10 eyes from patients with diabetes for less than 5 yr. Statistical analysis of the comparison between the vitreous fluorophotometry values in these groups showed a significant difference ($t = 3.1$; Df = 11; $P < 0.005$). Values in patients with diabetes for 5 yr or more were significantly higher.

In the group of noninsulin-dependent diabetic patients under better metabolic control (Table 2), there were 12 eyes from patients with diabetes for less than 5 yr and 26 eyes from patients with diabetes for 5 yr or more. Statistical analysis of the comparison between the vitreous fluorophotometry values in these two groups showed a significant difference ($t = 2.6$; Df = 36; $P < 0.01$). Vitreous fluorophotometry readings were significantly higher in patients with diabetes for 5 yr or more who were noninsulin dependent and were under relatively good metabolic control.

No significant differences, however, were observed in vitreous fluorophotometry values between patients with diabetes for less than 5 yr or those with diabetes for 5 yr or more who were either insulin or noninsulin dependent and were under poor metabolic control (Tables 1 and 2).

DISCUSSION

The results confirmed the presence of an alteration of the blood-retinal barrier in every diabetic eye examined.[2,4] Breakdown of the blood-retinal barrier appears to be, therefore, the earliest clinically detectable change to occur in the retina in diabetics.

Quantitative measurement by vitreous fluorophotometry of the breakdown of the blood-retinal barrier allowed us to look for any correlation between the alteration of the blood-retinal barrier and the metabolic control of diabetes. A close examination of the influence of metabolic control on the early breakdown of the blood-retinal barrier appeared to be of particular interest for two reasons. First, it was considered important to discover whether any correlation exists between the early breakdown of the blood-retinal barrier that occurs in diabetes and the metabolic deviation, which is kept to

a minimum when the patient is under relatively good metabolic control. Second, such a correlation would have additional interest because, at least initially, the breakdown of the blood-retinal barrier appears to be a functional alteration that is most probably reversible.[5]

Another relevant aspect of this study is the relatively low number of variables examined. Only one alteration of the retina is examined, at a time when it appears to be the only one present.

Our results show that a more marked alteration of the blood-retinal barrier, as evidenced by vitreous fluorophotometry measurements, is present in diabetic patients whose diabetes is under poor control. These values are significantly higher than those in similar groups of diabetic patients whose diabetes is under better control.

A correlation was also observed between vitreous fluorophotometry values and duration of diabetes. The patients who had had diabetes for longer periods of time showed higher vitreous fluorophotometry values than those recorded in patients with diabetes of shorter duration. This difference was particularly significant in patients under relatively good metabolic control.

Results of this study show, therefore, an association between the early breakdown of the blood-retinal barrier, which is present in the earliest stages of retinal alteration in diabetes, and two important aspects of diabetes—departure from adequate metabolic control and duration of the disease.

In a recent report by Waltman et al.,[5] a breakdown of the blood-retinal barrier, similarly evidenced by vitreous fluorophotometry, was the first change to occur in the retinas of rats with streptozotocin-induced diabetes. The degree of breakdown improved immediately after institution of appropriate diabetes treatment.

It appears that our results here and from previous work[2,6] show good support of the view that two kinds of processes take part in the pathogenesis of diabetic retinopathy.[7] First, a generalized disorder of the small blood vessels, at present of an unknown cause, appears to be specific to diabetes and, at least in part, is related to the degree and duration of departure from metabolic normality. Second, a group of local responses specific to the retina and conditioned by its vascular characteristics are, once initiated, largely self-perpetuating or at least little affected by the diabetic state. In the retina, the breakdown of the blood-retinal barrier may act as a link between the two sets of processes.

ACKNOWLEDGMENT

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