Published in final edited form as:

Retina. 2008 October ; 28(8): 1097–1103. doi:10.1097/IAE.0b013e318177f8ec.

Correlation Between Microaneurysms and Retinal Thickness in Diabetic Macular Edema

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Abstract

Background or Purpose—Microaneurysms commonly are believed to be related causally to retinal thickening in diabetic retinopathy, especially by leaking. We tested the hypothesis that thicker areas of retina in diabetic retinopathy have more microaneurysms per unit area than areas that are not as thick.

Methods—Retinal thickness analysis was performed with a prototype instrument in 27 eyes of 27 diabetic patients and in 22 normal eyes of 22 healthy subjects. Maps of retinal thickness were created, and microaneurysms were counted in zones having four levels of retinal thickness.

Results—There was no increase in either total microaneurysms or apparent leaking microaneurysms per unit area with increasing levels of retinal thickness ($P=0.77$ and 0.87 , respectively).

Conclusion—Some microaneurysms may not cause thickening, or other factors may contribute to retinal thickening in diabetic retinopathy. The results may have implications on the pathogenesis of diabetic macular edema.

Keywords

Blood-retinal barrier; Diabetic retinopathy; Macular edema; Microaneurysms; Retinal thickness analysis

INTRODUCTION

Diabetic macular edema is an important cause of visual loss in our society,^{1,2} and it is characterized by retinal thickening. Microaneurysms commonly are believed to be related causally to this retinal thickening, especially by leaking. Until recently it has not been possible to measure the thickness of the retina with high spatial resolution across large areas of the macula. Therefore, it has not been possible to quantitatively correlate areas of thickened retina with the location of microaneurysms.

Retinal thickness analysis was first described by Zeimer and coworkers in 1989.³ We have described how this method can be extended to map the retinal thickness in a 6 mm × 6 mm

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None of the authors has any proprietary interests related to the study.

area of the macula with a depth resolution of 50 μm .⁴ Although optical coherence tomography can measure retinal thickness with a depth resolution of 10 μm ,⁵ currently available algorithms allow measurements only in six radial lines passing through the fovea or in a circle concentric with the fovea. Thus, the thickness in large areas of central macula is not directly measured, but interpolated. However, spectral domain optical coherence tomography recently has become available that allows higher speed image acquisition and thus is overcoming this limitation.^{6,7} Retinal tomography and topography can be performed with the scanning laser ophthalmoscope, but its depth resolution is only 300 μm .⁸

Nishiwaki and coworkers found no increase in retinal thickness near microaneurysms in diabetic patients with very mild retinopathy (only 6.2 posterior pole microaneurysms on average).⁹ We were surprised by this result and suspected that this would not be the case in more severe retinopathy. Thus, we formed the hypothesis that thicker regions of retina in diabetic macular edema have more microaneurysms per unit area than regions that are not as thick. We used retinal thickness analysis to test our hypothesis.

METHODS

This investigation was approved by the Institutional Review Board of the University of Illinois. We studied 27 eyes of 27 diabetic patients referred for fluorescein angiography in the course of evaluation and management of macular edema. Diabetic patients were included if they met the following criteria: type 1 or type 2 diabetes, clear media, good fixation, good pupillary dilation, five or fewer drusen, no previous macular laser treatment, and no other diseases that might change retinal thickness. Included as controls were 22 normal eyes of 22 healthy subjects.

Retinal thickness was performed with a custom-built version of the retinal thickness analyzer according to methods described previously.^{4,10,11} To summarize briefly, a laser slit beam measuring 2 mm $\times$ 15 μm ($\lambda = 543 \text{ nm}$) was projected on the retina. Ten slit images were acquired in each of nine 2 mm $\times$ 2 mm regions. The retinal thickness at each 50 μm segment along the length of each slit was calculated using a computer algorithm. From these data the retinal thickness at each 200 μm $\times$ 200 μm location of the 6 mm $\times$ 6 mm region (that is, 900 locations) was determined. The normal retina varies in thickness by location. Thus, the relative retinal thickness at each location was calculated as the thickness at a given location divided by the mean thickness at the same location in the normal subjects. We divided the measured area of retina into four levels of relative retinal thicknesses: 1.10, 1.11–1.30, 1.31–1.50, or >1.50 times normal. These levels were chosen before the study to divide retinal areas into those having thicknesses near the mean, higher than the mean or possible slight thickening, definite but mild thickening, and substantial thickening, respectively. Maps depicting these levels were superimposed on the fluorescein angiograms of the diabetic patients (Fig. 1).

Optical coherence tomography was not performed in most eyes. When performed, standard methods as recommended by the manufacturer (Stratus, Carl Zeiss Meditec, Dublin, CA) were utilized (Fig. 2).

Two experienced vitreoretinal specialists counted the microaneurysms in areas having the relative retinal thickness levels of 1.10, 1.11–1.30, 1.31–1.50, or >1.50 in each eye. Punctate foci between 20 and 125 μm in diameter at least as fluorescent as the major vessels in the early venous phase were counted as microaneurysms. Diffuse leakage from dilated capillaries and clear-cut capillary nonperfusion were rare in the eyes in this study. It was difficult to quantify the magnitude of leakage from the microaneurysms, but microaneurysms that did not leak could be identified on the late-phase angiograms. We counted both the total number of microaneurysms and the number of nonleaking microaneurysms. We calculated the number of apparent leaking microaneurysms as the total microaneurysms minus the nonleaking microaneurysms.

Also, we calculated the concentration of microaneurysms in each of the four levels of retinal thickness in each eye. We did this by dividing the number of microaneurysms in each thickness level by the area of retina having that thickness level. This calculation was made for both the total microaneurysms and the apparent leaking microaneurysms.

The data were examined statistically using the chi square, Student's *t*-test, analysis of variance, and linear regression analyses as appropriate. The results were considered significant if the probability that they could have arisen by chance alone was less than 5%.

RESULTS

The demographic characteristics of the two groups are presented in Table 1. The means and SD of the ages were similar in the two groups: 57 ± 10 and 57 ± 12 years in the normal subjects and diabetic patients, respectively. There were slightly fewer males among the normal subjects (8 of 22) than among the diabetic patients (14 of 27), but this difference was not statistically significant. Although there was a preponderance of blacks among the diabetic patients (12 of 27) as compared with the normal subjects (2 of 22), we have not found substantial differences in retinal thickness by race in previous studies.¹² Eleven of 22 right eyes in the normal subjects and 13 of 27 right eyes in the diabetic patients were included in the analysis.

Additional information was available from 25 diabetic patients. The mean age at onset of diabetes $\pm$ SD was 42 ± 13 years. The mean duration of diabetes $\pm$ SD was 15 ± 9 years. Of the patients, 23 had type 2 diabetes, and 12 of them were being treated with insulin. Nineteen had systemic hypertension, and none was noted to have renal disease.

There was a high correlation between the counts of both total and nonleaking microaneurysms by the two observers ($r = 0.94$ and 0.90 , respectively) (Figs. 3 and 4). We used the mean of the counts from the two observers for further calculations. The mean number of microaneurysms $\pm$ SD in the $6 \text{ mm} \times 6 \text{ mm}$ measured region of eyes of diabetic patients was 63 ± 45 . Retinal thickness values greater than 1.25 times normal were outside the 95% confidence limit for normal thickness and were considered to be abnormal thickness. Of the 27 eyes of diabetic patients, 24 had at least two locations that were abnormally thickened. The mean number of thickened locations in each eye of diabetic patients $\pm$ SD was 115 ± 141 (range, 0–466) from the 900 locations measured in each eye.

The means $\pm$ SD of the areas of retina having retinal thickness levels of 1.10, 1.11–1.30, 1.31–1.50, and >1.50 were 21.1 ± 10.5 , 11.6 ± 7.2 , 4.4 ± 3.7 , and 2.3 ± 3.2 mm², respectively (Table 2). Analysis of variance indicated that the probability that these means were equal was extremely low. The values of total and apparent leaking microaneurysms per mm² for each of these thickness levels in each eye are presented in Figures 5 and 6. The means $\pm$ SD of total microaneurysms per mm² in areas of retina having retinal thickness levels of 1.10, 1.11–1.30, 1.31–1.50, and >1.50 were 1.7 ± 1.5 , 2.0 ± 2.2 , 1.5 ± 1.4 , and 1.5 ± 1.8 , respectively (Table 2). By analysis of variance, none of these means differed from any of the others statistically ($P=0.77$). The means and SD of apparent leaking microaneurysms per mm² in areas of retina having retinal thickness levels of 1.10, 1.11–1.30, 1.31–1.50, and >1.50 were 1.1 ± 1.1 , 1.4 ± 1.6 , 1.1 ± 1.1 , and 1.1 ± 1.3 , respectively (Table 2). By analysis of variance, none of these means differed from any of the others statistically ($P=0.87$).

DISCUSSION

We found no statistically significant increase in concentration of either total microaneurysms or apparent leaking microaneurysms in the thicker retinal regions in eyes with diabetic retinopathy, and there was no trend toward such a relation in the data. This finding was unexpected and raises two issues. First, it conflicts with the notion prevalent among many who are familiar with fluorescein angiography and biomicroscopy of the retina that the concentration of microaneurysms tends to be higher in areas of macular edema. Second, it suggests that the concentration of microaneurysms is not a major contributor to the causation of retinal thickening in diabetic macular edema.

There are several possible explanations for the discrepancy between our findings and the dominant current view. First, the sample size may have been too small. However, a sample size of 27 patients should have shown at least a trend if the relation between microaneurysm concentration and retinal thickness actually is strong.

Second, the microaneurysms were not counted with masking as to the thickness of the areas counted. We strongly expected to find that thicker areas had higher concentrations of microaneurysms. To the extent that this bias is present, the relation between microaneurysm concentration and retinal thickness actually is even weaker than we have stated.

Third, the method of evaluating retinal thickness may have been flawed. This seems unlikely since the method has been developed, established, and applied by our group for more than 10 years.^{9-11,13-23} Furthermore, retinal thickness results have been shown to be highly correlated with those of optical coherence tomography^{24,25} and the confocal scanning laser ophthalmoscope.²⁶

Fourth, some of the hyperfluorescent foci interpreted as microaneurysms actually may have been drusen or retinal pigment epithelial window defects. However, we excluded patients in whom more than five of these lesions were identified on color photography or clinical examination. Even if we had mistakenly included some of these lesions, they would have been counted as nonleaking microaneurysms since they do not leak on fluorescein

angiography. However, when we calculated the concentration of apparent leaking microaneurysms, there still was no preponderance in the zones of greater thickening.

Consideration of the pathophysiology of diabetic macular edema may help clarify the lack of correlation between the concentration of microaneurysms and retinal thickening. First, it may be that it is not how many leaks there are but rather how severe the leakage is from the individual leaks. Regrettably, it is notoriously difficult to quantify leakage on fluorescein angiograms.

Second, regions of fluorescein leakage and retinal thickening appear to overlap imperfectly. Some regions may have leakage without thickening and other regions may have thickening without leakage. We previously found a substantial number of retinal areas in diabetic patients with normal thickness but with leakage on fluorescein angiography.¹⁵ Lobo and coworkers used retinal thickness analysis and a sophisticated method of mapping the leakage of fluorescein from the retina into the vitreous in diabetic patients.²⁷ In addition to areas with both retinal thickening and fluorescein leakage, they found sizeable areas that had leakage without thickening. Thus, some microaneurysms, even if they leak, may not cause or be associated with thickening.

Conversely, factors other than microaneurysms and leakage may contribute to retinal thickening in diabetic retinopathy. One effect of injury is cellular swelling, termed cytotoxic edema.²⁸ This differs from vasogenic edema, which is caused by breakdown of the blood-retinal barrier, and in which the fluid is predominately extracellular.²⁹ Cytotoxic edema could result from a variety of perturbations in diabetes, such as ischemia. This type of edema would not be expected to coincide closely with microaneurysms or leakage. Lobo and colleagues, in their study, also found substantial areas that were thickened without leakage and discussed cytotoxic edema.²⁷

Another factor that may contribute to retinal thickening is reduced removal of fluid from the retina. The retinal thickness is related to a balance between the entry and exit of fluid from it. Regrettably, little is known about this latter process in diabetes, and we lack methods to assess it in patients. We look forward to improved methods capable of determining the relative contribution of these factors to retinal thickening in diabetic macular edema.

Acknowledgments

Supported in part by research grant EY 10314 and core grant EY 1792 from the National Eye Institute, Bethesda, MD; the Illinois Eye Fund, Chicago, IL; the Department of Veterans Affairs, Washington, DC; and an unrestricted grant from Research to Prevent Blindness, Inc, New York, NY.

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Summary Statement

We used retinal thickness analysis to test whether thicker areas of retina in diabetic retinopathy have more microaneurysms per unit area than do thinner areas. The finding that thicker areas did not have more microaneurysms may have implications on the pathogenesis of diabetic macular edema.

Figure 1.

Fluorescein angiogram from a representative patient showing microaneurysms. A. Square outlines the area in which retinal thickness was measured. B: Same angiogram with relative retinal thickness data (retinal thickness/normal thickness at the same location) superimposed. Locations with relative thickness levels of ≤ 1.10 , 1.11–1.30, 1.31–1.50, and >1.50 are blue, green, yellow, and red, respectively.

Figure 2.

Optical coherence tomography from the same patient shown in Figure 1. A. Angiogram with line depicting site of optical coherence tomogram. B. Tomogram with superior temporal aspect to the left and inferior nasal aspect to the right. Note marked thickening with intraretinal reflectile foci consistent with hard exudates to the left. C. Topography with color indicating the retinal thickness according to the key on the horizontal axis. Note the high degree of agreement with retinal thickness analysis in depicting thickness.

Figure 3.

Counts of total microaneurysms by two observers on fluorescein angiograms from diabetic patients.

Figure 4.
Counts of microaneurysms without leaking by two observers on fluorescein angiograms from diabetic patients.

Figure 5.
Concentration of total microaneurysms in regions with relative thickness levels of ≤ 1.10 , 1.11–1.30, 1.31–1.50, and >1.50 .

Retina. Author manuscript; available in PMC 2015 December 17.

Table 1

Demographic Characteristics of Controls and Diabetic Patients

	Controls	Diabetic Patients	<i>P</i>
No. of eyes	22	27	
Mean age (y) $\pm$ SD	57 $\pm$ 10	57 $\pm$ 12	0.89
Sex, no.			
Male	8	14	0.28
Female	14	13	
Race, no.			
White	17	15	0.007
Black	2	12	
Asian	3	0	
Right	11	13	0.90
Left	11	14	

Table 2

Area and Concentration of Microaneurysms by Relative Retinal Thickness Level in Diabetic Retinopathy

Relative Retinal Thickness Level	<1.10	1.11–1.30	1.31–1.50	>1.51	P
No. of eyes	27	27	16	6	
Mean area (mm ²) ±SD	21.1 ± 10.5	11.6 ± 7.2	4.4 ± 3.7	2.3 ± 3.2	<0.001
Mean concentration of total microaneurysms (microaneurysms/mm ²)±SD	1.7 ± 1.5	2.0 ± 2.2	1.5 ± 1.4	1.5 ± 1.8	0.77
Mean concentration of apparent leaking microaneurysms (microaneurysms/mm ²)±SD	1.1 ± 1.1	1.4 ± 1.6	1.1 ± 1.1	1.1 ± 1.3	0.87