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CLINICAL INVESTIGATION

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Progression of retinopathy and alteration of the blood-retinal barrier in patients with type 2 diabetes: a 7-year prospective follow-up study

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Abstract ● Background: The study was carried out to evaluate the correlation between blood-retinal barrier (BRB) permeability and the progression of diabetic retinopathy (DR), defined by development of "need for photocoagulation", over a 7-year period by means of vitreous fluorometry (VF). ● Methods: Forty type 2 diabetic patients with minimal or no retinopathy, aged 40–65 years (mean 53.9 + 7.3 years), were followed up prospectively for 7 years. Investigations including standard ophthalmological examination, fundus photography, fluorescein angiography and VF were performed at entry and 1, 4, 5 and 7 years later. Only one eye per patient was included in the study. Need for photocoagulation was based on Early Treatment Diabetic Retinopathy Study protocols and decided by the attending ophthalmologist. ● Results: After 7 years of follow-up a total of 22 of the 40 eyes had received photocoagulation. The eyes that needed photocoagulation were those that had higher VF values at the entry of the study and showed higher rates of deterioration (initial values 5.1 + 1.9 vs 2.8 + 1.5×10^{-6} min^{-1}, $P<0.001$; annual increase in leakage for the first year, 1.5 + 0.8 vs 0.5 + 1.0×10^{-6} min^{-1} , $P<0.001$,). The eyes that did not need photocoagulation during the 7 years of follow-up showed stable VF readings (-0.1 + 1.2×10^{-6} min^{-1}, difference between initial values and 7 years later). ● Conclusions: Abnormally high VF values and their rapid increase over time are good indicators of progression and worsening of the retinopathy in diabetes type 2.

Introduction

Retinopathy is the most frequent microvascular complication of diabetes mellitus. At present, the only proven treatment is photocoagulation, which is performed when the disease process is already irreversible [4]. Photocoagulation is recommended for two major events associated with loss of vision and worsening of the retinopathy, namely growth of new vessels or development of clinically significant macular edema. The latter, macular edema, is particularly frequent in diabetes type 2. For prevention and improved treatment of diabetic retinopathy (DR) it is fundamental to know the evolution of the earliest changes that occur in the patient with diabetes and how they are related to progression of the retinopathy.

One of the earliest changes occurring in the diabetic retina is an alteration of the blond-retinal barrier. This is demonstrated well by fluorescein angiography but requires vitreous fluorometry to be quantified in a reproducible manner.

Using fluorescein angiography and vitreous fluorometry, we evaluated prospectively a series of patients with adult-onset diabetes mellitus with moderate or no retinopathy. The patients were examined at entry into the study and at 1, 4, 5 and 7 years after the initial examination. A need for photocoagulation, as determined by pre-established guidelines, was considered a primary outcome indi-

cating worsening of the retinopathy. The results of the first 4 years of follow-up have been published [2]. We have now extended the follow-up of the patients to a total of seven 7 years, and the final results are now reported.

Material and Methods

Forty patients with adult-onset diabetes, type 2 (age at diagnosis 30 years or more) with retinopathy no greater than level 3 of the modified Airlie House classification [3] in the macular area, were followed up for the period of the study. Forty-three patients were initially enrolled but three did not complete the first year of follow-up. Thereafter there were no more losses to follow-up. Only one eye per patient was included in the study. All the results reported here are from the same eye for each patient.

Ophthalmological examination, fundus photography, fluorescein angiography and vitreous fluorometry were performed on entry into the study and 1, 4, 5 and 7 years later. Photocoagulation was performed by the attending ophthalmologist whenever it was considered needed and following Early Treatment Diabetic Retimopathy Study guidelines [2]. Vitreous fluorometry was performed only in patients who had not undergone photocoagulation.

The 40 patients covered an age range of 40–65 years (mean 53.9 ± 7.3 years), and all had had diabetes for more than 4 years at the start of the study (mean 9.9 ± 4.7 years). The patients were followed up in the diabetes clinic and remained within the limits of acceptable good control. The glycemic control at entry was fasting blood glucose 10.89 ± 4.39 mmol/l and HbA (%) 9.97 ± 3.74, (mean ± SD). Approval for the study was obtained from the ethics committee of Coimbra University Hospital, and all patients gave informed consent prior to enrollment.

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 kg body weight) was then injected intravenously. The fluorescein angiography was performed according to a set protocol [2]. Microaneurysms were graded semi-quantitatively into four categories: 0, absent or questionable; 1, one to five microaneurysms; 2, five to ten microaneurysms; 3, more than ten microaneurysms.

Ocular fluorometry

The measurements were made with a commercial fluorophotometer, the Fluorotron Master (Ocumetrics, alif: USA). Scans were taken before the administration of fluorescein and 60 min after injection. Details of the procedure and determination of the vitreous fluorometry penetration ratio (VFPR), which reflects blood-retinal barrier permeability, are described in the previous 4-year report of this study [2]. The normal value of the vitreous VFPR in a series of 22 persons below 60 years is $2.7 \pm 0.8 \times 10^{-6}$ min^{-1} (mean + 2SD, 4.3×10^{-6} min^{-1} , upper value of normal range with 95% degree of confidence) [7].

Evaluation parameters and statistics

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

Results

All but three patients enrolled in the study had had known diabetes for between 5 and 10 years. Only three had had diabetes for more than 10 years. The microaneurysm gradings at entry into the study were well distributed within the four categories: 9 eyes with retinopathy grade 0, 13 with retinopathy grade 1, 7 with retinopathy grade 2 and 11 with retinopathy grade 3 (table 1).

The VFPRs are presented in Table 1. At entry the mean value was $4.1 \pm 2.0 \times 10^{-6}$ min^{-1}. The difference from normal is statistically significant ($P<0.001$). Seventeen eyes (43%) had a VFPR higher than 4.3×10^{-6} min^{-1} (upper value of normal with 95% degree of confidence).

Of the 40 eyes in the study, 16 were photocoagulated during the period between 1 and 4 years after the initial examination, another 2 received photocoagulation after the 4th year and before the 5th year examination, and a further 4 needed photocoagulation before the 7th year follow-up examination. In total, 22 of the 40 eyes (55%), enrolled were photocoagulated during the 7th year follow-up period.

BRB permeability and development of need for photocoagulation

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 ($5.1 \pm 1.9 \times 10^{-6}$ min^{-1} vs. $2.8 \pm 1.5 \times 10^{-6}$ min^{-1}; respectively; $P<0.001$) (Fig. 1).

Furthermore, the eyes that needed photocoagulation showed a clear increase in VFPR in the one 1-period of follow-up immediately before the development of need for photocoagulation ($P<0.001$). For the 16 eyes that were photocoagulated before the 4-year examination, the increase in VFPR during the first year of follow-up was $1.5 \pm 0.8 \times 10^{-6}$min^{-1} ($P<0.0001$) and for the four eyes that were photocoagulated before the 7-year examination the increase in VFPR during the year between the 4 and 5-years examination was $2.8 \pm 1.5 \times 10^{-6}$ min^{-1} ($P<0.06$). This is in clear contrast ($P<0.001$) with the differences in VFPR values in the same periods for the eyes that did not need photocoagulation, which remained remarkably stable (respectively, $0.4 \pm 1.0 \times 10^{-6}$ min^{-1} and $0.1 \pm 1.2 \times 10^{-6}$ min^{-1}).

The 18 eyes that did not need photocoagulation during the entire 7-year period of follow-up, showed stable VFPR values ($-0.1 + 1.2 \times 10^{-6}$ min^{-1} , $P<0.8$).

When using for comparison the value of 4.3×10^{-6} min^{-1}, corresponding to the normal mean value plus two standard deviations, it was observed that of the 22 eyes that developed need for photocoagulation, 19 had higher VFPR values in the last vitreous fluorometry examination

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

Patient	Duration (years)	Retinopathy grading	Baseline	One year	Four years	Five years	Seven years
1	11	1	8.27	10.01	P[a]	–	–
2	20	0	4.87	3.00	5.70	2.00	2.32
3	10	0	2.94	2.91	3.38	2.41	2.10
4	6	2	7.72	8.99	P	–	–
5	8	3	4.01	3.79	P	–	–
6	5	0	3.40	3.88	2.81	5.00	4.02
7	10	3	11.22	12.60	P	–	–
8	14	1	2.95	3.70	2.66	2.46	2.12
9	6	0	4.60	5.50	3.38	5.93	P
10	10	3	5.10	7.31	P[a]	–	–
11	14	2	5.22	5.85	5.10	7.36	P
12	10	1	6.32	8.32	4.65	3.67	4.30
13	8	3	4.90	7.07	P[a]	–	–
14	25	2	2.32	2.37	1.24	2.49	2.31
15	10	1	4.56	6.60	P	–	–
16	22	3	2.35	3.00	2.85	2.84	2.16
17	12	3	5.80	8.04	P[a]	–	–
18	5	2	3.21	2.59	2.24	2.24	1.86
19	7	1	4.63	5.90	P	–	–
20	12	3	1.86	3.40	4.05	3.05	4.20
21	5	1	1.85	3.90	1.59	2.06	3.00
22	11	0	2.71	4.07	P[a]	–	–
23	7	3	2.07	2.58	1.81	1.30	2.51
24	14	3	3.53	3.71	4.13	2.02	2.99
25	5	0	3.71	3.02	2.91	7.82	P
26	10	0	3.12	4.78	4.87	P	–
27	15	1	3.26	5.46	4.26	5.79	P
28	9	3	1.49	2.91	0.70	1.79	0.97
29	9	2	4.93	5.31	P	–	–
30	5	1	5.92	7.75	P	–	–
31	4	1	3.74	4.23	3.32	P	–
32	5	3	3.97	4.33	3.50	2.57	3.75
33	10	2	1.78	2.37	1.83	1.83	1.38
34	9	1	4.72	6.79	P	–	–
35	9	1	5.73	8.07	P	–	–
36	5	1	1.45	1.63	1.80	1.47	2.27
37	15	2	1.91	1.41	2.96	1.37	3.46
38	6	1	4.79	6.15	P	–	–
39	10	0	3.67	3.79	P	–	–
40	8	0	2.72	2.24	3.52	2.70	4.15

[a] Eyes that received both focal and panretinal photocoagulation

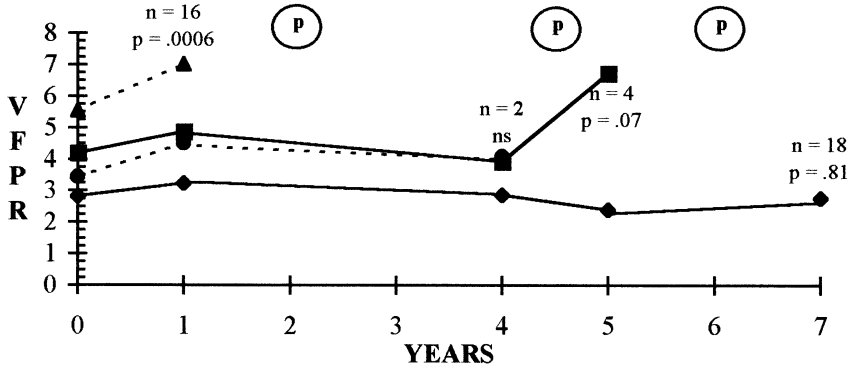

Fig. 1 VFPR (10^{-6} min^{-1}) evaluation over the 7-year period (—◆— eyes that did not need photocoagulation over the whole period, - - ▲ - - eyes that needed photocoagulation between 1st- and 4th-year examination, - - ● - - eyes that needed photocoagulation between 4th- and 5th-year examination, —■— eyes that needed photocoagulation between 5th- and 7th-year examination)

Table 2 Retinopathy grading vs photocoagulation

Initial retinopathy grading	No. of eyes	No photocoagulation	Photocoagulation
0	9	4	5 (= 55 %)
1	13	4	9 (= 69 %)
2	7	4	3 (= 43 %)
3	11	6	5 (= 45 %)

before photocoagulation (86%), whereas only three eyes (14%) presented lower VFPR values in the last examination before need for photocoagulation was established ($P<0.0001$).

Retinopathy grading and need for photocoagulation

Classification of the eyes according to microaneurysm count showed initially 9 eyes with level 0, 13 at level 1, 7 at level 2 and 11 at level 3. This type of retinopathy grading did not show any correlation with the development of need for photocoagulation ($P>0.35$), the primary end-point of the study (table 2).

Discussion

The present prospective study was undertaken to establish whether there is a correlation between BRB permeability and the clinical course of retinopathy in type 2 diabetes mellitus. Need for photocoagulation following pre-established guidelines according to the ETDRS protocol is the best available indicator for progression and worsening of the retinopathy. Establishment of "need for photocoagulation" is the result of the development in the diabetic retina of clinically significant macular edema or proliferative retinopathy and was, therefore, considered the primary end-point of the study. Only eyes with moderate or no retinopathy were included. The diabetic patients in the study had blood pressure within normal limits and were under acceptable metabolic control.

A first report published after 4 years of follow-up showed a positive correlation ($r^2 = 0.11$, $P=0.02$) between high BRB permeability and development of need for photocoagulation, even among patients with the same retinal morphology [2]. A positive correlation was also registered between fast rates of increase in BRB permeability during the first year of follow-up and future need for photocoagulation ($r^2 = 0.13$, $P=0.001$).

By the end of 4 years 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 deterioration of the BRB during the first. year of follow-up. Microaneurysm counts remained stable during the same period, showing that a morphological classification was less effective than BRB permeability quantification for recording progression of retinal damage in short periods of follow-up.

We have now extended the follow-up of the same study population to a total of 7 years, performing examinations 5 and 7 years after the beginning of the study. The results obtained confirm previous findings and further strengthen the observation that high BRB permeability values are highly correlated with development of need for photocoagulation, i.e., progression and worsening of diabetic retinopathy. The 16 eyes that had been photocoagulated at the 4 year examination showed faster rates of increase in BRB permeability during the first year of follow-up. The same finding was clearly repeated in the four eyes that needed photocoagulation before the 7- year follow-up examination. These eyes had statistically significant faster rates of increase in BRB permeability during year of follow-up, between the 4- and 5-year examinations. Compared with the eyes that never developed need for photocoagulation and in which the vitreous fluorometry values remained remarkably stable, it may be stated that in patients with diabetes type 2, high fluorometry values and/or a rapid increase in vitreous fluorometry values over periods of 1 year are good predictors for progression of the retinopathy and development of need for photocoagulation.

Furthermore, in this 7-year follow-up study vitreous fluorometry values appear to be clearly better predictors of progression of retinopathy than fluorescein angiography grading or duration of the disease.

Microaneurysm counts for grading of the retinopathy at entry into the study did not show any specific correlation with future development of need for photocoagulation (Table 2).

It is interesting to verify that, although the rate of development of need for photocoagulation, particularly in association with the occurrence of clinically significant macular edema, is faster in diabetes type 2 than in diabetes type 1 [6], a consideratle proportion of patients (45%) showed remarkably stable vitreous fluorometry values during the 7 year period of follow-up. In a few cases (nos. 12 and 28) there were important decreases in VFRV values during the course of the follow-up. No clear explanation could be found for this finding, probably due to the limited member of cases showing such behaviour.

Our study confirms in diabetes type 2 the observations made by other authors in diabetes type 1 showing, in the earlier stages of the retinopathy, a correlation between higher BRB permeability and retinopathy progression [1, 5].

The present study, using need for photocoagulation as the primary end-point, suggests that high BRB permeability is of clinical significance by virtue of identifying the eyes at risk for retinopathy progression. The results indicate that eyes of patients with type 2 diabetes who present higher VFPR and faster rates of deterioration of the BRB are at risk and should be followed up more closely in order to ensure appropriate treatment and at the best time. New forms of prevention and treatment of diabetic retinopathy should be tested primarily in this group of eyes.

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