Alterations of the Blood-Retinal Barrier and Retinal Thickness in Preclinical Retinopathy in Subjects With Type 2 Diabetes

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Objective: To identify alterations of the blood-retinal barrier by mapping retinal fluorescein leakage into the vitreous and changes in retinal thickness occurring in the macular region in preclinical diabetic retinopathy.

Methods: Ten eyes from 10 patients with type 2 diabetes and no lesions visible on fundus photography (level 10 of Wisconsin grading) were examined with the retinal leakage analyzer (RLA) (Confocal Scanning Laser Ophthalmoscope [modified]; Carl Zeiss Inc, Thornwood, NY) and the retinal thickness analyzer (RTA) (Talia Technology, Mevaseret Zion, Israel). The maps of retinal leakage and retinal thickness were aligned and integrated in the same image to correlate leakage with thickness. Data from the group of individuals with diabetes were compared with those of a healthy control population (N=14; mean age, 48 years; range, 42-55 years) and used to establish reference maps for the RLA and RTA.

Results: Areas of abnormally increased fluorescein leakage were detected in 9 of 10 eyes examined. The increased leakage in 6 (67%) of 9 eyes reached values higher than 40% more than the mean +2 SD RLA control value. Areas of abnormally increased thickness were found in 7 of 10 eyes examined. For the most part, the increases in retinal thickness were not severe (ie, <15% increase in 5 eyes and an 18% increase in 1 eye). The eyes with the most extensive leakage (cases 1, 3, and 9) showed relatively good coincidence between the location of the areas of increased leakage and the location of the areas of increased thickness. In 4 eyes (cases 2, 5, 7, and 8), no such correlation was apparent. The 3 remaining eyes showed little coincidence between these locations. Characteristically, the latter 3 eyes had areas of abnormally increased thickness that were much larger than the areas of increased fluorescein leakage, which were relatively moderate or absent of any leakage.

Conclusions: Localized sites of increased fluorescein leakage and zones of increased retinal thickness were found in most eyes in a series of 10 eyes in the preretinopathy stage from 10 patients with type 2 diabetes. Increases in retinal thickness may be observed that do not coincide with sites of retinal leakage. Two types of increased retinal thickness may, therefore, be present in the preretinopathy stage of diabetic retinopathy, one directly associated with an alteration of the blood-retinal barrier, and another occurring without apparent breakdown of blood-retinal barrier.

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DIABETIC retinopathy, which often is found in patients with diabetes, remains one of the leading causes of visual disability and blindness in economically developed countries. It is a particularly challenging condition since a sharp increase in the prevalence and incidence of diabetes (especially type 2 diabetes) is expected in the next decade.

Studies such as the Diabetes Control and Complications Trial,[1] the United Kingdom Prospective Diabetes Study,[2] the Diabetic Retinopathy Study,[3] and the Early Treatment of Diabetic Retinopathy Study[4] validated methods that are now considered standard in treating diabetic retinopathy. These methods include tight control of blood glucose levels to prevent retinopathy from worsening, and laser photocoagulation to halt progression after the development of clinically significant macular edema or proliferative retinopathy. These landmark studies clearly changed the expected outcomes of diabetic retinopathy by offering better approaches to its management.

Photocoagulation and good glycemic control are important improvements in the management of diabetic retinopathy. They are clearly more effective than what was available 15 years ago. However, despite the success of these approaches, preventable blindness still occurs. Photocoagulation offers a relatively poor treatment alternative because it is a technique that achieves results by destroy-

PATIENTS AND METHODS

PATIENTS

Ten eyes from 10 patients (6 men, 4 women; mean age, 54 years; range, 48-58 years) with type 2 diabetes but no evidence of retinopathy on slitlamp examination and stereo fundus photography (level 10 of Wisconsin grading) were examined with the RLA[6] and the RTA[7] (Talia Technology Ltd, Mevaseret Zion, Israel). Their duration of diabetes ranged from 1 to 10 years (mean duration, 5.6 years). Their glycosylated hemoglobin (HbA_{1C}) value ranged from 0.06 to 0.08 (mean HbA_{1C} value, 0.067) (**Table 1**). The patients were included in a series of patients with type 2 diabetes who had relatively good metabolic control (HbA_{1C} level < 0.08), between the ages of 40 and 60 years (similar to a healthy control population previously examined with RTA and RLA), and with blood pressure levels at or below 155/85 mm Hg.

Stereo fundus photography was performed according the Early Treatment of Diabetic Retinopathy Study protocol.[4] Stereoscopic pairs of fields were obtained using a 30° fundus camera to classify as level 10.

Tenets of the Declaration of Helsinki were followed, and the approval of the institutional review board was obtained.

RETINAL LEAKAGE ANALYZER

The RLA is a new method that quantifies localized fluorescein leakage in humans from the retina into the vitreous across the BRB.[6]

In brief, the instrumentation consists of a confocal scanning laser ophthalmoscope (Carl Zeiss Inc, Thornwood, NY) that was modified to obtain fluorescence measurements in the vitreous. Data and images obtained with this instrument are of a 20° field of view that corresponds to a scanned area 3150 µm wide × 2700 µm high. To build a map showing the permeability index of the BRB to fluorescein, or a retinal leakage map (RLmap), 2 scans are needed to quantitate fluorescein into the vitreous. The first scan is taken less than 5 minutes after the intravenous administration of 14 mg/kg of 20% sodium fluorescein, and the second scan is done 30 minutes after injection. Scans are aligned and the common areas are processed as previously reported.[6]

The RLA obtains an image of the fundus with real 3-dimensional information. Two types of information are obtained simultaneously, one for optical imaging and one representing fluorescence measurements being scanned.

Axial graphics of the fluorescein measurements obtained from the vitreous and representing a volume of 75×75×2550 µm were converted into RLmaps. Multiple measurements of retinal leakage can be graphically assembled in a false-color RLmap that represents the distribution of the BRB permeability indices in any chosen area of the total area of the posterior pole under examination. Intravisit reproducibility and intervisit reproducibility of the method are ±10.2% and ±13%, respectively.[6]

RETINAL THICKNESS ANALYZER

The RTA is a quantitative and reproducible method to evaluate retinal thickness.[5] The optical system is similar to that of a slitlamp biomicroscope, using a helium-neon laser (543 nm) as the illumination source. The system acquires optical images of the retina in sections by projecting the laser onto the retina at an angle, allowing the reflection or scattering of the laser light from the vitreoretinal and chorioretinal interfaces to be viewed. The separation between the reflections (and scatter) from these 2 interfaces is a measure of the retinal thickness. The intersection of the laser slit with the retina—the optical cross-section of the retina—is recorded using a video camera, then digitized. Each measurement covers a 2×2-mm area for a total of 10 optical sections, each separated by 200 µm. Nine scans are performed, covering the central 20° of the posterior pole. The fundus photograph is obtained simultaneously with a separate 60°-field clinical fundus camera. The data are analyzed and the retinal thickness can be represented as a numerical value or a color-coded map in 2 or 3 dimensions.

The total area scanned with the RTA is 6000×6000 µm (30×30 pixels). Each pixel represents an area of 200×200 µm.

Continued on next page

ing the retina. Other forms of therapy, targeted at the earliest stages of retinal disease when the disease process may still be reversible, are urgently needed.

New methods with which to examine the retina, such as the retinal thickness analyzer (RTA)[5] and the retinal leakage analyzer (RLA),[6] have been recently introduced. These systems are able to measure reproducibly in a clinical setting changes in the thickness of the retina and localized alterations of the blood-retinal barrier (BRB). We have applied these methods to the study of diabetic eyes with preclinical retinopathy, looking for changes that may be present before overt ophthalmoscopic retinopathy develops.

RESULTS

Areas of abnormally increased fluorescein leakage were detected in 9 (90%) of 10 eyes examined (**Table 3**). The increased leakage in 6 (67%) of 9 eyes reached values higher than 40% more than the mean RLA control + 2 SD. In 5 (56%) of 9 eyes, the areas of increased leakage covered 50% or more of the retina under examination. In 8 (89%) of 9 eyes, the areas of increased leakage extended to the fovea.

Areas of abnormally increased thickness were found in 7 (70%) of 10 eyes examined (Table 3). For the most part, the increases in retinal thickness were not severe (ie, <15% in 5 eyes and 18% in 1 eye). One eye with substantially increased retinal thickness of 50% also had the most retinal leakage. Areas of increased thickness covering 50% or more of the retina were found in 4 of 7 eyes also with increased leakage. In 5 (71%) of 7 cases, the areas of increased thickness did not involve the fovea. Increased retinal thickness in the central 500 µm of the foveola was found in only 2 eyes when the retinal thickening covered most of the retina examined (63% and 100% increases, respectively) and when intense leakage was associated. One eye had abnormally increased retinal thickness without associated abnormal increase in leakage. This patient had diabetes for 2 years and had a HbA_{1C} value of 0.06.

DATA ANALYSIS

Data from the group of individuals with diabetes were compared with those of a healthy control population (N=14; mean age, 48 years; range 42-55 years) used to establish reference maps for the RLA and RTA (**Figure 1** and **Figure 2**).

Owing to differences in resolution between the RLA and RTA, a value of leakage is obtained for an area of 75×75 µm, while a single value of thickness covers an area of 200×200 µm. Therefore, the smallest area that can be constructed that contains integer numbers of leakage and thickness values is 600×600 µm (ie, 8×8 values of leakage and 3×3 values of thickness).

To compare information from both the RLA and RTA using the more detailed data from the leakage maps, we chose areas of 300×300 µm. The maps, therefore, are composed of 63 (9 × 7) values, each representing an area of 300×300 µm, for a total of 2700×2100 µm. For each 300×300 µm area, the value of leakage is the mean and SD of 16 (4 × 4) values.

To obtain a value of thickness for an equivalent area of 300 × 300 µm, we performed a 2-step procedure. First, values of thickness were computed as the mean and SD of 9 (3×3) values, corresponding to an area 600 × 600 µm. Afterward, this area is split into 4 adjacent areas of 300 × 300 µm, and the mean and SD values obtained for the 600 × 600 µm area are now given to each of these 4 adjacent areas.

In this healthy control population, the mean values of the 63 mean values for leakage and thickness are 21.29 × 10^{-7} cm per second and 168.13 µm, respectively.The mean SDs for leakage and thickness are 6.19 × 10^{-7} cm per second and 22.56 µm, respectively. The relatively large SD for leakage, registered after averaging 14 RLmaps, is expected considering the resolution of the system associated with regional variations in vascular distribution. The center of the fovea corresponds to the point of intersection of the thicker central lines in the reference maps. Figures 1 and 2 present reference values for leakage and thickness for the right eye of the healthy control population. By comparing values from the patients with those of the controls, we can now compute maps of increased leakage or increased thickness as percentages.

All of the increases are computed considering the reference mean +2 SD. For instance, if a particular area of the sample population has a mean value of 150 and an SD of 15, the computed limit is 150 +2 × 15=180. For that particular area, a value of 207 would represent an increase of 15% over the reference value.

Both the grid and the circles depict the relative positions of the morphological structures, the permeability index shown in the RLmaps, and the retinal thickness shown in the RTA maps.

With these methods, we are able to identify and quantitate sites of increased leakage and increased thickness and measure their distance from the fovea.

To compute the distance from the fovea of altered areas (areas of either increased leakage or increased thickness), we have taken into account that the resolution of the RLmap is more than 7 times the resolution of the thickness map. For leakage, we have considered the distance in microns from the center of the fovea to the nearest corner of an altered area.

For thickness, the minimum distance of an altered area is computed as the distance in microns from the center of the fovea to the center of the altered area.

Correlations were established between the location and severity of the abnormal increases in both maps.

To compute "areas of coincidence" and "areas of no coincidence" we have to consider 4 possible situations: (1) for a particular area there are alterations both in leakage and in thickness (area of coincidence); (2) for a particular area there is an alteration in leakage but not in thickness (area of no coincidence); (3) for a particular area there is an alteration in thickness but not in leakage (area of no coincidence); and (4) for a particular area there is no alteration either in leakage or in thickness.

For patients in whom there is an alteration in leakage, thickness, or both that covers the entire map, the sum of cases 1, 2, and 3 is 100% (patient 3, **Table 2**). For the other patients, for whom there are areas of neither increase in leakage nor in thickness, the sum of the same cases (1, 2, and 3) is less than 100%, and the sum for all possible situations (1, 2, 3, and 4) is 100%.

Table 1. Characteristics of Diabetic Population in Study

Patient No.	Age, y	Duration of Diabetes, y	HbA_{1C}	Blood Pressure, mm Hg
1	56	5	7.0	140/70
2	51	4	4.9	140/80
3	53	7	6.0	150/70
4	54	6	7.4	140/70
5	48	5	7.9	120/80
6	58	7	6.1	150/85
7	53	2	5.5	140/80
8	55	1	7.6	130/80
9	55	9	6.9	120/70
10	56	10	7.4	155/85

Eyes with extensive leakage (cases 1, 3, and 9) showed relatively good coincidence between the location of the areas of increased leakage and the location of the areas of increased thickness (**Figure 3**). In 4 eyes (cases 2, 5, 7, and 8) no such correlation was apparent (Table 2). The 3 remaining eyes (cases 4, 6, and 10) showed little coincidence between these locations (**Figure 4**).

Two of these latter 3 eyes had areas of abnormally increased thickness much larger than the areas of increased fluorescein leakage, which were relatively moderate. Furthermore, the center of the fovea was spared in all of these eyes and the increase in retinal thickness remained outside a 500-µm radius centered on the foveola.

COMMENT

Localized sites of increased fluorescein leakage and zones of increased retinal thickness were found in most eyes in a series of 10 eyes in the preretinopathy stage from 10 patients with type 2 diabetes. The diabetes in this group of patients was reasonably well controlled (average HbA_{1C} value, 0.07).

Abnormal increases in fluorescein leakage were present in 90% of examined eyes and abnormal increases in

36	34	34	31	29	29	30	34	36
36	33	34	33	31	29	28	33	32
31	34	34	33	29	28	30	33	35
29	31	37	33	25	25	28	32	36
35	38	38	35	31	26	30	32	40
32	35	36	36	37	33	36	37	41
30	37	42	39	39	40	39	40	44

Figure 1. *Retinal leakage analyzer reference map ($\times 10^{-7}$ cm per second).*

212	212	207	207	209	209	213	213	216
217	217	205	205	191	191	206	206	227
217	217	205	205	191	191	206	206	227
206	206	190	190	169	169	189	189	230
206	206	190	190	169	169	189	189	230
212	212	206	206	202	202	214	214	230
212	212	206	206	202	202	214	214	230

Figure 2. *Retinal thickness analyzer reference map ($\times 10^{-7}$ cm per second).*

Table 2. Coincidence Between Increased Leakage and Increased Thickness*

Patient No.	Area of Coincidence	Area of No Coincidence	Area of Increased Leakage Without Increased Thickness	Area of Increased Thickness Without Increased Leakage
1	48	47	31	16
2	0	3	3	0
3	96	4	0	4
4	27	54	10	44
5	0	63	63	0
6	3	36	33	3
7	0	33	0	33
8	0	51	51	0
9	41	56	54	2
10	19	49	6	43

**Areas of coincidence and no coincidence are presented as percentages of the examined area.*

Table 3. Data for Leakage and Thickness in Each Eye Examined*

	Leakage			Thickness		
Patient No.	Abnormally Increased, % of Examined Area	Maximum Increase, %	Distance From the Fovea, µm	Abnormally Increased, % of Examined Area	Maximum Increase, %	Distance From the Fovea, µm
1	79	80	0	63	10	0
2	3	9	670	0	0	. . .
3	96	85	0	100	50	0
4	36	42	0	71	14	600
5	63	71	0	0	0	. . .
6	37	36	0	6	6	1200
7	0	0	. . .	33	9	600
8	51	60	0	0	0	. . .
9	95	87	0	43	8	600
10	25	27	0	62	18	600

**Ellipses indicate not applicable.*

retinal thickness were found in 70% of the eyes examined, even though no other abnormalities were visible in the retina or detected by ophthalmoscopy or 7-field stereo fundus photography.

The increases in fluorescein leakage were, in general, marked with sites reaching values 80% above normal mean + 2 SD values obtained from a healthy control population within a similar age range. Most eyes with increases in retinal thickness were about 15% above the normal mean + 2 SD values registered in the control population.

These findings have clear clinical value, since increased retinal fluorescein leakage identifies an alteration of the BRB and increased retinal thickness characterizes the presence of retinal edema. Retinal edema, the major cause of visual loss in people with type 2 diabetes,[8] has been often associated in the retina with an alteration of the BRB. In our study, this finding occurred in only 6 of 10 eyes examined, and only when the alteration of the BRB was most marked. In the other 4 eyes, however, there was no direct correlation between the

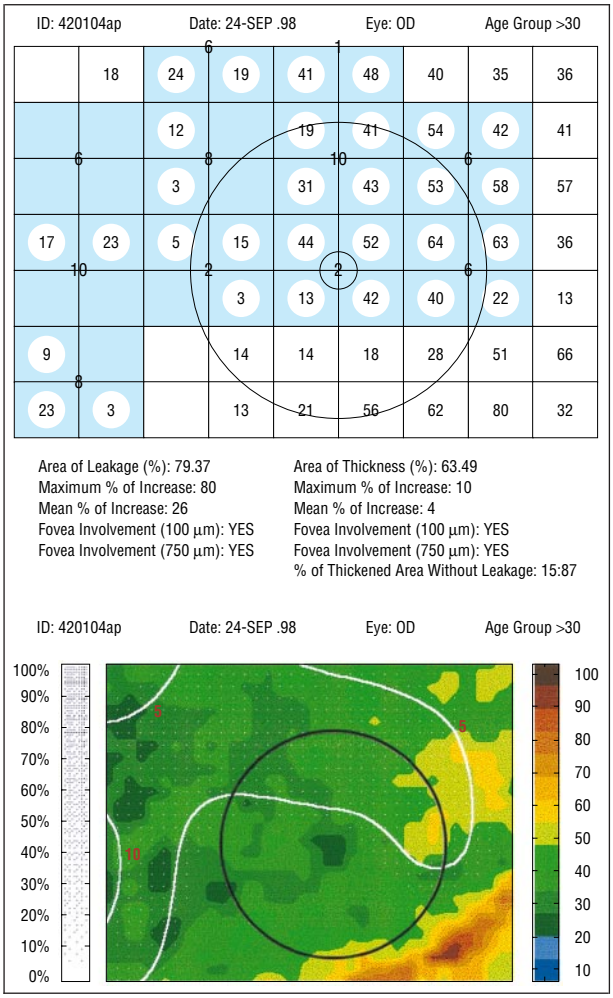

Figure 3. *Patient 1. Top, Values map. The gray areas represent increased thickness. Percentages of increase are represented in bold at intersecting lines. Each value represents 4 adjacent squares. Percentages of increased leakage are represented by numbers in the squares. Also represented are 2 concentric circles of 100-µm and 750-µm radii centered on the fovea. Bottom, Same eye with background representing the leakage using a false color code. Units are $\times 10^{-7}$ cm per second. Percentage of increased thickness is represented in white dots with varying density. The density bar on the left shows the relation between density and percentage of increase in thickness. Lines in white represent equal percentage of increase in thickness, having the corresponding value plotted in red. The black circle is centered on the fovea with a 750-µm radius.*

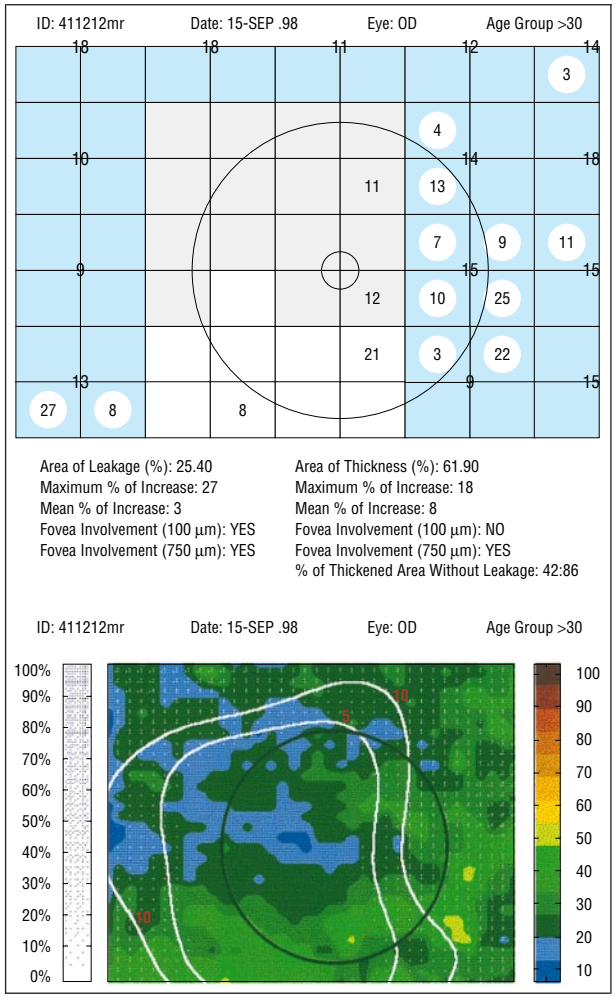

Figure 4. *Patient 10. Top, Values map. The gray areas represent increased thickness. Percentages of increase are represented in bold at intersecting lines. Each value represents 4 adjacent squares. Percentages of increased leakage are represented by numbers in the squares. Also represented are 2 concentric circles of 100-µm and 750-µm radii centered on the fovea. Bottom, Same eye with background representing the leakage using a false color code. Units are $\times 10^{-7}$ cm per second. Percentage of increased thickness is represented in white dots with varying density. The density bar on the left shows the relation between density and percentage of increase in thickness. Lines in white represent equal percentage of increase in thickness, having the corresponding value plotted in red. The black circle is centered on the fovea with a 750-µm radius.*

zones of increased retinal thickness and the sites of abnormal fluorescein leakage. Furthermore, areas of increased retinal thickness were detected in the absence of abnormal fluorescein leakage.

Retinal edema should be considered as any increase of water of retinal tissue resulting in an increase in its volume (ie, thickness). This increase in water content of the retinal tissue may, as with the brain, be classified by type as vasogenic or cytotoxic.[9] In the first case, vasogenic edema, the increase is the result of breakdown of the BRB with extracellular deposition of macromolecules. The primary defect is in the BRB and the accumulation of fluid is extracellular. There is, in this situation, increased retinal thickness associated with increased fluorescein leakage. The fluorescein leakage itself, particularly when examined within 30 minutes after fluorescein injection, is considered the result of increased inward BRB permeability rather than owing to a defective active outward transport.[10]

The cytotoxic type of edema, extensively described and studied in brain abnormalities, has been associated with excitatory release of glutamate or lactic acidosis and occurs in the absence of barrier breakdown.[11] Its presence in the retina in the initial, preclinical stages of diabetic retinal disease is suggested by our findings, but is still open to question. Alternative explanations must be considered. Sites of localized retinal edema without an associated site of breakdown of the BRB could represent areas of residual extracellular edema of the vasogenic type, resulting from previous leaking sites that have stopped leaking at the moment of the present examination. Areas of retinal edema may also result from alterations of the BRB occurring at relatively distant sites and without

an apparent direct correlation. Preferential pathways for fluid movement may occur in the retina, leading to accumulation of fluid in particular zones of the retina. Another alternative explanation for simultaneous observation in the same retina of areas of coincidence and areas of no coincidence between sites of leakage and areas of increased retinal thickness may be derived from the possibility of varying rates of retinal fluid reabsorption in areas of extracellular vasogenic edema. Only a prospective longitudinal study designed to follow in successive visits the sites of breakdown of the BRB and associated changes in retinal thickness may clarify these questions.

In summary, this study indicates that in the initial stages of diabetic retinal disease, breakdown of the BRB and retinal tissue thickening may occur simultaneously, either in association with each other or independently. From this study it is not clear which occurrence precedes the other. The BRB damage may modulate and play a role in the development of retinal tissue thickening, but the opposite is also a possibility.

Further studies of the natural history of the earliest stages of diabetic retinal disease, using new clinical methods of examination such as the RLA and the RTA in a prospective fashion, are expected to contribute to our understanding of the development of diabetic retinopathy.

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