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ORIGINAL ARTICLES

Retinal microcirculation in patients with diabetes mellitus: dynamic and morphological analysis of perifoveal capillary network

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Abstract

The new scanning laser technique allows one to quantify the retinal microcirculation. A digital image analysing system was used to study capillary blood flow velocities and morphological parameters of perifoveal intercapillary areas and foveal avascular zones in normal and diabetic subjects. Diabetic patients showed a significant reduction in capillary blood cell velocities in comparison with normal subjects. Perifoveal intercapillary areas and foveal avascular zones were significantly increased in all stages of diabetic retinopathy, and both parameters increased with progressing diabetic retinopathy. Significant changes in the perifoveal intercapillary areas were observed between normal subjects and patients with no retinopathy.

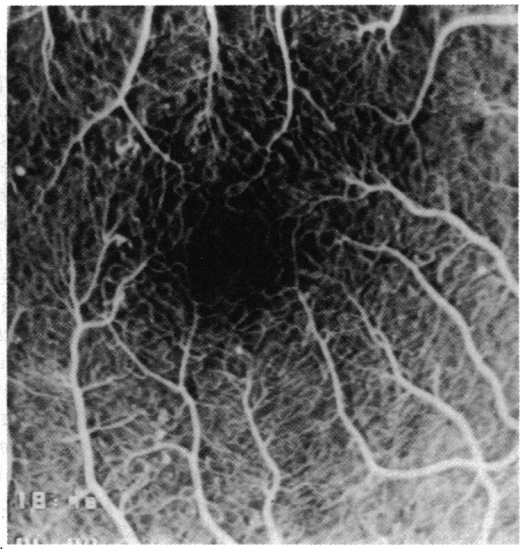

Figure 1 Digital fluorescein angiogram of the perifoveal network of a 28-year-old diabetic man with mild background retinopathy. Duration of diabetes mellitus was 10 years.

The combined vascular and haemodynamic pattern in diabetic patients is characteristic, though no single feature in the vascular bed has any absolute relation to the diabetic condition. The earliest detectable morphological changes in diabetic retinopathy are microaneurysm and capillary closure.[1] Development of diabetic retinopathy is, at least in part, due to progressive capillary occlusion and decreasing capillary perfusion.

The scanning laser technique[2,3] in combination with an image analysing system was used to assess the morphological and haemodynamic changes in diabetic retinopathy. Quantitative measurements of flow velocities in perifoveal capillaries[4] and morphological data of the perifoveal capillary network were obtained by this technique. To investigate whether retinal blood flow velocities and morphological parameters change in more severe retinopathy we determined capillary blood flow velocities (v), foveal avascular zones (FAZ), and perifoveal intercapillary areas (PIA). Forty eight patients with diabetes mellitus were included in this study.

Materials and methods

SUBJECTS

In 48 diabetic patients (23 male and 25 female, age 19 to 67 years) 48 eyes were examined. There were 25 insulin dependent and 23 non-insulin dependent patients. Eighteen patients had a history of systemic hypertension. The duration of diabetes was from 1 to 34 years. The eyes studied had a visual acuity of 6/12 or better, minimal or no refractive error (between +1·0 and −1·0 D), and intraocular pressure of less than 20 mm Hg. Different stages of diabetic retinopathy were found and defined by means of ophthalmoscopy, fundus photography, and angiography. Four retinopathy levels were used: (1) no retinopathy (NDR); (2) mild to moderate non-proliferative retinopathy (microaneurysms and dot haemorrhages only) (BDR); (3) preproliferative retinopathy (multiple cotton-wool spots, intraretinal microvascular abnormalities, venous beading, or areas of non-perfusion) (PPDR; and (4) proliferative retinopathy (PDR). Patients with ischaemic diabetic maculopathy were excluded from this study. Glucose metabolism was assessed by the blood level of haemoglobin Alc (glycosylated haemoglobin) (normal range: 4·3 – 6·0%) in all subjects.[5]

The characteristics of apparently healthy subjects[6] and diabetic patients are summarised in Table 1. The controls had no history of serious ocular or systemic disease. No significant differences between apparently healthy and

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Table 1 Details of patients

	No.	Sex M/F	Age (years)	BP syst. (mm Hg)	BP diast. (mm Hg)	Duration of diabetes (years)	HbA1c (%)
Healthy volunteers	21	10/11	26 (4)	126 (12)	79 (11)	–	–
Diabetic patients	48	23/25	42 (14)	143 (23)	86 (14)	14 (8)	8·1 (1·3)
			NS	NS	NS		
NDR	7	2/5	39 (18)	144 (26)	98 (21)	4 (3·2)	6·9 (0·8)
BDR	17	10/7	43 (13)	136 (19)	82 (13)	13 (9)	8·6 (0·9)
PPDR	10	4/6	46 (15)	145 (24)	86 (14)	15 (8)	7·9 (1·9)
PDR	14	7/7	40 (12)	145 (25)	83 (8)	18 (6)	8·2 (1·3)
Significance			NS	NS	NS	p<0·01	NS

NDR: no retinopathy. BDR: mild to moderate non-proliferative retinopathy. PPDR: preproliferative retinopathy. PDR: proliferative retinopathy.
Standard deviations in parentheses.

diabetic subjects for the demographic data were observed. Between the four diabetic retinopathy stages only the duration of diabetes showed significant differences.

Ophthalmological examination included best-corrected visual acuity, slit-lamp biomicroscopy, Goldmann applanation tonometry, indirect and direct funduscopy, and colour fundus photography. In all subjects video fluorescein angiography was performed by means of the scanning laser ophthalmoscope.

METHODS

The measuring technique used is presented in detail elsewhere.[4 6] In the digital video fluorescein angiograms segments of low and high fluorescence can be observed moving through the perifoveal network. The segments of low fluorescence may correspond to erythrocytes (rouleaux formations), the high fluorescence segments to cell-free plasma. The sequences were processed off-line to evaluate the following parameters[6]:

(1) (*a*) capillary blood flow velocity (v); (*b*) coefficient of variation of capillary blood flow velocity (homogeneity index): CV (v); (2) foveal avascular zone (FAZ); (3) perifoveal intercapillary area (PIA).

The measurement of the capillary blood flow velocity in the perifoveal network is calculated off-line by frame to frame analysis. The measurement of flow velocity is based on the determination of transit time Δt between two measuring points, separated by a known distance Δs. The velocity of the moving hypofluorescent segments was calculated as $v = \Delta s/\Delta t$ by image analysis. At least 15 vessels of each patient were measured for evaluating v. The velocities of 10 segments of low fluorescence were calculated in each vessel in order to decrease the influence of vasomotion, even if the flow appeared to be not pulsatile. Therefore, every value of the mean blood flow velocity (v) was the result of 150 single measurements. These 150 measurements were performed within in a time period of about 5 seconds in the arterial phase of the angiograms. In addition, the coefficent of variation for the blood flow velocity, CV(v), was calculated from these measurements. This parameter characterised the homogeneity of the perifoveal capillary blood flow velocities of each patient.

The area of the foveal avascular zone and the perifoveal intercapillary area in the perimacular network were calculated by image analysis. For the quantification of these morphological parameters five consecutive images were superimposed by the image analysing system. No segments of low fluorescence were visible in these images. The foveal avascular zone was determined by drawing round the foveal arcade with the cursor in the digital image. The area described by the cursor was calculated with the picture analysing system. After the FAZ was quantified, the perifoveal intercapillary areas were marked interactively by the same method. Within a circle of 5° round the fovea the area of 60 different intercapillary areas were measured in each angiogram. For each patient the mean

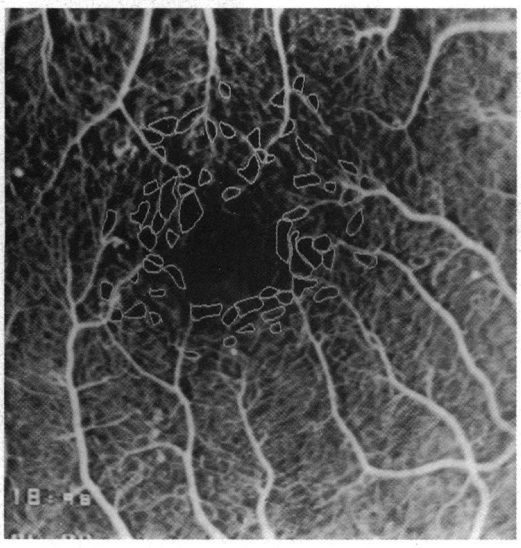

Figure 2 Perifoveal network of a patient with mild background retinopathy and interactively marked perifoveal intercapillary areas.

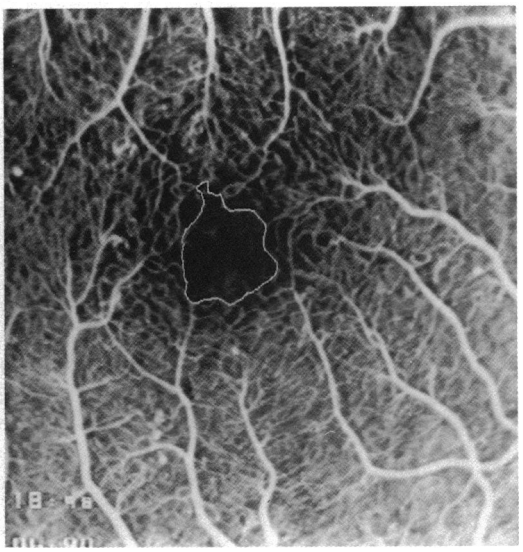

Figure 3 Perifoveal network of a patient with mild background retinopathy and interactively marked foveal avascular zone.

Table 2 Retinal capillary blood flow velocities (v) and homogeneity index of apparently healthy subjects[6] and diabetic patients

	No.	Blood flow velocity (mm/s)	Homogeneity index (%)
Healthy volunteers	21	3·28 (0·45)	22 (5)
Diabetic patients	48	2·43 (0·51)	22 (10)
Significance		p<0·01	NS

SD in parentheses.

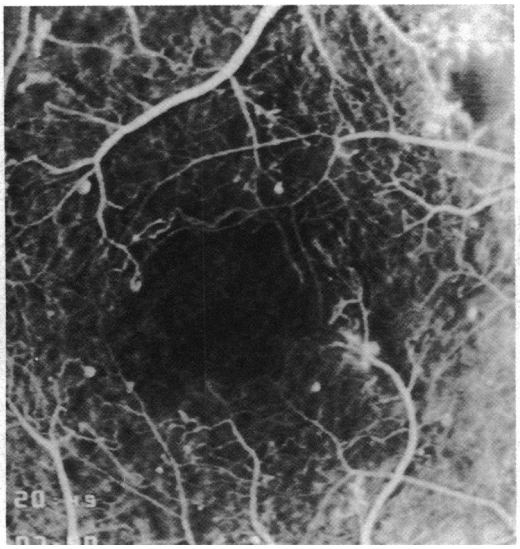

Figure 4 Perifoveal network and foveal avascular zone of a 37-year-old diabetic man with preproliferative retinopathy. He had been suffering from diabetes mellitus for 22 years.

area of perifoveal intercapillary areas was calculated from these 60 measurements.

In Figs 1-6 pictures of video fluorescein angiograms of two diabetic patients are shown. In these pictures the interactively marked parameters of perifoveal intercapillary areas (Figs 2, 5) and the foveal avascular zone (Figs 3, 6) are presented.

Table 3 Perifoveal intercapillary areas (PIA) and foveal avascular zones (FAZ) of normal subjects and diabetic patients

	No.	PIA (μm^2)	FAZ (mm^2)
Healthy volunteers	21	3900 (381)	0·231 (0·06)
Diabetic patients	48	8275 (2769)	0·424 (0·24)
Significance		p<0·01	p<0·05

SD in parentheses.

Table 4 Capillary blood flow velocity (v) and homogeneity index of diabetic patients divided in subgroups according to the retinopathy level

	No.	Blood flow velocity (mm/s)	Homogeneity index (%)
Healthy volunteers	21	3·28 (0·45)	22 (5)
NDR	7	2·51 (0·88)	25 (8)
BDR	17	2·42 (0·40)	24 (13)
PPDR	10	2·28 (0·16)	19 (7)
PDR	14	2·27 (0·58)	17 (5)
Significance		NS	NS

NDR: no retinopathy. BDR: mild to moderate non-proliferative retinopathy. PPDR: preproliferative retinopathy. PDR: proliferative retinopathy.
SD in parentheses.

Table 5 Perifoveal intercapillary areas (PIA) and foveal avascular zones (FAZ) and stage of diabetic retinopathy

	No.	PIA (μm^2)	FAZ (mm^2)
Healthy volunteers	21	3900 (381)	0·231 (0·06)
NDR	7	6092 (0176)	0·276 (0·08)
BDR	17	7119 (1415)	0·318 (0·18)
PPDR	10	8106 (1782)	0·513 (0·29)
PDR	14	10990 (3219)	0·590 (0·31)
Significance		p<0·01	p<0·05

NDR: no retinopathy. BDR: mild to moderate non-proliferative retinopathy. PPDR: preproliferative retinopathy. PDR: proliferative retinopathy.
SD in parentheses.

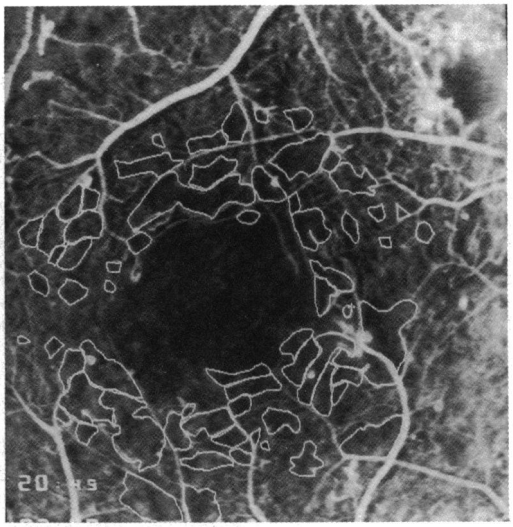

Figure 5 Interactively marked perifoveal intercapillary areas. These zones show an enlargement compared with those of the patient with mild retinopathy (Fig 2).

STATISTICS

The mean value and standard deviation are given for all samples. To assess the significance of the results non-parametric tests were used. For multiple group test statistics the Kruskal-Wallis test with following sequential rejective multiple test procedure[7] was used. Findings with an error probability value less than 0·05 were considered to be statistically significant.

Results

The clinical and demographic data of the apparently healthy and diabetic subjects are presented in Table 1. The microcirculatory parameters (Tables 2 and 3) in diabetics were compared with reference data of controls published elsewhere.[6] The capillary blood flow velocity (v) in the retinal capillaries of diabetics was significantly (p<0·01; U test) reduced compared with normal subjects.

The coefficient of variation of the capillary blood flow velocity (homogeneity index) of the capillary blood flow velocities showed no signifi-

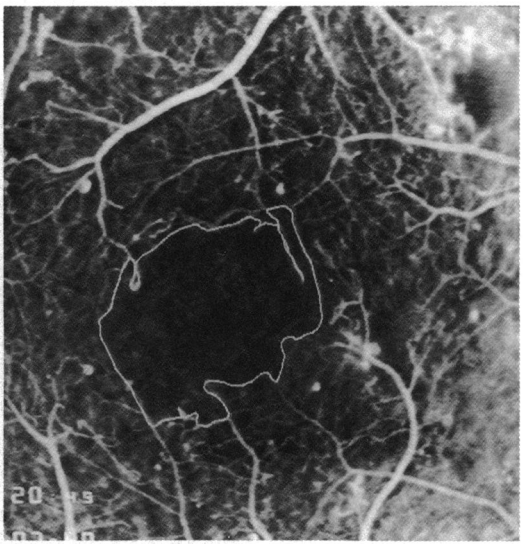

Figure 6 Interactively marked foveal avascular zone. This is larger than in the patient with mild retinopathy (Fig 3). The normal arcade round the fovea is destroyed.

Table 6 Statistical significant differences of perifoveal intercapillary areas (PIA) and foveal avascular zones (FAZ) between the four stages of retinopathy and the healthy subjects

PIA						*FAZ*					
	HS	*NDR*	*BDR*	*PPDR*	*PDR*		*HS*	*NDR*	*BDR*	*PPDR*	*PDR*
HS	-	0·01	0·01	0·01	0·01	HS	-	NS	0·05	0·05	0·01
NDR		-	NS	NS	0·01	NDR		-	NS	NS	NS
BDR			-	NS	0·05	BDR			-	NS	0·05
PPDR				-	NS	PPDR				-	NS
PDR					-	PDR					-

HS: healthy subjects. NDR: no retinopathy. BDR: mild to moderate non-proliferative retinopathy. PPDR: preproliferative retinopathy. PDR: proliferative retinopathy. NS: not significant.

cant differences between diabetic and normal subjects (Table 2). The mean area of perifoveal intercapillary areas was more than doubled and the foveal avascular zone was significantly enlarged in the diabetic patients as compared with healthy subjects (Table 3).

Table 4 shows the mean capillary blood flow velocity in perifoveal capillaries of the eyes studied, graded by retinopathy level. There was a slight but not significant decrease in the capillary blood flow velocity with more severe retinopathy level. All subgroups showed a significant ($p<0·05$) reduction of flow velocities as compared with the healthy volunteers. No significant differences of the homogeneity index were observed.

Table 5 shows the morphological data for the four subgroups of diabetic patients. The perifoveal intercapillary areas and the foveal avascular zones were significantly different in the four groups. Both parameters enlarged according to the retinopathy level. The mean area of perifoveal intercapillary areas was significantly (two-group statistics) enlarged in all subgroups as compared with the healthy volunteers. Only patients with any diabetic retinopathy (BDR; PPDR; PDR) showed a significant (two group statistics) enlargement of the foveal avascular zone as compared with the healthy subjects.

Table 6 shows the significant levels between the subgroups of diabetic patients and the healthy subjects.

Discussion

Different techniques have been used to evaluate retinal blood flow in diabetes mellitus.[8-14] The results of this study showed a significant decrease in retinal capillary blood flow velocity in patients with diabetes mellitus compared with normal subjects. This confirms the findings of previous studies,[10 11 15 16] which showed increased arteriovenous passage times in video fluorescein angiograms as a result of a decrease of flow velocities.

The capillary blood flow velocity in perifoveal capillaries showed a slight but not significant decrease at more severe retinopathy levels. Grunwald *et al* found reduced blood flow velocities in eyes with no, mild, or proliferative retinopathy by means of laser Doppler velocimetry.[12] Bertram *et al* showed a significantly decreased mean dye velocity and increased arteriovenous passage times with more severe diabetic retinopathy.[10] Other investigators presented different tendencies of blood flow velocities in more severe diabetic retinopathy. They found a decrease or increase in blood flow velocity, but most can confirm a decrease in blood flow velocities in proliferative retinopathy.[9 14 17-21]

The reduction in capillary blood flow velocity in patients with diabetes mellitus may be due to morphological changes of the vascular bed[22] in combination with the missing capability of vascular autoregulation[23 24] and the decrease of blood fluidity.[25] In particular the influence of plasma viscosity seems to be important for the microcirculation in diabetes mellitus.[26] A reduction in capillary blood flow velocities in diabetes mellitus, too, was observed in the conjunctiva bulbi[16] and the nailfold.[27]

The blood flow velocity in perifoveal capillaries is much higher than in cutaneous[28-30] or conjunctival capillaries.[31 32] This may be due to the high metabolic rate of the retinal tissue. The coefficient of variation of capillary blood flow velocity showed no significant differences between the healthy subjects and the patients with diabetes mellitus and with more severe retinopathy level. This indicates that there is no increase of inhomogeneity in blood flow velocities in patients with diabetic retinopathy and with more severe stages of retinopathy. The reduction of capillary blood flow velocity in diabetics and an unchanged homogeneity index may indicate chronic disturbances of the entire microcirculation in the retinal tissue.

The intercapillary areas are of great importance for the metabolic supply of the retinal tissue. Increasing values of capillary free zones result in increased O_2 diffusion time and may cause chronic hypoxia. Lübbers[33] discussed a reduction of oxygen supply in diabetics which is caused by conditions such as reduced red blood cell velocity, narrowing of capillary vessel diameters, and decrease in density of capillary vessels, which is identical with prolonged O_2 diffusion length.

In this study patients with diabetes mellitus showed a significant extension of intercapillary areas compared with healthy volunteers; even in patients with no retinopathy an enlargement of the intercapillary areas by about 60% was found. With increasing severity of diabetic retinopathy the intercapillary areas nearly doubled. That the perifoveal capillaries are actually occluded is supported by correlative histopathological studies, in which the capillaries in the non-perfused zone appear as acellular strands.[34 35] Angiographically Sleightholm *et al*[36] and Bresnick *et al*[37] described vascular closure in perifoveal capillaries and increased perifoveal non-perfusion areas in more severe stages of diabetes mellitus.

In agreement with other authors[37-39] we found a significant increase in the mean areas of foveal avascular zones in diabetics as compared with normal subjects. The foveal avascular zones in diabetic patients differ by 40% compared with healthy subjects. With more severe changes in diabetic retinopathy the values rise about 115%. In agreement with other investigators[37 40] we found a significant enlargement of FAZ in progressive diabetic retinopathy.

In conclusion, we found a reduction in capillary blood flow velocities in diabetic patients. In particular, the reduction of flow velocities in patients with no retinopathy indicates that

changes of the retinal blood flow are prior to more severe morphological changes. With more severe retinopathy only a slight reduction in capillary blood flow velocities was observed. The quality of glycaemic control (glycosylated haemoglobin: HbAlc) showed no significant differences between the stages of retinopathy evaluated in this study (Table 1).

Histological studies showed that microaneurysm formation, loss of intramural pericytes, and acellular (non-perfused) capillaries are the earliest detectable morphological changes.[34 35] The sequence of these abnormalities is not clear. In this study the perifoveal intercapillary areas showed a marked increase in diabetic patients, even in those with no retinopathy. It can be concluded that capillary closure appears to precede microaneurysm formation in diabetic patients. Depending on the stage of diabetic retinopathy, the perifoveal intercapillary areas and the foveal avascular zones increase significantly. The reduction of capillary blood flow velocities and the increased diffusion times may lead to chronic hypoxia of the retinal tissue. This chronic hypoxia is suspected to be the cause of capillary leakage and neovascularisation.

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