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Blood-retinal barrier permeability and its relation to progression of retinopathy in patients with type 2 diabetes. A four-year follow-up study

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Abstract. Forty patients with late-onset diabetes (age at diagnosis 30 years or more) and minimal retinopathy as found by fundus photography were followed prospectively by repeated examination (baseline, 1 year, and 4 years). The study shows that early retinopathy changes are not permanent or invariably progressive. In the 1st year of follow-up microaneurysms worsened in 25%, improved in 10%, and remained stabilized in 65%. Vitreous fluorometry was able to detect an overall increase of $0.84 \pm 1.06 \times 10^{-6}\ min^{-1}$ in blood-retinal barrier (BRB) penetration ratios. After 4 years, 16 of the 40 patients had undergone photocoagulation (focal photocoagulation in 11 and pan retinal photocoagulation in 5). The eyes that needed photocoagulation were the eyes that had higher fluorometry penetration ratios at the patient's entry into the study and showed a higher rate of deterioration during the 1st year of the study (5.54 ± 1.97 vs $3.11 \pm 1.22 \times 10^{-6}\ min^{-1}$, $P < 0.001$, initial values; 1.52 ± 0.76 vs $0.45 \pm 0.99 \times 10^{-6}\ min^{-1}$, $P < 0.001$, annual increase in leakage). The eyes that did not need photocoagulation, 24 out of 40, showed stable fluorometry readings within the 4-year period of follow-up ($+0.02 \pm 0.98\ 10^{-6}\ min^{-1}$). Abnormally high vitreous fluorometry values and their rapid increase over time appear to be good indicators of rapid progression and worsening of the retinopathy.

Introduction

Retinopathy is a frequent microvascular complication of diabetes mellitus. At present, the only proven treatment is photocoagulation [6]. This is, however, a destructive treatment and is not always effective. Future developments in the prevention and treatment of diabetic retinopathy will depend on a better understanding of the evolution of the early changes that occur in the retina, when they may still be reversible.

The initial changes occurring in the diabetic retina are characteristically located in the small retinal vessels of the posterior pole of the retina, in the macular area. These are capillary closure in the arterial side of the capillary net and microaneurysm formation in the venous side [1]. Fluorescein angiography demonstrates particularly well these early changes and the associated alteration of the blood-retinal barrier (BRB). This last alteration may also be quantified, in a reproducible manner, by vitreous fluorometry.

Using fluorescein angiography and vitreous fluorometry we evaluated prospectively a series of patients with adult onset diabetes mellitus and moderate or no retinopathy. The patients were examined at entry into the study, 1 year after the initial examination, and reexamined at the end of 4 years. A need for photocoagulation as determined by preestablished guidelines was considered a primary outcome indicating worsening of the retinopathy.

Materials and methods

Forty patients with adult-onset diabetes, type 2 (age at diagnosis 30 years or more, with retinopathy no greater then level 3 of the modified Airlie House Classification [5] in the macular area) completed the study. Forty-three patients were initially enrolled but three did not complete the 1st year of follow-up and were excluded. Thereafter there were no more losses to follow-up. Excluded from the study were patients with the following conditions: more than level 3 of the modified Airlie House classification of diabetic retinopathy in the macular field; vitreous pathology; cloudy ocular media or refraction greater than 5 diopters; vascular, neurological, or dermatological complications of diabetes, heart disease, or hypertension (diastolic blood pressure greater than 90 mmHg or systolic blood pressure greater than 160 mmHg). The retinopathy exclusion criteria determined that the eyes to be included in the study should have not more microaneurysms and hemorrhages than reference photograph 2 A, no more exudates than in reference photograph 3, and no evidence of IRMAs (Intraretinal microvascular

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abnormalities) or cotton-wool spots [1]. Only one eye per patient was included in the study and this was called the study eye. As stated previously, only patients without clinically significant macular edema and with no signs of neovascularization were enrolled in the study.

Visual acuity testing, slip-lamp biomicroscopy, ophthalmoscopy, fundus photography, fluorescein angiography, and vitreous fluorometry were performed on entry into the study and 1 and 4 years later, within a period of 44–52 months after the initial examination. During this period of 4 years the patients were reexamined at 6-month intervals by slit-lamp biomicroscopy and ophthalmoscopy. Photocoagulation was performed by the attending doctor whenever it was considered to be needed and following preestablished guidelines. The guidelines for photocoagulation were that focal photocoagulation was performed whenever a diagnosis of clinically significant macular edema was made. Clinically significant macular edema was diagnosed when retinal thickening at or within 500 μm of the center of the macula and or hard exudates associated with thickening of the adjacent retina were seen on slip-lamp examination. Panretinal photocoagulation was performed whenever the ophthamological examination showed clear evidence of neovascularization involving the optic disk. This diagnosis was always confirmed by fluorescein angiography.

On the 4-year examination vitreous fluorometry was only performed in patients who had not undergone photocoagulation.

The 40 patients covered an age range of 40–65 years and all had had diabetes for more than 4 years at the start of the study (mean age 53.9 ± 7.3 years, mean diabetes duration 9.9 ± 4.7 years).

The patients here reported were followed closely by the Diabetes Clinic and remained within the limits of acceptably good control. Their data in relation to diabetes duration, glycated hemoglobin, and fasting blood glucose levels are presented in Table 1. Approval for the study was obtained from the Ethics Committee of the Coimbra University Hospital and all patients gave informed consent prior to enrolment.

Retinopathy grading

After maximal dilation of the pupil fundus, photographs were taken of both eyes in fields 1 and 2 as defined by the Diabetic Retinopathy Study [3]. A solution of 20% sodium fluorescein (14 mg per kilogram body weight) was then injected intravenously. The fluorescein angiography was performed according to a set protocol. A series of 20 photographs of field 2 of the study eye were started before the capillary phase of the angiogram. A sequence of four photographs of field 2 were also performed after 50 s. At 1 min, stereophotographs of fields 1 and 2 were taken of both eyes. Late photographs (10 min) were again taken of the study eye.

Microaneurysms were evaluated on fluorescein angiography, according to a semiquantitative grading system. Microaneurysms were classified according to presence and number in the macular area into four categories: absent or questionable; 1: 1–5 microaneurysms; 2: 5–10 microaneurysms; 3: more than 10 microaneurysms. The evaluation was performed by two senior ophthalmologists who did not know to whom the angiograms belonged.

Ocular fluorometry examinations

The measurements were made with a commercial fluorophotometer, the Fluorotron Master TM. Details of the procedure have been previously described [5]. Scans were taken before the administration of 14 mg fluorescein/kg body weight and 60 min after injection. The fluorophotometric data were saved on magnetic diskettes and then processed to correct the influence of the autofluorescence and to provide the numerical values of the amount of fluorescein that had penetrated into the vitreous. This subtraction minimizes the contribution of natural fluorescence, mainly that of the lens. Based on previous experience we estimated the mean of the measure between 2 and 4 mm to obtain posterior vitreous values (Pv_{2-4}). We then proceeded to determine the penetration ratio (VFPR) which reflects the blood-retinal barrier permeability by using the following formula:

$$\text{VFPR} = \frac{Pv_{2-4}(60') - Pv_{2-4}(0')}{\text{Plasma fluorescein integral } (3\text{–}60')}$$

The normal value of the vitreous fluorometry penetration ratio in a series of 22 persons below 60 years is $2.7 \pm 0.8 \times 10^{-6}$ min^{-1} (mean $\pm$ 2SD: 4.3×10^{-6} min^{-1}). This value is apparently not influenced by age or refraction. Reproducibility of vitreous fluorometry is 12% [10].

Plasma fluorescein measurements

The decay of plama fluorescein was expressed as a logarithmic function: log (plasma concentration) $= a + b + \log(t)$, where a and b are constants that can be determined by two or more measurements and t is the time after injection. All the technical procedures have been described in detail elsewhere [3]. We collected blood samples on which we performed measurements at 10, 15, and 50 min.

Evaluation parameters and statistic

The purpose of the study was to compare initial permeability values and the longterm clinical course. As parameter for evaluation of the clinical courses need for photocoagulation was chosen. Data were analyzed using the one-way ANOVA (analyses of variance) and the unpaired Student's t test.

Results

BRB permeability, initial values and characterization of the patient group

Table 1 shows the results of fasting blood glucose and hemoglobin (HFbA1) in relation to duration of diabetes. It shows that the majority of patients enrolled in the study had had known diabetes for up to 10 years. The duration groups show comparable levels of fasting blood glucose and HbA1.

The vitreous fluorometry penetration ratios (VFPR) at entry are presented in Table 2. They show a mean value of $4.08 \pm 1.97 \times 10^{-6}$ min^{-1}. When these are compared with normal values registered in a series of 22 normal subjects with an upper age limit of 60 years ($2.7 \pm 0.8 \times 10^{-6}$ min^{-1}), the difference is statistically significant ($P < 0.001$). There were 17 eyes (43%) that showed a VFPR higher than the normal mean $\pm$2SD.

No linear correlation was observed between the initial penetration ratios and duration of diabetes.

Correlation between BRB permeability and microaneurysm gradings at entry

Classification of the eyes according to microaneurysm grading showed 9 eyes at level 0.13 at level 1.7 at level 2, and 11 at level 3 (Table 3). The VFPR in the 9 eyes graded at level 0 was $3.3 \pm 1.1 \times 10^{-6}$ min^{-1}; in the 13 eyes graded at level 1 it was $3.8 \pm 2.0 \times 10^{-6}$ min^{-1}; in

Table 1. Clinical data of the 40 patients with type 2 diabetes

Duration of diabetes	Number of patients	Age (years) (mean ± SD)	Fasting blood glucose (mmol/l) (mean ± SD)	HbA1 (%) mean ± SD
5–10 years	28	54.2 ± 6.8	10.9 ± 3.3	9.6 ± 1.9
11–15 years	9	54.6 ± 5.1	11.9 ± 2.1	11.4 ± 2.8
16–20 years	1	58	9.6	10
21–30 years	2	44 and 50	13.6 and 5.5	7.2 and 10.3

Table 2. Vitreous fluorometry results (VFPR, 10^{-6} min^{-1})

No.	Age	Sex	Duration (years)	Initial VFPR		VFPR difference		VFPR Difference	
						0–12 months		0–4 years	
1	57	F	11	8.27		+1.74		Photoc.	
2	58	F	20		4.87		−1.87		+1.33
3	43	M	10		2.94		−0.03		+0.44
4	60	M	6	7.72		+1.27		Photoc.	
5	47	F	8	4.01		−0.22		Photoc.	
6	53	M	5		3.40		+0.48		−0.59
7	61	M	10	11.22		+1.30		Photoc.	
8	60	F	14		2.95		+0.75		−0.29
9	57	F	6		4.60		+0.90		−1.22
10	53	M	10	5.10		+2.21		Photoc.	
11	45	M	14		5.22		+0.63		+0.12
12	58	M	10		6.32		+2.00		−1.67
13	48	M	8	4.90		+2.17		Photoc.	
14	65	F	15		3.26		+2.20		+1.00
15	60	M	25		2.32		+0.05		−1.08
16	64	F	10	4.56		+2.04		Photoc.	
17	64	M	22		2.35		+0.65		−0.50
18	60	F	12	5.80		+2.24		Photoc.	
19	42	F	5		3.21		−0.62		−0.97
20	56	F	7	4.63		+1.27		Photoc.	
21	53	F	12		1.86		+1.54		+2.19
22	44	M	5		1.85		+2.05		−0.26
23	59	M	11	2.71		+1.36		Photoc.	
24	53	F	7		2.07		+0.51		+0.35
25	52	M	14		3.53		+0.18		+0.60
26	44	M	5		3.71		−0.69		+0.80
27	60	M	15		1.91		−0.50		+1.05
28	52	F	9	4.72		+2.07		Photoc.	
29	43	F	9	5.73		+2.34		Photoc.	
30	40	F	10		3.12		+1.66		+1.75
31	45	M	4		3.74		+0.49		−0.42
32	55	M	9		1.49		+1.42		−0.79
33	58	F	9	4.93		+0.38		Photoc.	
34	65	F	5		1.45		+0.18		+0.35
35	47	F	5	5.92		+1.83		Photoc.	
36	56	M	10	3.67		+0.12		Photoc.	
37	42	F	5		3.97		+0.36		−0.47
38	61	F	6	4.79		+2.06		Photoc.	
39	61	F	8		2.72		−0.48		+0.80
40	53	M	10		1.78		+0.59		+0.05
Mean	53.9		9.4	5.54	3.11	1.54	0.45		0.02
SD	7.3		1.9	1.97	1.22	1.72	0.99		±0.98

the 7 eyes graded at level 2 it was 5.2 ± 1.4 10^{-6} min^{-1}; in the 11 eyes graded at level 3 it was 4.3 ± 2.6 10^{-6} min^{-1}. The eyes grouped at level 2, showing 5–10 microaneurysms, had a significantly higher level of alteration of the BRB, using analysis of variance, than level 0 or 1 ($P = 0.027$).

Evolution of BRB permeability and angiography gradings in 1 year

One year into the study none of the eyes followed had received photocoagulation. The vitreous fluorescein penetration ratios increased during the initial 12 months of

Table 3. Microaneurysm gradings

No.	Grading		
	Initial	1 year	4 years
1	1	3	Photocoagulation
2	0	2	2
3	0	0	0
4	2	2	Photocoagulation
5	3	3	Photocoagulation
6	0	1	1
7	3	3	Photocoagulation
8	1	1	2
9	0	1	3
10	3	3	Photocoagulation
11	2	2	2
12	1	1	0
13	3	2	Photocoagulation
14	2	1	2
15	1	1	1
16	3	3	Photocoagulation
17	3	2	2
18	2	2	Photocoagulation
19	1	1	1
20	3	3	Photocoagulation
21	1	1	2
22	0	1	2
23	3	3	Photocoagulation
24	3	3	3
25	0	1	0
26	0	0	0
27	1	1	0
28	3	3	Photocoagulation
29	2	3	Photocoagulation
30	1	0	0
31	1	2	3
32	3	3	3
33	2	2	Photocoagulation
34	1	1	1
35	1	2	Photocoagulation
36	1	3	Photocoagulation
37	2	3	3
38	1	1	Photocoagulation
39	0	0	2
40	0	0	0

follow-up as shown in Table 2 ($+0.84 \pm 1.1 \times 10^{-6}$ min^{-1};). Thirty-three eyes (83%) showed an increase in VFPR. After the 1st year of follow-up there were increased numbers of microaneurysms that resulted in a change of grading in only ten eyes (25%). In four patients the angiographic examination showed a decreased number of microaneurysms resulting in a decrease in grading (10%). The remaining 26 eyes (65%) remained stabilized.

BRB permeability and angiography gradings after 4 years

Sixteen of the 40 diabetic patients (40%) with diabetes type 2, who at entry had minimal or no retinopathy changes, had received photocoagulation treatment when reexamined 4 years after the initial examination (Table 2). Of the 16 eyes that underwent photocoagulation, 11 received focal treatment, and the remaining 5 received panretinal photocoagulation.

The 24 eyes that did not need photocoagulation showed that the VFPR values had remained stabilized, showing an increase of only $0.02 \pm 0.98 \times 10^{-6}$ min^{-1}; over the 4-year period.

Examination of the initial fluorophotometric values shows that the eyes that progressed to photocoagulation had clearly higher initial VFPR and more marked alteration of the BRB than the eyes that did not need photocoagulation ($5.5 \pm 2.0 \times 10^{-6}$ min^{-1} in the eyes that were later photocoagulated; $3.1 \pm 1.2 \times 10^{-6}$ min^{-1} in the eyes that didö not need photocoagulation; $P < 0.001$). Furthermore, the eyes that needed photocoagulation within the 4-year period of follow-up showed a more marked increase in vitreous fluorometry values during the 1st year than the eyes that did not need photocoagulation ($+1.54 \pm 0.72 \times 10^{-6}$ min^{-1} in the eyes that were later photocoagulated; $+0.45 \pm 0.99 \times 10^{-6}$ min^{-1} in the eyes that did not need photocoagulation; $P < 0.001$).

The 4-year examination of the 24 patients who did not need photocoagulation showed an advance in microaneurysm grading during this period in only 9 eyes (37%). The grading dropped in 4 eyes (17%) and remained stable in 11(46%).

No correlation could be found between change in gradings in the 1st year and later need for photocoagulation. For example, of the ten eyes in which microaneurysm grading went up during the 1st year, only four later needed photocoagulation, whereas the other six did not. A higher microaneurysm grading at entry was observed more frequently in eyes that later needed photocoagulation, but this finding did not reach statistical significance. Eight of the 16 eyes were graded at level 3, four at level 2, and another four at level 1.

Discussion

The present 4-year follow-up study was undertaken to establish whether there is a correlation between the BRB permeability and the clinical course of retinopathy in type 2 diabetes mellitus; evaluation of the course was defined according to the need for photocoagulation following preestablished guidelines. Only eyes with moderate or no retinopathy were included and the known duration of diabetes was generally between 4 and 10 years (95%). The diabetic patients included in the study had blood pressure within normal limits and were under acceptable good metabolic control.

The study showed that there is a positive correlation between high BRB permeability and a need for photocoagulation, even among patients with the same retinal morphology. A positive correlation was also registered between faster rates of increase in BRB permeability during the 1st year of follow-up and future need for photocoagulation.

At the end of the 4-year period of follow-up, 16 of the 40 study eyes had received photocoagulation, of the focal type in 11 and panretinal in 5. These eyes had higher fluorometric values for BRB permeability at entry

into the study and presented faster rates of deterioration of the BRB during the 1st year of follow-up.

The study did not show any clear correlation between alteration of the BRB and age at entry into the study or duration of diabetes, possibly because of the homogeneity of this group of patients.

Microaneurysm grading on fluorescein angiography was chosen as the representative parameter for changes in retinal morphology, considering that only eyes with a retinopathy of less than level 3 of the modified Airlie House Classification were included in the study. The microaneurysm gradings remained stable in 50% of the study eyes during the 1st year of the study, and thus appear to be less effective than BRB permeability quantification for recording progression of retinal damage in short periods of follow-up.

No clear difference was apparent between BRB permeability in eyes with microaneurysm grading levels 1 and 0. Similarly, only 43% of the eyes included in the study had VFPR that were higher than the mean value plus two standard deviations of a normal population used as control. Our results in this study of type 2 diabetic patients support the view that, although an alteration of the BRB permeability is an early phenomenon occuring in the diabetic retina, it is by no means a general one. This finding contradicts findings by ourselves [2] and confirms studies by other investigators [9].

The overlapping observed between the VFPR in the four groups of microaneurysm gradings suggests a certain degree of discrepancy between BRB permeability and retinal morphology, at least in the initial stages of diabetic retinopathy. It must be realized, however, that vitreous fluorometry values represent predominantly the fluorescein leakage occurring in the macular region [4], and that may also be modified by the presence of various degrees of capillary closure.

"Need for focal photocoagulation " is here synonomous with "occurrence of clinically significant macular edema" and "need for panretinal photocoagulation" with "disk neovascularization". The rate of occurrence of these events in this group of 40 patients with type 2 diabetes is high compared with previous epidemiological studies [8]. It also suggests that progression to clinically significant macular edema is much faster in type 2 than in type 1 diabetes [7]. It must not be forgotten, however, that duration of diabetes in type 2 diabetes is generally underestimated. Age may also play a role in the faster deterioration of the retinal vasculature and BRB.

Most studies aimed at the characterization of BRB permeability in the earlier stages of retinopathy have been performed in patients with type 1 diabetes. This study is the first to report a prospective follow-up of the BRB alteration in type 2 diabetes. It confirms generally the findings reported in an 8-year follow-up study of insulin-dependent (type 1) diabetic patients [7]. Our study suggests, however, that in type 2 diabetes there is a more rapid deterioration of the BRB, particularly in a specific group of patients whose eyes therefore appear to be at risk for retinopathy progression. This finding, if confirmed, is of major importance. It suggests that a high BRB permeability is of clinical significance for the development of the retinopathy.

Our study indicates that BRB permeability measured by vitreous fluorometry could be used to evaluate the degree of retinal damage, especially with respect to the need for later photocoagulation treatment. The eyes of patients with type 2 diabetes who present higher VFPR and faster rates of deterioration of the BRB should be followed more closely in order to be treated appropriately and at the best moment in time. Eyes with low and stable vitreous fluorometry readings need less frequent examinations, as they appear to have a much better prognosis for retaining good vision.

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